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**Amino Acids  
and Peptides**  
**VOLUME 21**

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# Amino Acids and Peptides

## Volume 21

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during 1988

Senior Reporter

**J. H. Jones**, *University of Oxford*

Reporters

**G. C. Barrett**, *Oxford Polytechnic*

**J. S. Davies**, *University College of Swansea*

**D. T. Elmore**, *Queen's University Belfast and  
University of Oxford*

**P. M. Hardy**, *University of Exeter*

**R. W. Hay**, *University of St Andrews*

**K. B. Nolan**, *The Royal College of Surgeons in Ireland*

**A. V. Stachulski**, *Beecham Pharmaceuticals*

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# Preface

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And so the series comes of age. The one and twenty years reviewed have seen a relentless increase in output, and a few volumes back it became necessary to jettison the coverage of protein chemistry which was part of the original brief, simply in order to stay afloat. The present volume is still longer than the first, has more literature citations, and is more compressed. The chapters on amino acids, and on peptide synthesis, for example, now cover on average between two and three times as many primary publications as they did initially. It has been an era of fundamental advance. Proteins have been synthesised, and really rational peptide-related drug design is now possible. Who knows what will have been achieved by the turn of the century? Purpose-built completely unnatural enzymes for industrial processes, perhaps. Such speculations are hostages to fortune, but today's dreams can become tomorrow's everyday realities. In the first volume of the series, I wrote "It may even be that advances in nucleotide chemistry will overtake improvements in peptide synthesis - the best way of obtaining synthetic enzyme analogues might then be by instruction of biological protein-synthesising systems with artificial messenger molecules." This seemed rather wildly improbable at the time, even to me as I wrote it - I was just trying to be smart, as young men will. From the standpoint of staid middle age, replete with retrospective wisdom, it sounds a rather obvious bit of philosophising. I must hasten to point out, however, that I have also got things wrong in a big way. My comments on solid phase peptide synthesis in the early volumes of the series were in general over critical, with more emphasis on the problems and failures of the approach than its merits and successes. In this I reflected the prevailing climate of opinion among those bred up on classical organic chemistry, and the precept that synthesis without fully characterised intermediates was somehow not really cricket. Now, of course, solid phase peptide synthesis dominates the field, and when used critically is a very powerful tool. Again, I ventured to air on a number of occasions thoughts to the effect that although the chemical synthesis of proteins was a magnificent thing, I could not conceive a major problem which might be sufficiently advanced by the synthesis of a relevant protein for the enormous labour and expense to be justifiable. This puts the limits of my imagination on record: AIDS was not then on the agenda. Therein lies the greatest current challenge to molecular biology, and there can be little doubt that peptide chemistry has an important part to play in meeting it.

Balliol College, Oxford

John Jones



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# *Abbreviations*

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Abbreviations for amino acids and their use in the formulation of derivatives follow, with rare exceptions, the 1983 Recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature, which are reprinted as an Appendix in Volume 16 of this title. Exceptions and additions are defined in the text as they occur.

# 1

## Amino Acids

---

BY G. C. BARRETT

### 1 Introduction

The literature on amino acids, taken as a whole, includes elegant lessons in biology for the chemist, and conversely provides insights for the biologist into relationships between molecular structure and properties. It will please this Reviewer if some sense of this duality continues to be conveyed in this Chapter, which has, as usual, been confined to the occurrence, chemistry and analysis of amino acids. The Chapter is arranged into sections as used in all previous Volumes of this Specialist Periodical Report.

Access to the literature for creating this Chapter has been by way of Chemical Abstracts (to Volume 110, issue 9) and Biological Abstracts (to issue 8 of Volume 85), supplemented by scanning major journals so as to cover adequately the literature of 1988. The abstracts coverage also nets a few citations published in 1987, and these are included to give continuity for the topic over the years in this series of Specialist Periodical Reports.

### 2 Textbooks and Reviews

One of the Tetrahedron 'Symposia-in-Print' series (which comprise collections of original research papers), is devoted to  $\alpha$ -amino acid synthesis, describing current themes and practice in this field.<sup>1</sup> Reviews have appeared on methionine sulphoxide<sup>2</sup> and cross-linking amino acids in proteins,<sup>3</sup> the latter in a Volume of that covers other amino acids in the protein context. Further reviews are cited in the relevant sections later in this Chapter.

A Russian language text<sup>4</sup> originates from a research group active in the amino acids field.

### 3 Naturally Occurring Amino Acids

**3.1 Isolation of Amino Acids.**— While this would be thought of as a routine topic, there is a salutary lesson in the comparison of five different extraction methods for Serpula lacrimans;<sup>5</sup> the amino acid profile determined for this fungus varies widely, depending on the extraction procedure.

The adsorption of N-acetyl cysteine from solution on to activated carbon has been described.<sup>6</sup>

**3.2 Occurrence of Known Amino Acids.** - There is a vast and continuing literature on familiar amino acids in familiar biological contexts, and this is almost entirely excluded from this Chapter. This Section is restricted, as in previous Volumes of this Specialist Periodical Report, to the occurrence of unusual amino acids, and other significant relevant observations.

Amino acids present in carbonaceous chondrite meteorites have been reviewed.<sup>7</sup> The content of ornithine in fossil bones increases with age;<sup>8</sup> in samples of known age (1 100 - 37 000 years), reasonable linearity of correlation of ornithine content with age has been established, which suggests a useful method for fossil dating, as an alternative to enantiomer-ratio age determination (see Section 6.1).

The simplest amino acids continue to be found as their betaines in natural sources. Glycine betaine occurs in *Echinacea purpurea* and *angustifolia*, then finds its way into pharmaceutical preparations;<sup>9</sup> L-alanine betaine occurs in the marine green alga *Cladophora*.<sup>10</sup> Hydroxylated analogues of simple amino acids include  $\beta$ -hydroxy-L-valine in fruiting bodies of *Pleurocybella porrigens*,<sup>11</sup> and  $\beta$ -hydroxyaspartic acid and N<sup>6</sup>-hydroxyornithine (with homoserine and citrulline) in pyoverdins and azotobactins.<sup>12</sup> The isolation of (S)-(+)- $\alpha$ -methylserine in *Sphagnum palustre* represents the first observation of the occurrence of this amino acid in plants.<sup>13</sup>  $\alpha$ -Hydroxymethylserine occurs (with L-citrulline) in "tianhuafen", the root tuber of *Trichosanthes kirilowii*.<sup>14</sup>

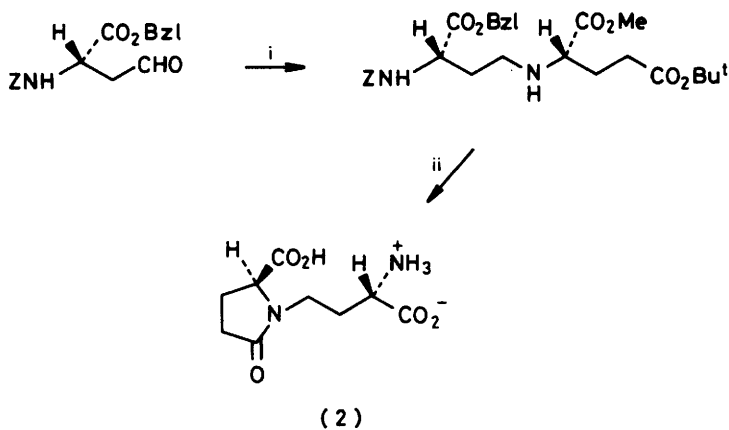
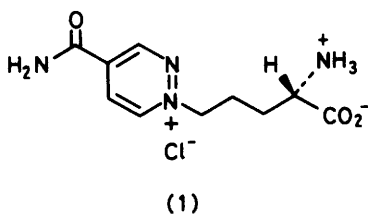
The continuing fascination of phycobiliproteins (see Vol.20, p.1) from cyanobacterium *Nastigocladus laminosus* is expressed in the report<sup>15</sup> that these contain three N<sup>6</sup>-methylasparagine residues. Occurrences of other relatively simple  $\alpha$ -amino acids and analogues include 1-(malonylamino)cyclopropane-1-carboxylic acid in soya bean (*Glycine soja*) seedlings,<sup>16</sup> N<sup>6</sup>-malonyl-D-tryptophan as the only D-amino acid that accumulates during wilting of tomato leaves,<sup>17</sup> (S)-4-chloro-tryptophan in seed protein of the pea plant (*Pisum sativum*),<sup>18</sup> and 4-amino-anthranilic acid in *Streptomyces flocculus*.<sup>19</sup> The last-mentioned ' $\beta$ -amino acid', not previously observed in Nature, is an important discovery as a part of a new shikimate pathway. Another "first observation" is of 2-acetylamino-3-hydroxy-4-methyloct-6-enoic acid in *Neocosmospora vasinfecta* E.F.Smith;<sup>20</sup> the amino acid itself is well known as a constituent of the peptide cyclosporin A (which is also produced by this fungus).

A careful study<sup>21</sup> has established the absence (contrary to previous reports) of  $\beta$ -leucine in human blood.

The report<sup>22</sup> of the presence of 1-amino-2-propanol in *Onopordum acanthium* (ca 400 mg g<sup>-1</sup> fresh weight) and 11 other Compositae is worth recording in this Chapter (it is not an amino acid, but is at least, a close relative).

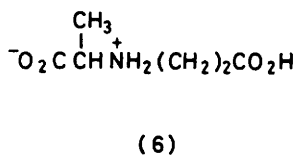
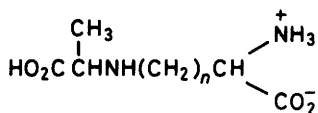
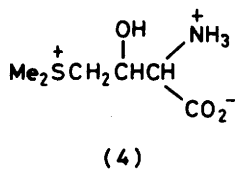
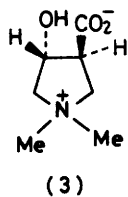
**3.3 New Natural Amino Acids.** - The L-ornithine-based  $\alpha$ -amino acid (1) is a new antifungal agent (from *Streptomyces violaceoniger griseofuscus* Tü 2557.<sup>23</sup> It is





Reagents: i, H-Glu(OBu<sup>t</sup>)OMe and NaBH<sub>3</sub>CN; ii, routine elaboration

**Scheme 1**



the first pyridazine discovered among microbial secondary metabolites. A compound from the mushroom *Lactarius piperatus*<sup>24</sup> that is, at first sight, similar to ( 1 ) is, however, on closer inspection, clearly shown to be an *N*-alkylated L-glutamic acid ( 2 in Scheme 1). The n.m.r. structure determination of ( 2 ) has been verified through its straightforward synthesis from  $\gamma$ -t-butyl-L-glutamic acid.<sup>24</sup>

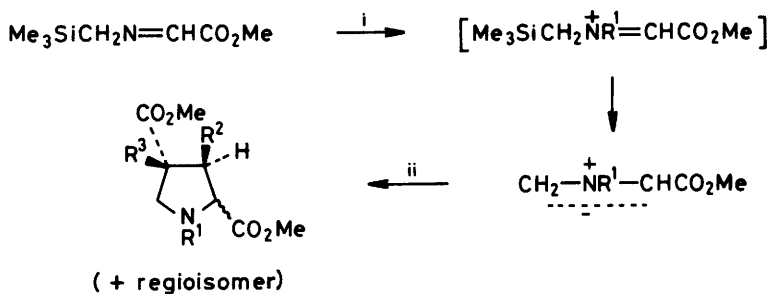
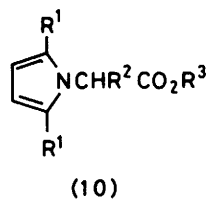
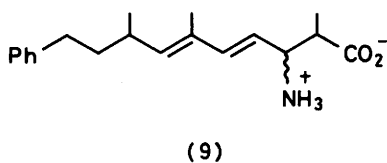
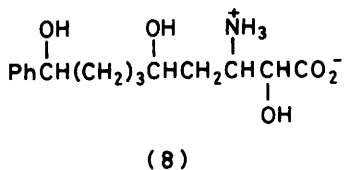
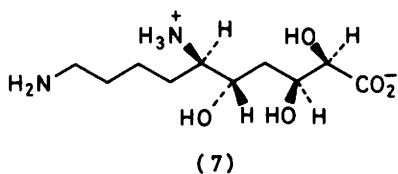
Further new amino acids showing similarities with ( 1 ), on the basis of their betaine or mixed zwitterion structures, are the hydroxyproline relative ( 3 ) and the L-methionine derivative ( 4 ).<sup>25</sup> Both ( 3 ) and ( 4 ) are from marine algae; ( 3 ) from *Grateloupia proteus*, and ( 4 ) from *Lophocladia lallemandi*.<sup>25</sup>

Making comparisons between amino acids on this superficial basis is not intended to indicate similarities in biosynthetic pathways, but the link between ( 2 ) and the opines [*N*-( $\alpha$ -carboxyalkyl)- $\alpha$ -amino acids] is less tenuous. New opines continue to be reported more frequently than many other types of  $\alpha$ -amino acid; can a rational explanation for the ubiquity of this class of compound be the subject of much longer gestation? In addition to the recently-discovered ornithine analogue ( 5a ) produced by *Streptococcus lactus* cultured on an arginine-deficient medium, (2*S*,8*S*)-*N*<sup>6</sup>-(1-carboxyethyl)lysine ( 5b ) has now been detected.<sup>26</sup> Among further new opines are  $\beta$ -alanopine, *N*-[(*R*)-1-carboxyethyl]- $\beta$ -alanine ( 6 ), from the adductor muscle of the blood shell *Scapharca broughtonii*;<sup>27</sup> and vitopine (details absent from *Chemical Abstracts* source of this citation).<sup>28</sup>

Full details are available for galantinamic acid ( 7 ), a component of galantin, shown to be (2*R*,3*S*,5*S*,6*R*)-6,10-diamino-2,3,5-trihydroxydecanoic acid.<sup>29,214</sup>

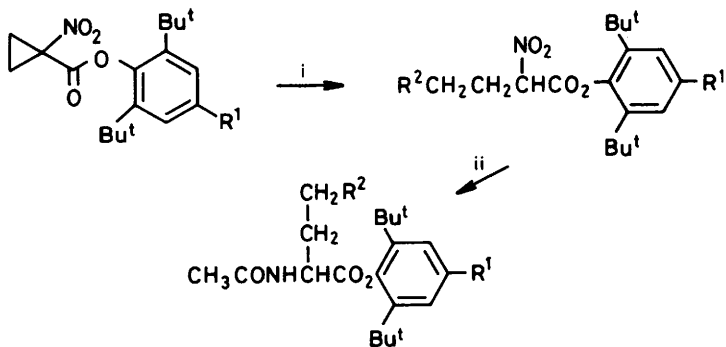
**3.4 New Amino Acids from Hydrolysates.**- New amino acids that have been discovered as residues in peptides and proteins are described in this Section, whether or not they are actually liberated as such by hydrolysis. Even so, the section would be substantial if it attempted to cover thoroughly, for example, newly-discovered compounds with amino acid side-chain - carbohydrate covalent links (as in glycoproteins); and no such comprehensiveness is intended. An example is asparagine, glycosidically-linked to rhamnose through the side-chain amide nitrogen atom, this moiety being released on hydrolysis of the surface glycoprotein of *Bacillus stearothermophilus* NRS 2004/3a.<sup>30</sup>

(2*S*,3*R*,5*S*)-3-Amino-2,5,9-trihydroxy-10-phenyldecanoic acid ( 8 ) is found (with other unusual amino acids) in hydrolysates of scytonemin A, a novel calcium antagonist from the blue-green alga *Scytonema*.<sup>31</sup> It is interesting to note the structural similarity between this new amino acid and 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid ( 9 ), present in cyanoginosin-LA, a cyclic heptapeptide toxin from *Microcystis aeruginosa*.<sup>32</sup>



Reagents: i,  $\text{R}^1\text{Br}$ , or TFAA (catalytic)  $\rightarrow \text{R}^1 = \text{H}$ ; ii,  $\text{R}^2\text{CH}=\text{CR}^3\text{CO}_2\text{Me}$

**Scheme 2**



Reagents: i,  $\text{R}^2\text{X}$  ( $\text{CN}^-$ ,  $\text{CH}(\text{CO}_2\text{R})_2$ ,  $\text{N}_3^-$ , etc.); ii,  $\text{Zn}-\text{AcOH}/\text{Ac}_2\text{O}$ , reflux 4 h

**Scheme 3**

#### 4 Chemical Synthesis and Resolution of Amino Acids

##### 4.1 General Methods for $\alpha$ -Amino Acid Synthesis.-

The full range of standard methods of  $\alpha$ -amino acid synthesis<sup>33</sup> continues to be gainfully employed, and in some cases, usefully developed. Later sections of this Chapter, particularly the next one (4.2 'Asymmetric Synthesis') and that (6.3) covering modifications of amino acid side chains (by which one amino acid can be prepared from another) are relevant for readers seeking an overall view of the recent literature on this topic.

The hydroformylation - amidoalkylation procedure has been reviewed,<sup>34</sup> and has been given a thorough mechanistic study using  $C_6F_5CH=CH_2$  and  $Co_2(CO)_8-Rh_6(CO)_{16}$  as the test-rig.<sup>35</sup> Distantly related amination procedures include reaction of  $\alpha$ -hydroxyalkanoic acid esters with hydrazoic acid, diethyl azodicarboxylate, and  $Ph_3P$ ,<sup>36</sup> and ammonolysis of 2-bromo-3-methyl-5,5,5-trifluoropentanoic acid for the synthesis of 5,5,5-trifluoro-DL-isoleucine and DL-alloisoleucine.<sup>37</sup>

$\alpha$ -Alkylation of glycine derivatives services most of the routine syntheses, diethyl 2-acetamidomalonate<sup>38-41</sup> continuing to be favoured; among these is a notable study<sup>39</sup> describing phase transfer-catalyzed alkylation 'without solvent' (although using solid KOH containing up to 15% water as base). Imines  $Ph_2C=NCH_2CO_2R$  have been converted into  $\alpha$ -methylene derivatives  $Ph_2C=NC(=CH_2)CO_2R$  to be used as synthons for  $\beta$ -substituted alanines (Michael addition or Lewis acid-catalyzed addition).<sup>42</sup> A related approach<sup>43</sup> employs radical addition ( $RHgX$  to  $CF_3CONHC(=CH_2)CO_2Me$ ). The carbon radical approach [irradiation of  $N$ -hydroxy-pyridine-2-thione esters of  $N$ -protected amino acids (Vol.20, p.12, ref.159) in the presence of alkenes] has been used for the synthesis of  $\omega$ -carboxyalkyl- $\alpha$ -amino acids.<sup>44</sup>

Alkylation of 2-(1-pyrrolyl)acetates (10) using an alkyl halide and lithium di-isopropylamide is a new glycine alkylation approach;<sup>45</sup> however, the alkyl group introduced in this way needs to be ozone-friendly if it is to survive 'deprotection'. The 'other-way-round' imines, e.g.  $Me_3SiCH_2N=CHCO_2Me$ ,<sup>46</sup> are the source of azomethine ylides suitable for 1,3-dipolar cycloaddition reactions (e.g. in a synthesis of substituted prolines, Scheme 2).

The classical route based on alkylation of 2-phenyloxazol-5(4H)-one (formed *in situ* from hippuric acid) is widely useful (e.g. in synthesis of  $\beta$ -di- and -trifluoromethylphenylalanines by condensation with corresponding phenyl fluoromethyl ketones, followed by reduction and hydrolysis<sup>47</sup>).

$\alpha$ -Alkylation of an alkyl  $\alpha$ -nitroacetate, in the various ways described above for analogous glycine derivatives,<sup>18,48,49</sup> is the starting point for another general amino acid synthesis. Reduction using  $H_2$ -Pt<sup>48</sup> or  $Zn$ -AcOH<sup>49</sup> completes the process, which, in the latter case, is part of an overall route to  $\gamma$ -substituted  $N$ -

acetylbutyrines through nucleophilic ring-opening of aryl 1-nitrocyclopropane-1-carboxylates (Scheme 3).

Substitution of the halogen atom of *N*-benzoyl- $\alpha$ -bromoglycine methyl ester<sup>80</sup> (see also ref.72) and the corresponding reaction with an  $\alpha$ -methoxy amino acid,<sup>81</sup> illustrate other general methods. Radical conditions with 2-functionalized allylstannanes are used in the former example, leading to  $\gamma\delta$ -unsaturated  $\alpha$ -amino acids (Scheme 4), and intramolecular electrophilic substitution in the latter.

A non-routine variant is employed in a single-step Strecker synthesis of methionine hydantoin (acrolein, ammonium carbonate, MeSH, HCN),<sup>82</sup> and a classical version (a ketone R'COR<sup>2</sup>, ammonium hydroxide or chloride, NaCN; then methacryloyl chloride, NaOH; then 12*N*-sulphuric acid) has been reported for the synthesis of *N*-(methacryloyl)amino acids CH<sub>2</sub>=CMeCONHCR'R<sup>2</sup>CO<sub>2</sub>H.<sup>83</sup>

#### 4.2 Asymmetric Synthesis of $\alpha$ -Amino Acids.-

Most of the methods employed are developments of standard general routes, though there seems to be no end to novelties of planning and execution. A review, with title identical with that of this Section, has appeared.<sup>84</sup> Later sections of this Chapter feature specific asymmetric syntheses, using both routine and novel methods.

A rare example, amounting to asymmetric  $\alpha$ -carboxylation of an amine, involves anodic  $\alpha$ -methoxylation (cf. Vol.20, p.18) of an *N*-protected (S)-phenylethylamine, followed by substitution by Me<sub>3</sub>SiCN and hydrolysis, to give a mixture of L-isoleucine and D-alloisoleucine in modest optical yield.<sup>85</sup> Poly[(R)-3-hydroxybutanoate], -(CHMeCH<sub>2</sub>COO)<sub>n</sub>-, is an inexpensive starting material to which a recently-introduced amination procedure (di-tert-butyl azodicarboxylate) has been applied.<sup>86</sup> This leads to D-allothreonine and L-threonine with only moderate diastereoselectivity, but conformational restriction (conversion of the starting material into a dioxanone analogue) raises this to 99%. Not too distantly related, and capable of very high diastereoselectivity, are asymmetric Ugi<sup>87a</sup> and Strecker<sup>87b</sup> syntheses, employing 2,3,4,6-tetra-O-pivaloyl- $\beta$ -D-galactopyranosylamine as chiral auxiliary (Scheme 5). The former route can yield enantiomerically pure D-amino acids. So does the Strecker route when the Lewis acid is ZnCl<sub>2</sub> in propan-2-ol, or SnCl<sub>4</sub> in THF, but the bias is towards L-amino acids using ZnCl<sub>2</sub> in CHCl<sub>3</sub>.<sup>87</sup>

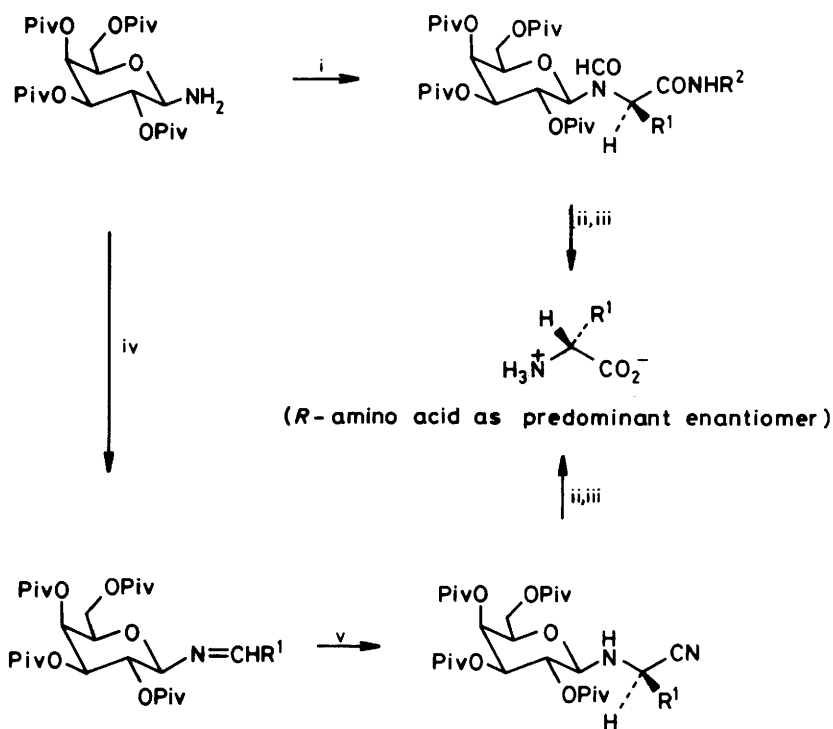
Photolysis of Cr-carbene complexes (11) containing a chiral, optically-active amino-alcohol moiety, gives lactones in high yields and with high diastereoselectivity (Scheme 6).<sup>88</sup> Hydrolysis of the products gives D-amino acids with return of the chiral auxiliary.

Ammonolysis of  $\alpha$ -keto-acids in the presence of  $\beta$ -cyclodextrin-functionalized pyridoxamines mimics the *in vivo* transaminase-catalyzed process with moderate success.<sup>89</sup>



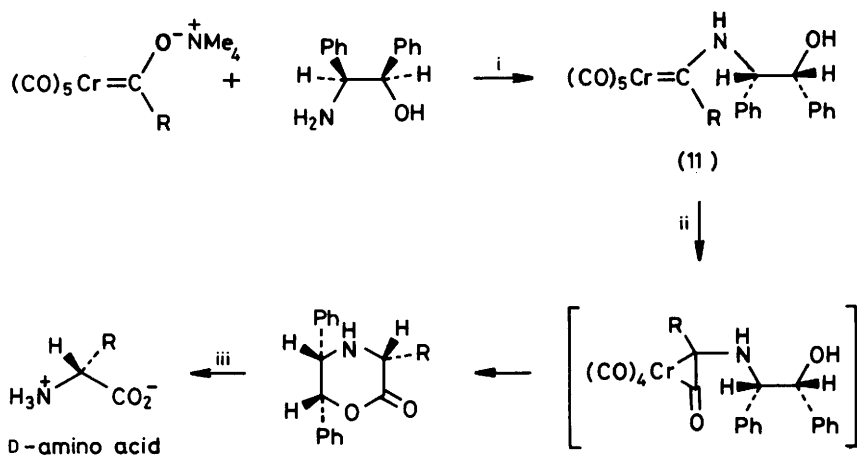
Reagents: i,  $\text{R}^1 = \text{Br}$ ; aza - isobutyronitrile, toluene,  $85^\circ\text{C}$ ; ii,  $\text{CH}_2=\text{CR}^2\text{CH}_2\text{SnR}_3$

**Scheme 4**



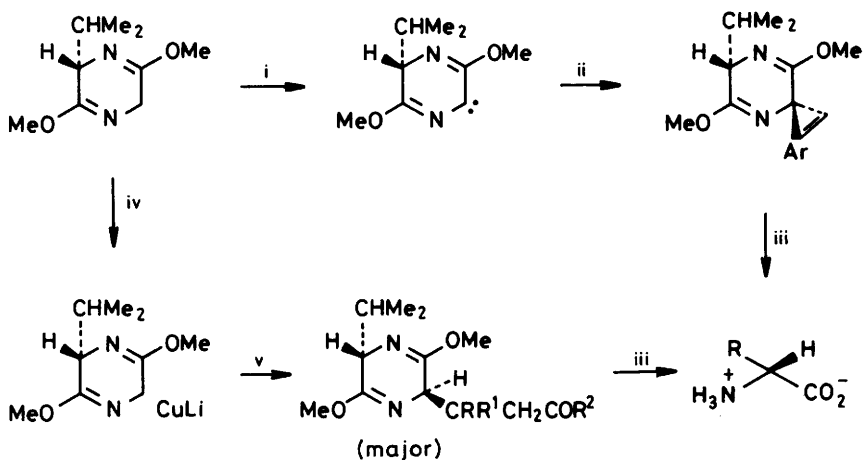
Reagents: i,  $\text{R}^2\text{NC}, \text{R}^1\text{CHO}$ ,  $\text{ZnCl}_2$ ,  $\text{HCO}_2\text{H}$ ; ii,  $\text{HCl}-\text{MeOH}$ ; iii,  $6\text{M}-\text{HCl}$ ,  $80^\circ\text{C}$ , then Amberlite IR 200; iv,  $\text{R}^1\text{CHO}$ ; v,  $\text{Me}_3\text{SiCN}$ ,  $\text{ZnCl}_2$  (or  $\text{SnCl}_4/\text{THF}$ )

**Scheme 5**



Reagents: i, AcBr, then MeOH, argon; ii, 450 W lamp; iii  $\text{H}_2$ -PdCl<sub>2</sub>

**Scheme 6**



Reagents : i, BuLi, PhSO<sub>3</sub>N<sub>3</sub>; ii, ArC≡CH; iii, hydrolysis ( $\text{H}_3\text{O}^+$ ); iv, BuLi, CuBr, Me<sub>2</sub>S;  
v, RR'C≡CHCOR<sup>2</sup>

**Scheme 7**

Synthesis of (S)-(-)- $\alpha$ -methylDOPA through an asymmetric Strecker route, and of (R)-(-)- $\alpha$ -methylserine and of D-serine by the 'bis-lactim ether' method, have been reviewed.<sup>60</sup> The latter method, amounting to the asymmetric alkylation of an enolized glycine moiety, has featured in every one of the last ten or more Volumes of this Specialist Periodical Report. It is featured again this year, in a showcase of papers from the research group that invented it.<sup>61-64</sup> Two of these reports are selected for display here, since they are both representative of the method and interesting (in different ways) in mechanistic terms (Scheme 7). In one project, (R)- $\alpha$ -Boc-amino-2-arylcyclopropene-1-carboxylates are formed through an asymmetric cycloaddition of a carbene to an alkyne;<sup>62</sup> in the other,<sup>64</sup> a bis-lactim ether-derived cuprate is added to  $\alpha\beta$ -unsaturated carbonyl compounds.

Further examples of diastereoselective substitution of glycine derivatives, the area in which most effort is being directed for asymmetric  $\alpha$ -amino acid synthesis, have been reported. A paper that appeared at the end of 1988<sup>65</sup> describes a new chiral glycine enolate synthon derived from (R)-2-phenyl-2-aminoethanol ("D-2-phenylglycinol") for asymmetric syntheses (Scheme 8). This approach is modelled on an earlier classic study<sup>66</sup> which has been extended further (Scheme 9).<sup>67,68</sup> The chiral auxiliary used for this route (12) is commercially available, and can be recycled after recovery at the end of the synthesis.

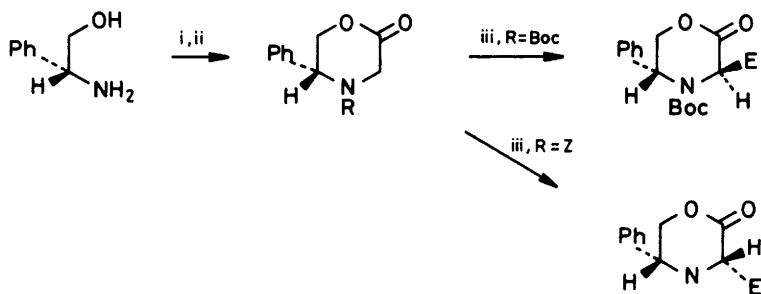
There are puzzling aspects as far as diastereoselectivity is concerned, in these studies. In the 'asymmetric nucleophilic glycine' approach, the  $\alpha$ -protecting group determines the bias as shown in Scheme 8; solvent and counterion,  $X^-$ , are also crucial.<sup>66</sup> Similar results in the 'asymmetric electrophilic glycine' approach<sup>67</sup> (Scheme 9) indicates that either retention or inversion accompanies substitution of the halogen atom, the anti-relationship resulting from substitution with inversion, being associated with easily crystallised products.

Alkynylstannanes  $Bu_3SnC\equiv CR'$  have been used with the  $\alpha$ -benzyloxycarbonyl analogue of (12) in an extension of the route in Scheme 9,<sup>69</sup> the resulting  $\alpha$ -alkynylglycine derivatives being susceptible to hydrogenation/hydrogenolysis ( $H_2/Pd$ ) to give  $\gamma$ -substituted D-butyrynes.

The basis of these routes is not new-found, but the natural conservatism that has made some workers reluctant to take up some of the earlier methods will be overcome by the thoroughness of studies such as these, and there should be much to report on them in future Volumes of this Report. A thoroughly-studied route with a longer history is based on the alkylation of chiral imidazolidinones (e.g. 13), and further results have been reported,<sup>70</sup> for the asymmetric synthesis of  $\alpha$ -alkylated ornithines, lysines, and tryptophans.

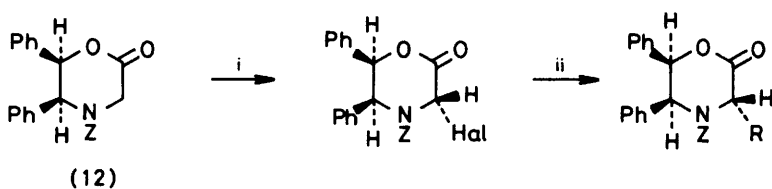
An analogous approach using the  $\alpha$ -acylated  $\alpha$ -hydroxyglycine (14)<sup>71</sup> is flawed by the requirement of an indirect cleavage method for the acyl group at the end of the synthesis if racemization is to be avoided. The use of Steglich's  $\alpha$ -bromo- $\alpha$ -Boc-glycine tert-butyl ester approach [but using the corresponding (-)-





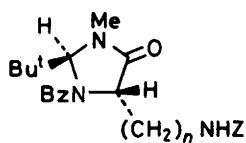
Reagents: i,  $\text{BrCH}_2\text{CO}_2\text{Ph}$ ; ii,  $\text{RX}$ ; iii,  $\text{EX}$

**Scheme 8**

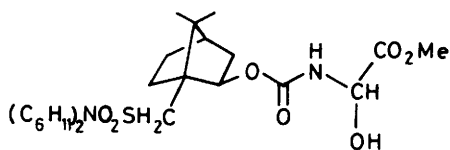


Reagents: i, NBS or  $\text{Bu}^t\text{OCl}$ ; ii,  $\text{R-M, ZnCl}_2$

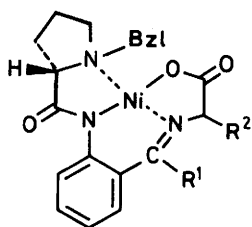
**Scheme 9**



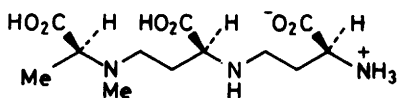
(13)



(14)



(15)



(16)

phenylmethyl ester], represents another chiral electrophilic glycine equivalent, and has been developed further (photolysis of *N*-bromosuccinimide in  $\text{CCl}_4$  for introduction of the bromine atom; substitution by Grignard reagents).<sup>72</sup>

Alkylation of the sodium enolate of the imine formed between glycine tert-butyl ester and (*R*)-camphor has been found to give low stereoselectivity, but other chiral ketones were no better from this point of view.<sup>73</sup> The enantiomeric excess, never more than 62%, secured in the allylation of the anion of the achiral imine  $\text{Ph}_2\text{C}=\text{NCH}_2\text{CO}_2\text{Me}$  in the presence of a  $\text{Pd}(\text{dibenzylidene acetate})$ -chiral diphosphine depends on the proportion of chiral ligand in the complex.<sup>74</sup> The analogous benzylation of ethyl *N*-benzylideneglycinate gives *D*-phenylalanine in 89.9% optical yield when conducted under phase transfer conditions in the presence of (-)-*N*-benzylcinchonidinium chloride.<sup>75</sup> The asymmetric aldol condensation between alkyl  $\alpha$ -isocyanoalkanoates and paraformaldehyde in the presence of ferrocenyl - chiral phosphine - gold complexes to give  $\alpha$ -alkyl serines has been studied.<sup>76</sup>

A substantial effort has been put into evaluating the use of the chiral nickel complexes (15), composed of an *N*-benzyl-L-prolinanilide moiety "overlapping" an *N*-arylidene-glycine<sup>77-79</sup> or -alanine<sup>78-80</sup> moiety. Alkylation by  $\alpha\beta$ -unsaturated aldehydes leads, after sodium cyanoborohydride reduction, to L-proline and its derivatives.<sup>77</sup> An alkyl chloride in DMF in the presence of solid NaOH leads to (2*S*,4*S*)-2,4-di-aminoglutaric acid (and the corresponding di-aminosuccinic acid)<sup>79</sup> and various  $\alpha$ -methyl- $\alpha$ -amino acids, enantioselectivities up to 80% being achieved.<sup>78</sup>

A different Russian group is continuing a substantial study of  $\text{PdCl}_2$  - (*S*)-(-)-phenylethylamine-catalyzed hydrogenolytic aminolysis of 4-arylidene-oxazol-5(4*H*)-ones ("azlactones").<sup>81,82</sup> One of these studies<sup>81</sup> was successfully directed towards the synthesis of (*S*)-*p*-fluorophenylalanine.

The remaining area, largely irrelevant as a practical asymmetric synthesis of  $\alpha$ -amino acids but of continuing interest for mechanistic insights that it can offer, is the homogeneous-catalyzed hydrogenation of 2-aminoalken-2-*oic* acid derivatives. Rhodium(I) - chiral phosphine complexes catalyze the hydrogenation of  $\alpha$ -acetamidocinnamic acid<sup>83,84</sup> with added triethylamine sharply reducing optical yields.<sup>83</sup> Less than 27% enantiomeric excesses were observed in an analogous study employing  $[\text{Ir}(\text{cod})(\text{PhCN})(\text{L})]^+\text{ClO}_4^-$  as catalysts, where L is a chiral phosphine ligand.<sup>85</sup>

The hydrogenation ( $\text{H}_2/\text{Pd-C}$ ) of oximes and Schiff bases of amides formed between pyruvic acid and a chiral amine gives, in the best case [*(R)*- $\alpha$ -ethylbenzylamine], a 70% enantiomeric excess of L-alanine.<sup>86</sup>

#### 4.3 Synthesis of Protein Amino Acids and Other Naturally-Occurring $\alpha$ -Amino Acids.

Several examples of the synthesis of protein amino acids have been given already in this Chapter, and this Section has featured non-protein amino acids almost exclusively in recent years. A title such as this demands mention of the vast

literature on biotechnological production of protein amino acids, but as usual, only representative papers are cited here [readers are reminded of the easy access to this topic through Section 16 of Chemical Abstracts (Biotechnology and Industrial Fermentations)].

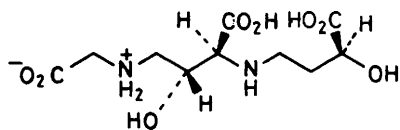
Reviews<sup>87-93</sup> have appeared, of large scale production of L-tryptophan,<sup>87,88</sup> L-phenylalanine,<sup>89</sup> and of natural  $\alpha$ -amino acids generally.<sup>90-93</sup> By large scale in one of these reviews is meant 200 tons y<sup>-1</sup> (of methionine, phenylalanine, or valine,<sup>91</sup> produced by acylase 'resolution' of N-acetyl-DL-amino acid esters).

The continuing importance of enzyme-catalyzed production of L-amino acids from DL-hydantoins,<sup>88,92,94,95</sup> and from keto-acids,<sup>93,96,97,98</sup> and more emphasis on unusual amino acids,<sup>90</sup> production of D-amino acids,<sup>90,94,96,99</sup> and reactor design,<sup>91,100</sup> is reflected in these reviews, and is also noticeable in the primary literature citations given. Particular commercial importance associated with L-DOPA,<sup>96</sup> glutamic acid,<sup>101</sup> arginine,<sup>102</sup> alanine,<sup>103</sup> and aspartic acid,<sup>103</sup> and several other protein amino acids, is reflected in these papers, but work on less common amino acids such as D- and L-pipecolic acid (the latter converted into L-aminoadipic acid)<sup>99</sup> is notable.

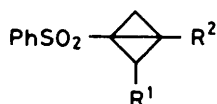
Just as the preceding citations are a small fraction of the total literature, research on amino acid biosynthesis pathways cannot be more than hinted at here. The first stage in conversion of crotonobetaine into L-carnitine by enterobacteria is hydroxylation of the double bond.<sup>104</sup> Further representative citations in this topic area can be found later in this Chapter (Section 6.2).

Reduction of methyl hydroximino-cyanoacetate  $\text{HO}=\text{C}(\text{CN})\text{CO}_2\text{Me}$  at a lead or carbon cathode gives the amine, which by acetylation and further reduction gives the protected viomycin constituent, 2,3-diaminopropionic acid.<sup>105</sup> Other syntheses of relatively simple natural (non-protein) amino acids include L-discadenine [three steps and a tenfold yield improvement<sup>106</sup>] from L-homoserine lactone,<sup>107,108</sup> 3,4-seconicotianamine (16)<sup>109</sup> from N-trifluoroacetyl-L-aspartic  $\beta$ -semialdehyde ethyl ester and N-methyl-L-alanine. The interesting iron-chelating amino acid 2'-epi-distichonic acid A (17)<sup>110</sup> has been synthesized through a similar reductive coupling procedure involving  $(\text{S})\text{-EtO}_2\text{CCH}(\text{OAc})\text{CH}_2\text{CHO}$ ,  $\text{NaBH}_4\text{CN}$ , and  $\text{NH}_2\text{CH}(\text{CO}_2\text{Bu}^+)-\text{CH}(\text{OAc})\text{CH}_2\text{NBocCH}_2\text{CO}_2\text{Bu}^+$  (for a further example of the same process, shown in Scheme 1, see Ref.24).

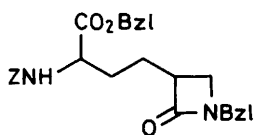
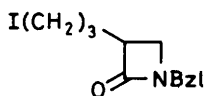
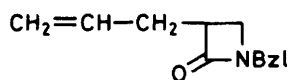
Synthesis of alicyclic  $\alpha$ -amino acids continue to engender ingenuity of planning (and also the application of routine methods). Previously-reported synthetic studies have been completed by establishment of (1S,2S)-stereochemistry for norcoronamic acid (1-amino-2-methylcyclopropane-1-carboxylic acid).<sup>111</sup>  $\alpha\beta$ -Dehydroglutamic acid has been subjected to standard cyclopropane synthesis (photolysis of the stable dihydropyrazole formed with diazomethane) to give "2,3-methanoglutamic acid".<sup>112</sup> Ring-opening of 1-arenesulphonyl bicyclobutanes (18;  $\text{R}^1 = \text{CO}_2\text{H}$ ) provides 1-amino-cyclobutane-1-carboxylic acids, including both *cis*- and *trans*-



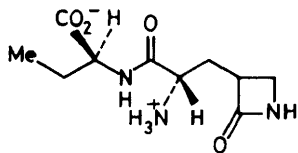
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(18)

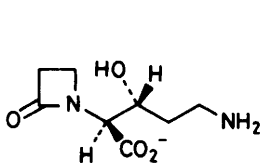
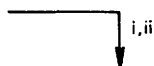


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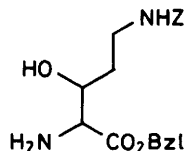
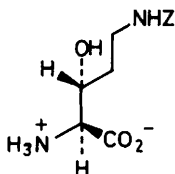


Reagents : i, dicyclohexylborane, then Chloramine T, NaI; ii, Z - Gly - OBzl dianion;  
iii, routine elaboration

Scheme 10



(20)



Reagents : i,  $(\text{Me}_3\text{Si})_2\text{NLi}$ ; ii, 2M HCl - ether, then  $\text{NaHCO}_3$ ; iii, *E. coli* acylase on  
N-PhCH<sub>2</sub>CO - derivative; iv, established methods

Scheme 11

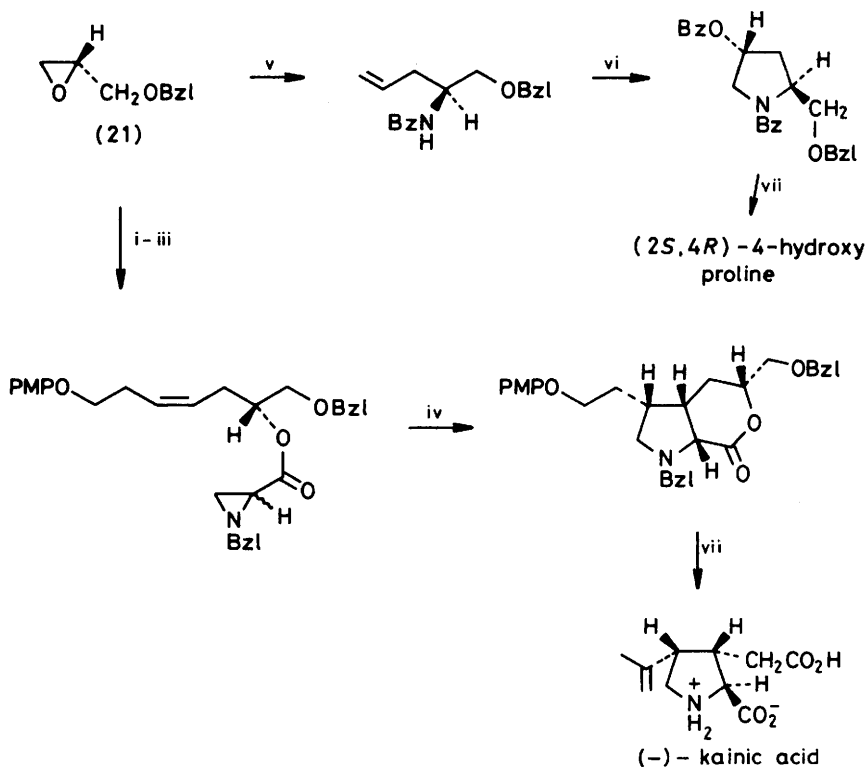
2,4-methanoglutamic acids.<sup>113</sup> A thorough coverage of the 1-aminocyclobutane-1-carboxylic acids from the legume *Atelia herbert smithii*, from the synthetic point of view,<sup>113,114</sup> has been extended with total syntheses of 2,4-methanoglutamic acid, 2,4-methanoproline, and *cis*-1-amino-3-(hydroxymethyl)-cyclobutane-1-carboxylic acid.<sup>114</sup> The last-mentioned compound was identical with one of the seed components [an example of the role of total synthesis in structure determination of trace constituents of plants], though the same structure for this constituent from the same plant, has been arrived at by another group (Vol.20, p.3).

This study<sup>114</sup> includes a synthesis of 2,4-methano-pyrroglutamic acid, a possible further component of the seeds of this species. Smaller-ring lactams desoxytabtoxine  $\beta$ -lactam ( 19 )<sup>115</sup> and (2*S*,3*R*)-proclavaminic acid ( 20 )<sup>116</sup> have been synthesized by the routes shown in Schemes 10 and 11, respectively. The synthesis of ( 20 ) involved construction of the azetidinone ring on L- $\beta$ -hydroxylysine, and led to the assigned stereochemistry.

Proline derivatives provide targets for novel cyclization methods, especially since some of these compounds show extraordinary physiological properties (e.g. acromelic acids as potent neuroexcitatory agents from the poisonous mushroom, *Clitocybe acromelalga*). (2*S*,4*R*)-4-Hydroxyproline emerges from the iodine-mediated cyclization of 2-aminoalk-4-enols (Scheme 12),<sup>117</sup> and the same starting material, (*S*)-2-(benzyloxymethyl)oxirane ( 21 ), is used in a concise enantioselective route to (-)-kainic acid (Scheme 12; cf. Vol.20, pp.13,15).<sup>118</sup> A further route, an intramolecular Diels-Alder reaction with novel diastereoselectivity, has been applied for the same purpose (Scheme 13).<sup>119</sup> Syntheses of acromelic acids A and B ( 22 ; cf. Vol.20, p.15) from (-)-kainic acid, have been fully described.<sup>120</sup> The cobalt-mediated route established earlier (Vol.20, p.17) for proline ring closure can be used (Scheme 14) to provide acromelic acids without the restrictive requirement for (-)-kainic acid as starting material.<sup>121</sup>

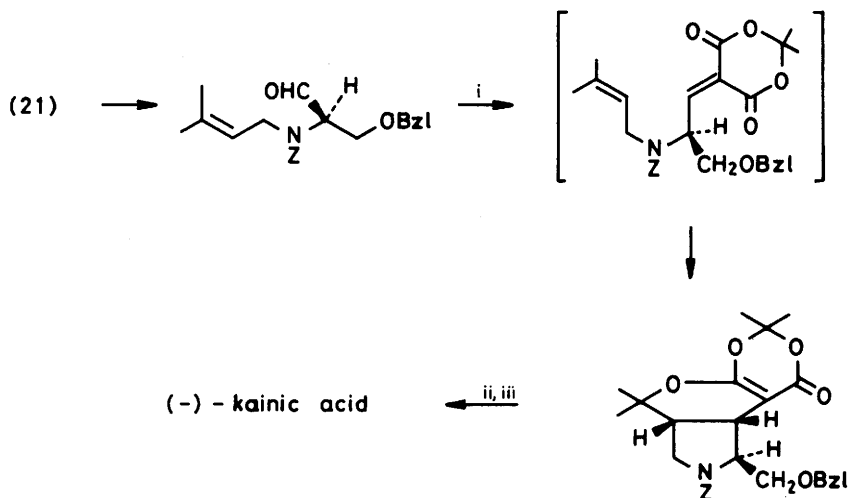
(2*S*,3*S*,5*S*)-3-Hydroxy-5-methyl-2-pyrrolidine-1-carboxylic acid ( 23 ; a component of actinomycin Z1), and its C-3 epimer, have been synthesized, starting with cycloaddition of MeC $\equiv$ NO to a protected L-vinylglycine (Scheme 15).<sup>122</sup> The acidic proline derivative ( 24 ) from the marine red alga *Schizymenia dubyi* has been synthesized<sup>123</sup> from Z-L-pyrroglutamic acid tert-butyl ester, the novel route (Scheme 16) being constructed using established synthetic operations. A similar starting point is used in a new synthesis of (-)-bulgescine (Scheme 17).<sup>124</sup>

Hydroxylated acyclic  $\alpha$ -amino acids support an increasing proportion of the literature under the heading for this Section, even after relegating discussion of  $\beta$ -hydroxyglutamic acid to the later Section 4.16 'Synthesis of  $\beta$ - and Other  $\omega$ -Amino Acids' because of its eligibility to be documented there, as well as here, coupled with its structural relationship with the statines discussed there. A conventional asymmetric glycine alkylation approach, in which H-(pyruvylideneglycyl)-D-phenylalaninato-copper(II) is condensed with imidazole-4-



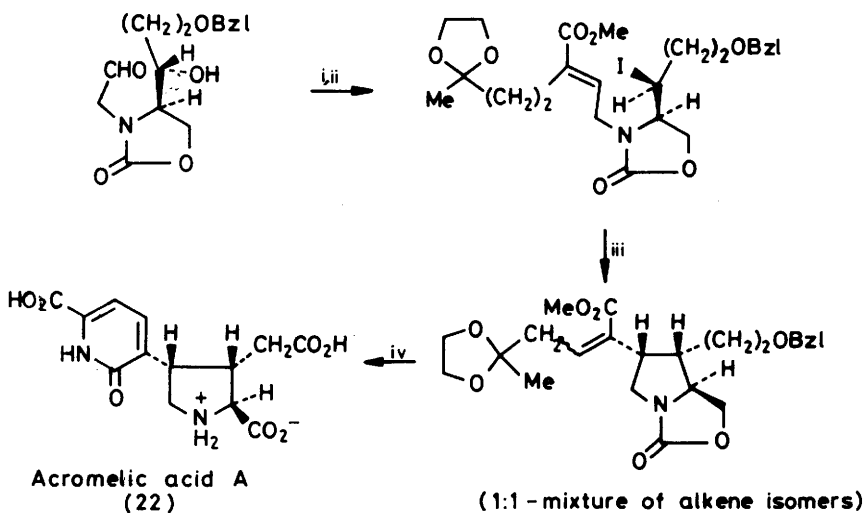
Reagents: i, MeLi, PMPO CH<sub>2</sub>CH=CH<sub>2</sub>; ii, H<sub>2</sub>-Pd; iii, 2,3-dibromopropionyl chloride - Et<sub>3</sub>N, then BzI-NH<sub>2</sub>; iv, 310°, xylene at high dilution, sealed tube; v, several steps including NaH - DMSO, H<sub>2</sub>-Pd, DEAD - phthalimide - Ph<sub>3</sub>P; vi, I<sub>2</sub>; vii, several routine manipulations

**Scheme 12**



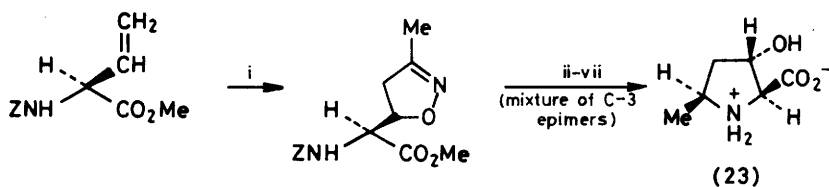
Reagents: i Meldrum's acid +  $(\text{CH}_2\text{NH}_2)_2 \cdot 2 \text{OAc}^-$ ; ii, aq. dioxan; iii, TBDMSCl, then  $\text{NaBH}_4$ ,  $\text{Ac}_2\text{O}$ ,  $\text{Bu}_4\text{NF}$ , Jones' oxidation

Scheme 13



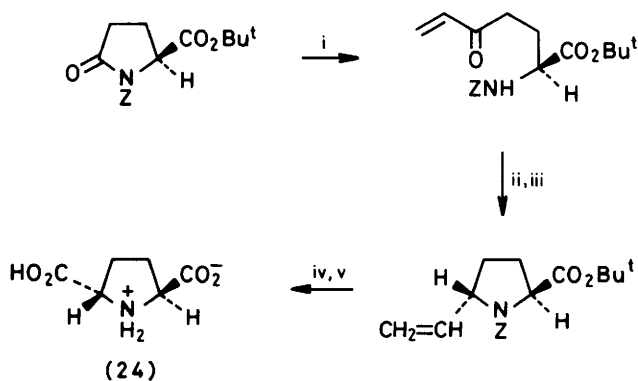
Reagents: i,  $\text{MeCO}(\text{CH}_2)_2\text{C}(\equiv\text{PPh}_3)\text{CO}_2\text{Me}$  protected as dioxalane; ii,  $(\text{CF}_3\text{SO}_2)_2\text{O}$ , py,  $\text{NaI}$ ; iii,  $\text{Co(I)}$ ; iv, construction of pyridone ring (for precedent, see vol. 20, p.15)

Scheme 14



Reagents : i,  $\text{MeC}\equiv\text{N}^+\text{O}^-$ ; ii, separate diastereoisomers (silica gel); iii, Raney Ni,  $\text{B}(\text{OH})_3$ , MeOH; iv,  $\text{H}_2$ -Pd; v,  $\text{NaBH}_3\text{CN}$ ; vi,  $\text{Ba}(\text{OH})_2$ ; vii, ion-exchange separation

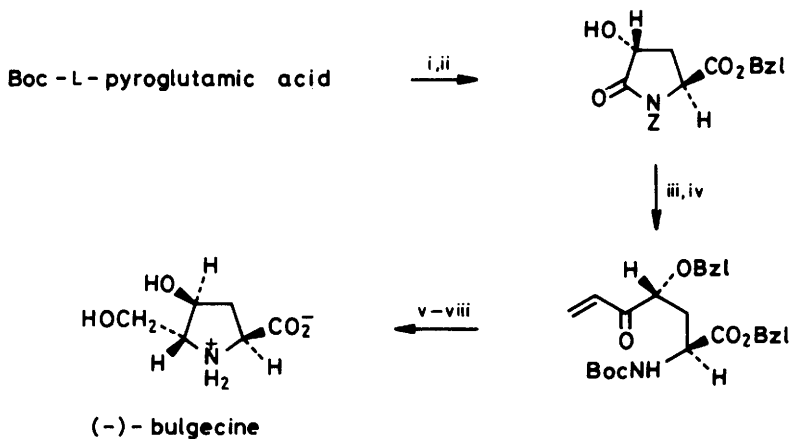
Scheme 15



Reagents : i,  $\text{CH}_2=\text{CHMgX}$ ; ii,  $\text{NaBH}_4$ ,  $\text{CeCl}_4$ ; iii,  $\text{MsCl}$ ,  $\text{Et}_3\text{N}$ ; iv,  $\text{O}_3$  /  $-78^\circ$ , MeOH; v, TFA

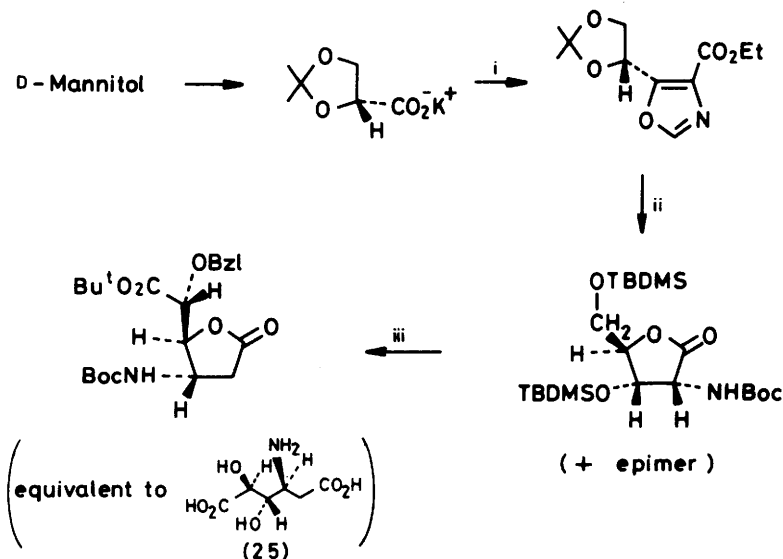
Scheme 16





Reagents : i,  $\text{LiN}(\text{SiMe}_3)_2$  ; ii, *N*-tosyl-3-phenyloxaziridine ; iii,  $\text{BzOH}$ ,  $\text{DEAD}$ ,  $\text{Ph}_3\text{P}$  ;  
iv,  $\text{CH}_2=\text{CHMgBr}$  ; v,  $\text{NaBH}_4$  ; vi,  $\text{MsCl}$  ; vii,  $\text{O}_3$  ; viii,  $1\text{M NaOH}$

Scheme 17



Reagents: i,  $\text{CNCH}_2\text{CO}_2\text{Et}$  ; ii,  $\text{MeSO}_3\text{H}$ , then  $(\text{Boc})_2\text{O}$ , then  $\text{H}_2$ -Rh catalyst,  $\text{TBDMSCl}$  ;  
iii,  $\text{CrO}_3$ -py, then  $\text{DIBAL}$ ,  $\text{Ph}_3\text{PCOME}$ ,  $\text{Hg}(\text{OAc})_2$ ,  $\text{NIS}$

Scheme 18

carbaldehyde, offers an efficient route to  $\beta$ -hydroxy-L-histidine, a component of the bleomycins.<sup>125</sup> The other  $\beta$ -hydroxylated  $\alpha$ -amino acids covered this year present fearsome stereochemical challenges; these have been well confronted, syntheses of the hydroxy-amino acid moiety of AI-77-B, a gastroprotective substance from *Bacillus pumilus* AI-77 (25) being notable (Scheme 18).<sup>126</sup>

A general asymmetric synthesis for  $\beta$ -hydroxy- $\alpha$ -N-methylamino acids<sup>127</sup> involving efficient Sharpless epoxidation of an appropriately located side-chain double bond (but depending on the presence of a  $\gamma$ -CH<sub>2</sub> group) has been developed as a result of the ongoing interest in routes to 'MeBmt', the non-protein  $\alpha$ -amino acid (4R)-4-[(E)-2-butenyl]-4-NN-dimethyl-L-threonine of cyclosporin. This approach is shown in Scheme 19; an alternative stereospecific synthesis<sup>128</sup> of this compound is shown in Scheme 20, involving the chiral auxiliary approach to generate the correct stereochemistry at the threonine side-chain moiety, and depending on this centre for all subsequent chirality (although inversion at C-2 was needed).

The syn relationship between  $\alpha$ -amino- and  $\beta$ -hydroxy-groups is completely secure in a route (Scheme 21) providing D-threonine,  $\gamma$ -hydroxy-L-threonine, and 5-Q-carbamoylpolyoxamic acid;<sup>129</sup> the latter compound has been synthesized from D-serine (Scheme 22), which gives a 6:1 bias in favour of the required diastereoisomer (26) in the initial stage.<sup>130</sup>

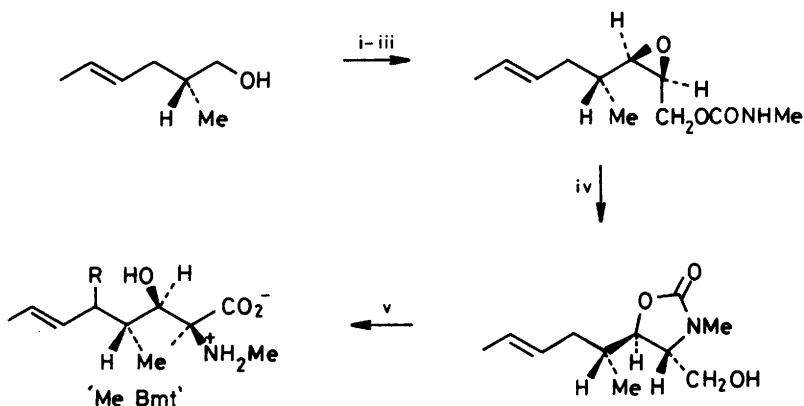
The less-common  $\gamma$ -hydroxy- $\alpha$ -amino acids are represented by the N-terminal residue of nikkomycin B, for which a synthesis has been accomplished that depends on intramolecular Diels-Alder addition of a glyoxylic acid N-benzoylimine (27 in Scheme 23) to introduce the required stereochemistry.<sup>131</sup>

Modifications of earlier routes (Vol.20, p.21) have led to improved syntheses of racemic ibotenic acids (28).<sup>132,133</sup>

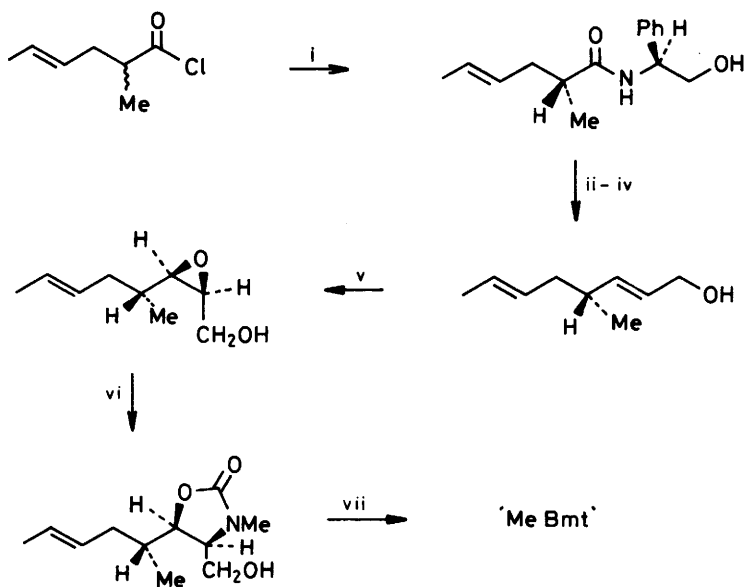
**4.4  $\alpha$ -Alkyl Analogues of Protein Amino Acids.**— General methodology (some of which has been referred to in earlier sections) for the synthesis of these homologues includes the Schmidt reaction (rarely used for the purpose) with ketones ( $R^1COCR^2R^3CO_2Et \rightarrow H_2NCR^2R^3CO_2Et$ ),<sup>134</sup> and alkylation of imines.<sup>135</sup> Michael addition of imines  $RCH=NCHR^1CO_2R^2$  to  $CH_2=CHR^3$  employs solid  $K_2CO_3$  and  $MeOH$ <sup>42</sup> to give  $\alpha$ -substituted  $\alpha$ -amino acids; with aldehydes as electrophile, the same substrate gives corresponding serine homologues.<sup>135</sup>

Variations of this latter process have been reported, such as the 'bis-lactim ether' route (Scheme 7) providing both enantiomers of 4-methylcycloserine.<sup>136</sup> Use of 4-trifluoromethyloxazoles for the synthesis (Scheme 24) of 2-substituted 3,3,3-trifluoroalanines<sup>137</sup> has been described.

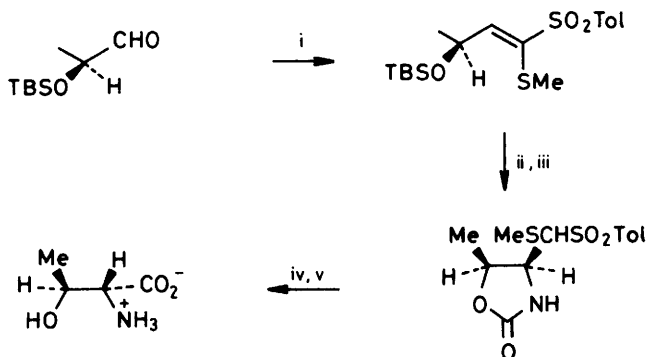
Three standard methods leading to  $\alpha$ -alkyltryptophans have been reported.<sup>138</sup> The direct approach, exemplified by the synthesis of  $\alpha$ -methyl-L-methionine from L-methionine via the derived 1,3-oxazolidin-5-one (13; O in place of NMe) can be



Scheme 19

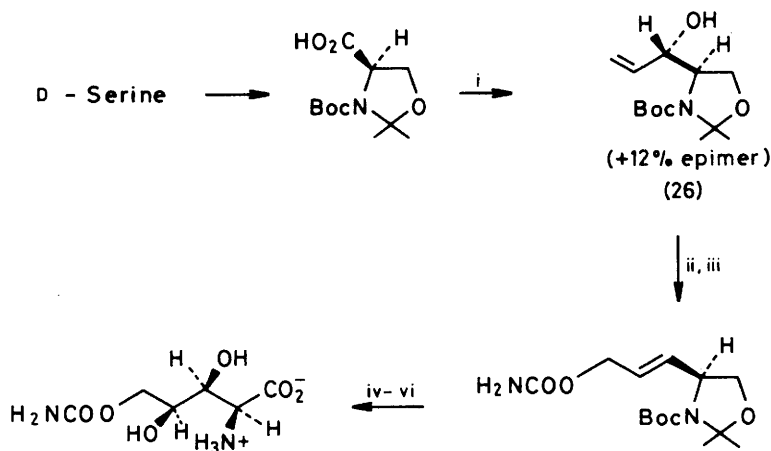


Scheme 20



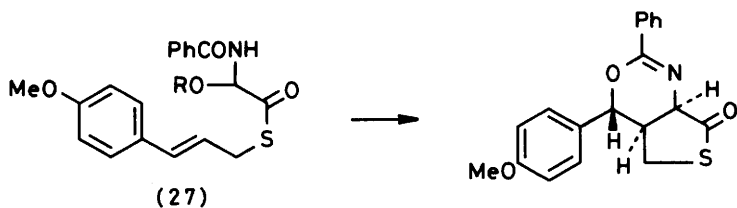
Reagents : i,  $\text{MeSCH}_2\text{SO}_2\text{Tol}$ ,  $\text{BuLi}$ ; ii,  $1\text{M HCl}$  then  $\text{CCl}_3\text{CONCO}$ ; iii,  $\text{K}_2\text{CO}_3\text{-MeOH}$ ;  
 iv,  $\text{MCPBA}$ , then  $\text{TFAA}$ ; v,  $\text{COSMe} \rightarrow \text{CO}_2\text{H}$

Scheme 21

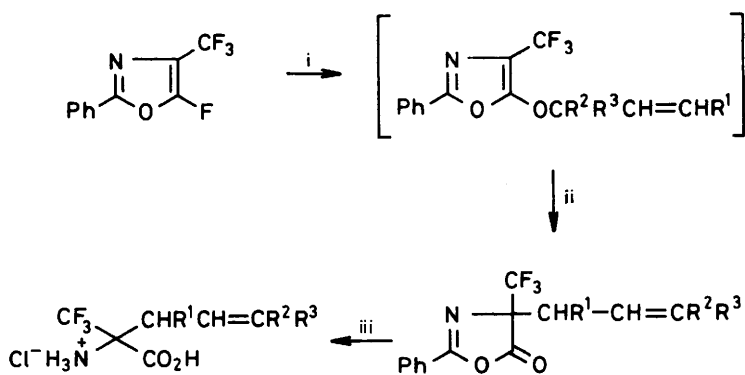


Reagents : i,  $\text{CH}_2=\text{CHMgBr}$ ; ii, separate,  $p\text{-NO}_2\text{-C}_6\text{H}_4\text{OCOCl}$ , then  $\text{NH}_3$ ; iii,  $\text{PdCl}_2$   
 (allylic rearrangement); iv,  $\text{TSOH}$ ,  $\text{MeOH}$ , then  $\text{KMnO}_4$ ; v,  $N\text{-bromo-urea}$  (oxidation); vi,  $\text{aq. HCl}$

Scheme 22

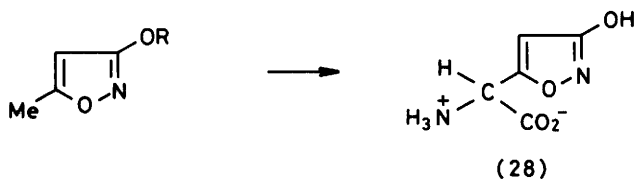


Scheme 23



Reagents: i,  $R^1CH=CHCR^2R^3OH$ ; ii, spontaneous hetero-Cope rearrangement;  
iii, boiling conc. HCl

Scheme 24



operated on a moderately large scale (45 g L-methionine gave 10 g of its  $\alpha$ -methyl homologue).<sup>139</sup>

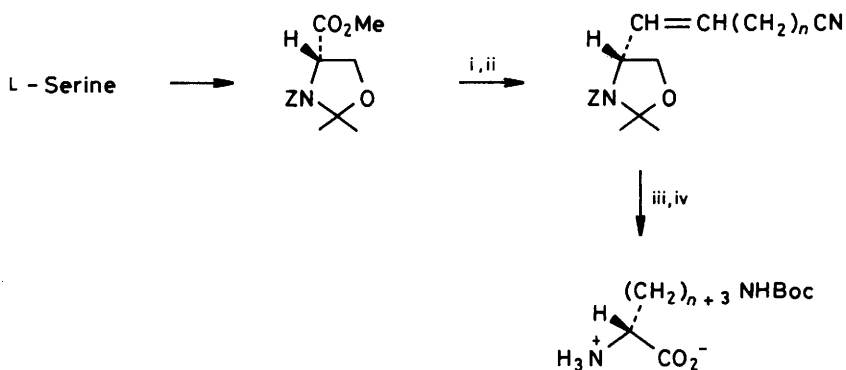
**4.5 Other Aliphatic and Saturated Heterocyclic  $\alpha$ -Amino Acids.**— This section collects syntheses of aliphatic amino acids that, it so happens, are not known in natural sources. However, most of the methods are complementary to, or identical with, those in the preceding Sections of this Chapter.

Routine elaboration of the *N*-protected oxazolidine from L-serine (Scheme 25) provides a general enantioselective synthesis of D- $\alpha,\omega$ -diamino-alkanoic acids,<sup>140</sup> but less routine is the cycloaddition of an ynamine to the oxazolone ( 29 ) leading to 3-substituted asparagines (Scheme 26).<sup>141</sup> All the other reports deal with cyclic structures, including enantioselective syntheses of 1-aminocyclopropane-1-carboxylic acids from the (-)-phenylglycinol-derived synthon ( 30 )<sup>142,143</sup> (Scheme 27),<sup>142</sup> and of  $\alpha$ -(carboxycyclopropyl)glycines from (S)-2-aminobut-3-enol (Scheme 28).<sup>144</sup> The fortunate capture of the halogen atom in a preparation of a 4-methyleneoxazolone and its retention during cyclopropanation with diazomethane (cf. also Ref. 112) provided a means of synthesis of 2,3-methanohomoserine and 2,3-methanomethionine.<sup>145</sup> Stereospecific synthesis of *cis*- and *trans*-1-amino-3-(hydroxymethyl)cyclobutane-1-carboxylic acids has been developed based on the easy availability of the bridged lactone ( 31 ).<sup>146</sup> All stereoisomers of 1-aminocyclopentane-1,3-dicarboxylic acid,<sup>147,148</sup> and of 1-aminocyclohexane-1,3-dicarboxylic acid,<sup>148</sup> have been prepared from corresponding 3-oxo-compounds. These, like several of the other cycloalkane-based amino acids mentioned above, attract interest as conformationally-restricted analogues of neurotransmitter amino acids; while *cis*-1R,3R-compounds mimic *N*-methyl-D-aspartic acid activity, the others are (-)- $\alpha$ -kainic acid-like in their effects.<sup>147</sup>

Gabriel-type synthesis of 3-phenylaziridine-2-carboxylates by amination of (E)-cinnamates is not stereospecific,<sup>149</sup> contradicting earlier assertions.<sup>150</sup> Ring expansion of these imino acids occurs (with retention of configuration) when they are treated with 2-chloroethanol and 2-chloroethanethiol, giving 4-oxa- and 4-thia-pipecolic acids respectively.<sup>151</sup> 4-Oxopipecolic acid is accessible through cyclization of tert-butyl 2-Boc-amino-4-oxo-hex-5-enoate.<sup>152</sup>

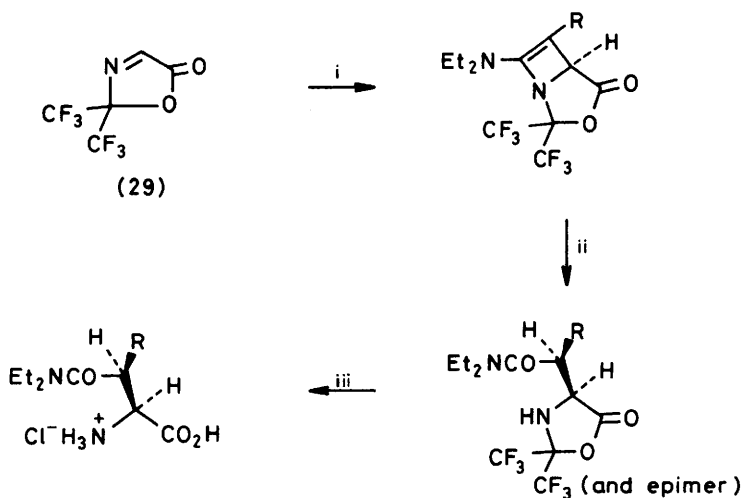
All stereoisomers of 3-ethylproline have been obtained through Michael addition of pent-2-enal or ethyl pent-2-enoate to an *N*-protected 2-aminomalonate, followed by routine elaboration, and resolution using (+)-dibenzyltartaric acid.<sup>153</sup>

Intramolecular Michael addition ( 32  $\rightarrow$  33 ) giving 3-carboxymethylproline<sup>154</sup> is a simple exercise following the pioneering routes that had to be overcome for the much more demanding problems of stereospecific synthesis of the kainic acids described in preceding Volumes of this Report. The *cis:trans* ratio of the product (30:70) was amenable to equilibration (NaOEt/refluxing benzene) to 5:95.



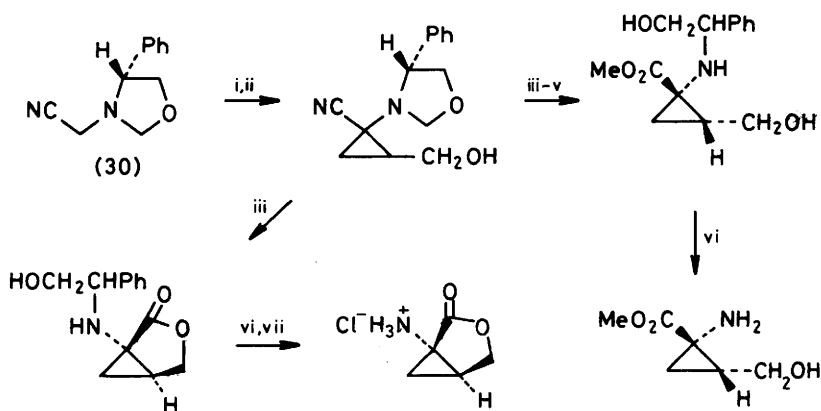
Reagents: i, DIBAL; ii,  $\text{Ph}_3\text{P}=\text{CH}(\text{CH}_2)_n\text{CN}$ ; iii,  $\text{NaBH}_4$  then  $(\text{Boc})_2\text{O}$ ; iv,  $\text{TsOH}$ , Jones' reagent,  $\text{H}_2$ -Pd

Scheme 25



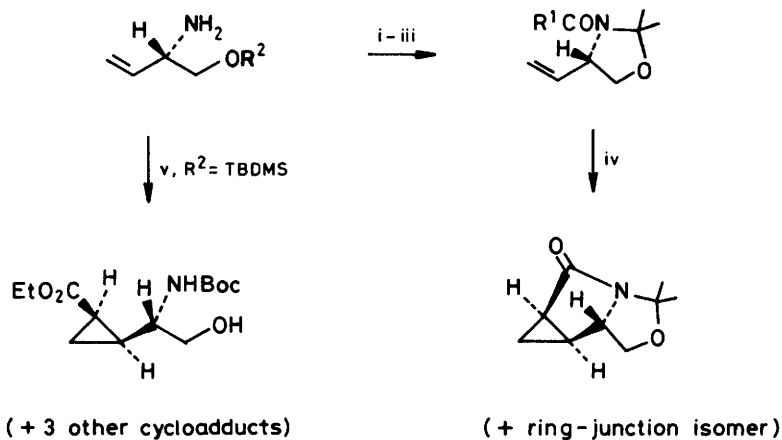
Reagents: i,  $\text{Et}_2\text{N}-\text{C}\equiv\text{CR}$ ; ii, 0.05M  $\text{HCl}$ ; iii, conc.  $\text{HCl}$

Scheme 26



Reagents: i, LDA - HMPA; ii, epibromhydrin; iii, chromatographic separation;  
iv, aq. NaOH; v,  $\text{SOCl}_2$  - MeOH; vi,  $\text{H}_2$  - Pd; vii, HCl - EtOH

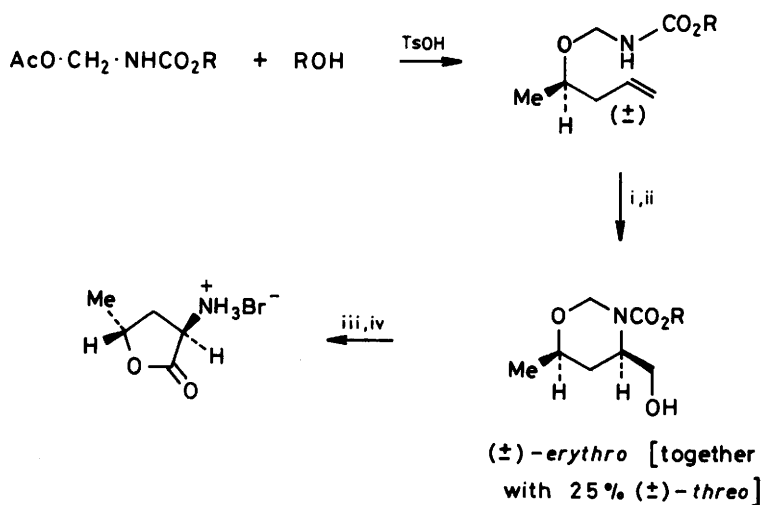
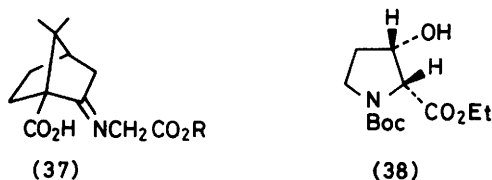
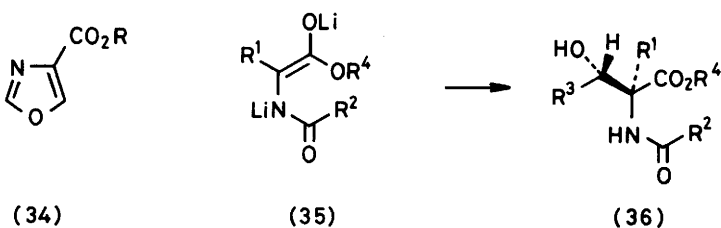
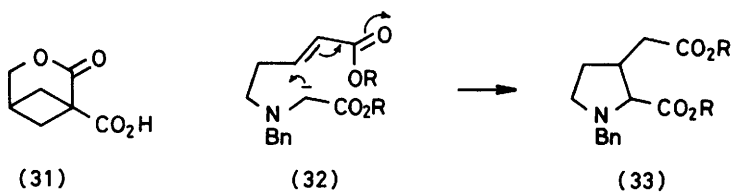
Scheme 27



Reagents: i *N*-Boc-Gly-OSu,  $\text{Et}_3\text{N}$ ; ii, acetone,  $(\text{MeO})_2\text{CMe}_2$ ;  
iii,  $\text{R}^1 = \text{BocNHCH}_2 \longrightarrow \text{CHN}_2$ ; iv,  $\text{Pd}(\text{OAc})_2$ ; v,  $\text{Boc}_2\text{O}$ , then  $\text{N}_2\text{CHCO}_2\text{Et}$ ,  $\text{Pd}(\text{OAc})_2$

Scheme 28





Reagents : i,  $\text{Hg}(\text{NO}_3)_2, \text{MeCN}$ ; ii,  $\text{KBr}, \text{O}_2, \text{NaBH}_4$ ; iii, Jones' reagent; iv,  $\text{HBr} - \text{AcOH}$

Scheme 29

**4.6 Prebiotic Synthesis of Amino Acids.**- Some familiar science can be detected among recent papers reporting attempts to simulate the contents and characteristics of the primordial environment. High energy discharge experiments continue with u.v. picosecond laser pulsing at 266 nm through ammonium acrylate solution to give  $\alpha$ - and  $\beta$ -alanines,<sup>155</sup> and electric discharge through  $H_2S$ ,  $CH_4$ ,  $N_2$ ,  $NH_3$ , and  $H_2O$  to give methionine, thirteen protein amino acids, and one non-protein amino acid.<sup>156</sup>

Experiments with aqueous solutions of cyanides<sup>157,158</sup> reveal that much milder treatment leads to amino acids when these solutions overlay some common minerals. 2.2M-Aqueous KCN, after 26 days at 70° over sepiolite or zeolites, contains aspartic and glutamic acids, glycine, serine, threonine, and  $\alpha$ - and  $\beta$ -alanines,<sup>157</sup> while a similar solution containing the ethyliminium cation over crystalline kaolinite, develops  $\alpha$ -aminopropionitrile, hydrolysis giving  $\alpha$ -alanine enriched in either L- or D-enantiomer, depending on the chirality at the adsorption site and the orientation of the cation at this site.<sup>158</sup> Clearly, this last-mentioned aspect is of extraordinary importance and will require confirmation.

The basis of a review<sup>159</sup> is that  $H_2S$  has special importance in prebiotic chemistry. A further example of the sort of armchair reasoning that has been regularly published on this topic discusses the fact that 16 of the protein amino acids possess a  $\beta$ - $CH_2$  group, and a further three possess a  $\beta$ -methine group;<sup>160</sup> it is proposed that this uniformly-sized "spacer group" has arisen through a form of prebiotic selection since properties of peptides and proteins are tailored to a purpose (here, the reasoning becomes more vague!) that is fulfilled by this structural feature.

**4.7 Halogenoalkyl Amino Acids.**- Noting some that some papers relevant to this section can be found in the preceding sections, since they also have relevance there, a conventional approach has been described for the synthesis of (2R,3S)- and (2R,3R)-3-bromobutyrynes from appropriate threonines and from L-threo-3-methyl aspartic acid.<sup>161</sup>

**4.8 Hydroxyalkyl Amino Acids.**- A review has appeared, covering 4-alkoxycarbonyloxazoles (34) for use in synthesis as latent  $\beta$ -hydroxy- $\alpha$ -amino acids.<sup>162</sup>

Dilithiated N-acylamino acid esters give threo- and erythro- $\alpha\beta$ -disubstituted serines through addition to aldehydes (35 + 36).<sup>163</sup> A similarly conventional approach has been used, but with the Schiff base (37) based on (+)-ketopinic acid as chiral auxiliary, for the synthesis of diastereoisomers of  $\beta$ -phenylserine.<sup>164</sup>

A new general method (see also Ref. 204) for the stereoselective synthesis of threo- or erythro-1,3-amino alcohols from a single precursor, based on intramolecular amidomercuration, has been used for synthesis of both

diastereoisomers of  $\gamma$ -hydroxy-DL-norvaline<sup>166</sup> (from 4-penten-2-ol; Scheme 29).

Continuation of studies (Vol. 20, p.15) employing monosaccharide lactones for stereoselective synthesis, is described in reports on (2R,3S,4R)-3,4-dihydroxyproline<sup>166</sup> and its enantiomer.<sup>167</sup> Protected (2R,3S)-3-Hydroxyproline (38) is obtained in better than 99% optical purity by yeast-mediated reduction of the corresponding 3-oxo-proline.<sup>168</sup>

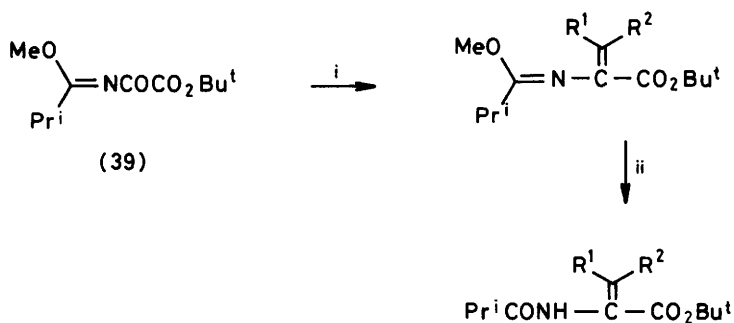
**4.9 Aminoalkyl Amino Acids.-** While noting that earlier sections have claimed mention of reports that could have been located here, one out-of-the-ordinary study, of the analogue of S-adenosyl-L-methionine in which the sulphur atom is replaced by the tertiary amine function (N≡ in place of \*S≡), is noteworthy.<sup>169</sup>

**4.10 Amino Acids with Unsaturated Side Chains.-**  $\alpha\beta$ -Unsaturated  $\alpha$ -amino acids have been reviewed.<sup>170</sup> Standard routes based on azlactones (see also Refs. 81,82,185) include an unusual application<sup>171</sup> of DMF-imines  $RN=CHNMe_2$  (R = various 6-membered diaza-heterocycles) for the preparation of corresponding  $\beta$ -RNH-substituted-methylene- $\alpha$ -amino acids. The first report of Wittig-Horner olefination of the cationic amino acid synthon, ethyl N-carbethoxyoxamate (39 in Scheme 30) has appeared,<sup>172</sup> as well as a conventional Wittig approach employing N-acyl  $\alpha$ -(diethoxyphosphonyl)glycine ethyl esters.<sup>173</sup>

Interesting new routes to these 'dehydro-amino acids' involve N-carboxyanhydride formation from a 2-azido-3-substituted alkanoid acid,  $COCl_2$ , and  $NaReO_4$ ,<sup>174</sup> and preparation of trifluoroacetyl N-methyl derivatives (Scheme 31).<sup>175</sup>

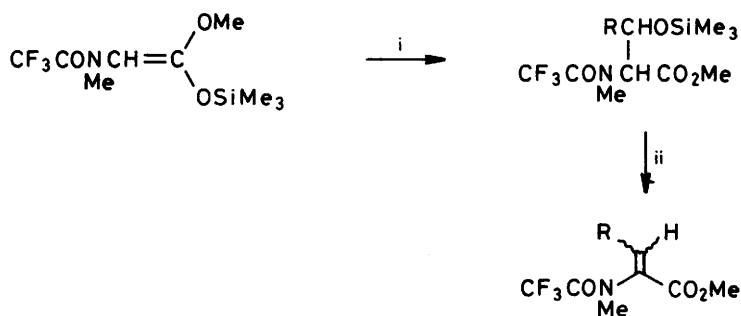
Some routes to DL-vinylglycine give disappointing overall yields, far lower than the 52.2% for the base-catalyzed condensation of diethyl 2-acetamidomalonate with phenyl vinyl sulfoxide in DMSO at elevated temperatures.<sup>176</sup> Alkylation of acetamidomalonates with tert-butyl propargylate gives 'trans- $\beta$ -v-dehydro-glutamates', from which, by routine elaboration, correspondingly unsaturated L-ornithine and L-arginine have been prepared.<sup>177</sup> General routes to these  $\beta$ -unsaturated amino acids have been reviewed;<sup>178</sup> this article essentially covers reductive elimination of 5-chloromethyloxazolines catalyzed by Vitamin B<sub>12</sub> or cobalt esters for the purpose, also the highly stereoselective amidoalkylation of (E)- or (Z)-alkenylsilanes with *in situ*-generated acyliminium cations.<sup>179</sup>

**4.11 Aromatic and Heteroaromatic Amino Acids.-** Some conventional studies, involving conversion of protein amino acids with aromatic or heteroaromatic groups in their side chains, into homologues through substitution reactions, are featured every year in this section (and also in the later Section 6.3). Oxygenation of L-phenylalanine, to give DOPA (and other hydroxylated phenylalanines, total yield ca. 50%) can be effected under Udenfriend conditions ( $Fe^{2+}$  -  $O_2$  with electrochemical reduction of  $Fe^{3+}$  formed in the process).<sup>179</sup> Both 2- and 3-



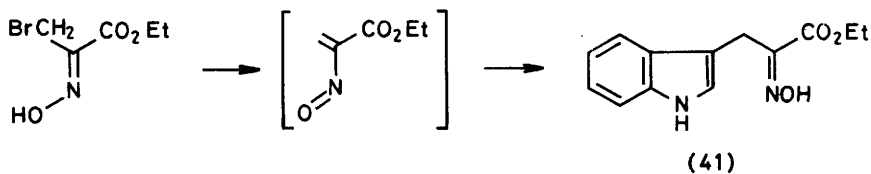
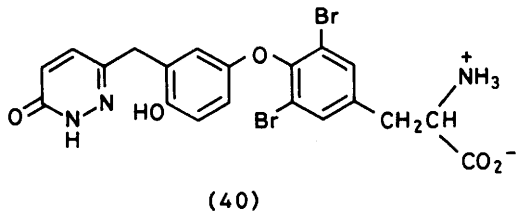
Reagents: i,  $\text{Ph}_3\text{P}=\text{CR}^1\text{R}^2$ ; ii, aq. HI

### Scheme 30



Reagents: i,  $\text{RCHO}$ ,  $\text{ZnBr}_2$ ; ii,  $100-110^\circ$  with  $\text{CF}_3\text{SO}_3\text{H}$  or  $(\text{MeSO}_2)_2\text{O}$  - DMAP

### Scheme 31



monofluorotyrosines are formed through direct ( $F_2$ ) fluorination of *O,N*-diacetyl L-tyrosine methyl ester, the short time being the crucial feature recommending the route for use in synthesis of isotopically-labelled analogues.<sup>180</sup>

Synthesis of ( 40 ; alias SK & F L-94901, a novel thromimetic and, from the point of view of this Section, a mixed aromatic - heteroaromatic system) has been described (see also Ref.225).<sup>181</sup>

Most of the 'heteroaromatic interest' focuses on tryptophan analogues. 8-Aza-tryptophan has been prepared through the bislactim ether approach, usually used (cf. Section 4.2) for asymmetric synthesis but unaccountably used in this study for the preparation of the racemate.<sup>182</sup> Oximes ( 41 ) formed through cycloaddition of indoles with  $\alpha$ -nitroso-acrylates can be reduced ( $Zn/AcOH$ ) to 2-substituted tryptophans.<sup>183</sup> Nitration of *N*-trifluoroacetyltryptophan methyl ester with  $HNO_3$  gives 60% 6-nitro- and 6.7% 2-nitrotryptophans.<sup>184</sup>

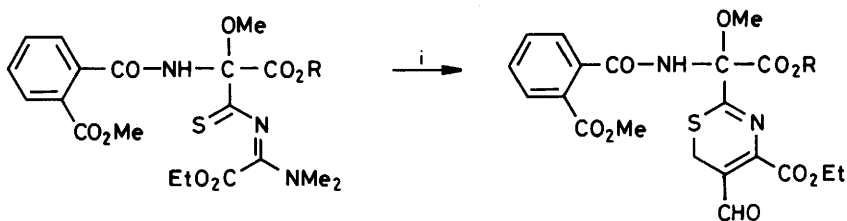
$\gamma$ -*N*-Alkylhistidines have been prepared through the azlactone route (condensation of 1-alkyl-4-formyl-1*H*-imidazoles with hippuric acid and acetic anhydride).<sup>185</sup> Interest in the thiazine ring (present in peptide-based cephalosporins) has been of long standing, and an approach based on earlier routes (Scheme 32) for the synthesis of amino acids containing this side-chain moiety has been reported.<sup>186</sup>

**4.12 *N*-Hydroxy Amino Acids.** - Oxidation of Schiff bases with *m*-chloroperbenzoic acid and opening of the resulting oxaziridines with hydroxylamine is a serviceable route to these derivatives.<sup>187</sup>

**4.13 Amino Acids containing Sulphur or Selenium.** - Sulphenyl chlorides  $RSOCl$  add to acrylic esters to give  $\alpha$ -chloro- $\beta$ -alkylthioalkanoates as major regioisomers, from which, by conventional azidolysis but unconventional reduction of the resulting azides (with  $H_2S$ -pyridine or  $H_2S-Re_2S_7$ ), correspondingly substituted cysteines are obtained.<sup>188</sup> A similar addition approach underlies the formation of thiazoline-4-carboxylic acids from  $\alpha$ -chloro-acrylic acid and *N*-methylthiobenzamide,<sup>189</sup> *N*-formyl- $\beta$ -alkylthio- $\alpha$ -amino acid esters from ' $\alpha\beta$ -dehydroamino acids' and alkanethiols,<sup>190</sup> and ring-opening of *cis*- and *trans*-2-phenylaziridine-3-carboxylic acid (-)-menthyl esters with  $H_2S$  to give  $\beta$ -mercaptophenylalanine enantiomers.<sup>144</sup>

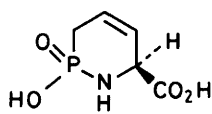
(*S*)- $\beta$ -2- and 3-Selenienylalanines have been prepared by two standard asymmetric synthesis methods, Rh-chiral phosphine-catalyzed hydrogenation or the use of the bis-lactim ether method.<sup>191</sup> The enzymic resolution approach continues to show its breadth of application, with use of *Q*-acetylhomoserine sulphydrylase for the preparation of L-selenocysteine and L-selenohomocysteine from  $Na_2Se_2$  and acetyl derivatives of serine and homoserine.<sup>192</sup> The former of these has been used for the preparation of the new selenium-containing amino acid L-selenodjenkolic acid.

**4.14 Phosphorus-containing Amino Acids.** - Methyl  $\alpha$ -methoxyhippurate and the *N*-

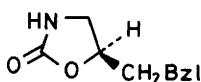


Reagents: *i*,  $\text{CH}_2=\text{CHCHO}$ , Amberlyst 15

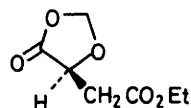
**Scheme 32**



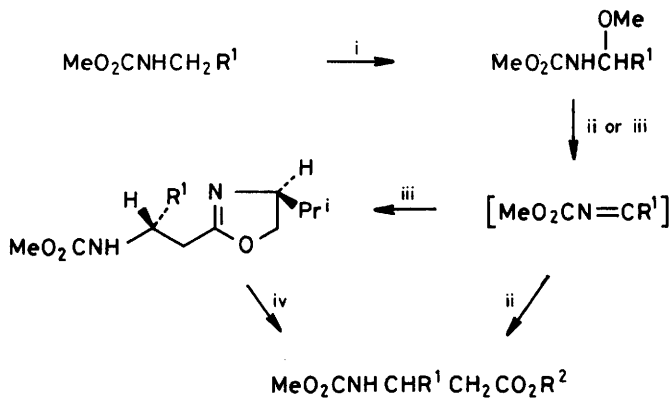
(42)



(43)



(44)



Reagents: *i*,  $\text{e}^-$ ,  $\text{MeOH}$ ; *ii*,  $\text{MeCO}_2\text{R}^2$ ,  $\text{LDA}$ ,  $\text{THF}$ ,  $-70^\circ\text{C}$ ; *iii*, (S)-4-isopropyl-2-methyloxazoline,  $\text{LDA}$ ,  $\text{THF}$ ,  $-70^\circ\text{C}$ ; *iv*,  $\text{IM-HCl}$ , then  $\text{HCl}-\text{MeOH}$

**Scheme 33**

benzyloxycarbonyl analogue yield the corresponding  $\alpha$ -phosphonylglycines, and these have been converted into phosphites with Lewis acids.<sup>133</sup>

Diethyl 2-(*p*-chloromethylbenzyl)-2-acetamidomalonate has been shown to be susceptible to Arbuzov reaction with triethyl phosphite, as well as to other halogen-substitution processes (e.g.  $\rightarrow \text{CH}_2\text{SO}_3\text{Na}^+$ ).<sup>134</sup> The plumbicin A and B (N-1409) component (42) has been synthesised by Ugi 4-component condensation and other general methods using the aldehyde  $\text{OCHCH}=\text{CHP}(\text{O})(\text{OEt})\text{OR}$  and straightforward elaboration of the resulting protected 3,4-didehydro-5-phosphono-norvaline.<sup>135</sup>

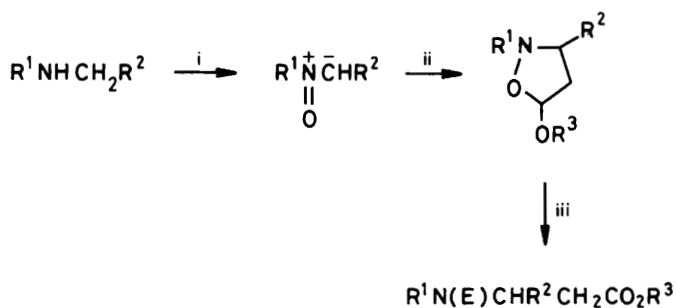
**4.15 Amino Acids Synthesized for the First Time.**— No reader searching for newly-synthesized amino acids should concentrate only on this Section, since several such compounds for which other points of interest are discernible, are mentioned elsewhere in this Chapter. New di- and trichlorophenylalanines,<sup>38</sup> ring-substituted phenylalanines and tryptophans,<sup>136</sup> and thyroxines,<sup>41</sup> have been reported.

**4.16  $\beta$ - and Higher Amino Acids.**— A new  $\beta$ -amino acid synthesis<sup>137</sup> based on nucleophilic addition of enolate anions of alkyl acetates to *N*-methoxycarbonyl imines is displayed in Scheme 33. Use of chiral optically-active 2-methyl-oxazolines instead of alkyl acetates leads to 72-90% enantiomeric excesses. A high yield route (Scheme 34) is based on alkylation of amine oxides with vinyl ethers.<sup>138</sup> Hydrocyanation of phthalimido-alkynes catalyzed by nickel powder leads to mixtures of  $\beta$ - and  $\gamma$ -amino acids.<sup>139</sup>

Particular examples of synthesis targets include (*S*)-isoserine  $\text{NH}_2\text{CH}_2\text{CH}(\text{OH})\text{CO}_2\text{H}$ , for which three independent studies claim the first enantioselective synthesis. (*S*)-Malic acid, *via* (43) formed with  $(\text{PhO})_2\text{P}(\text{O})\text{N}_3$  and  $\text{Et}_3\text{N}$  in refluxing benzene,<sup>200</sup> or *via* Curtius rearrangement of (44),<sup>201</sup> and (-)-8-phenylmenthyl glyoxylate monohydrate,<sup>202</sup> are the chosen starting materials. In the latter study,<sup>202</sup>  $\text{KF}$ -promoted addition to  $\text{MeNO}_2$  gives only 50% yield after work-up, but the product is optically-pure. Enantiomers of cucurbitine (45 in Scheme 35) and the near relative, (-)-3-amino-tetrahydrothiophen-3-carboxylic acid (45; *S* in place of *NH*), have been synthesized through a 1,3-dipolar cycloaddition sequence followed by pig liver esterase-catalyzed hydrolysis.<sup>203</sup>

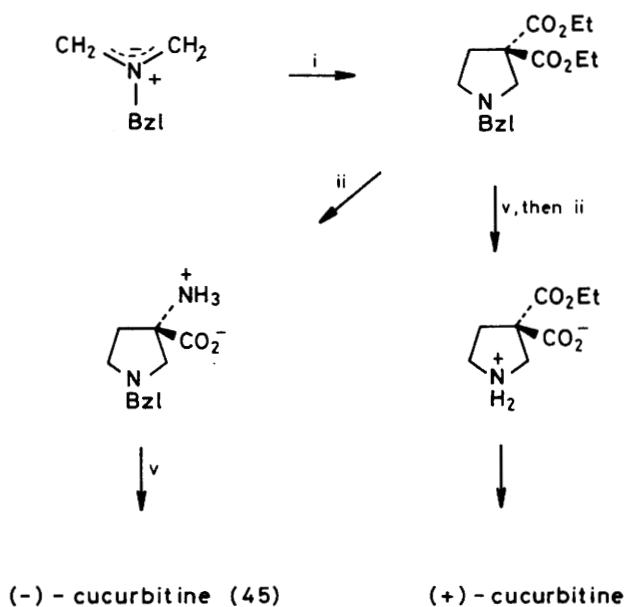
Stereoselective synthesis of racemic *threo*- $\gamma$ -hydroxy- $\beta$ -lysine lactone<sup>204</sup> from  $\text{PhI}(\text{CH}_2)_2\text{CH}(\text{OCH}_2\text{NHCOOC}_6\text{H}_{11})\text{CH}=\text{CHCH}_2\text{OBzl}$  provides a further example of cyclization by the amidomercuration method (see Scheme 29; ref.165).

$\gamma$ -Amino acids are represented by simple examples (46)<sup>205</sup> and (*R*)-carnitine (Scheme 36)<sup>206</sup> as far as stereospecific synthesis is concerned, but pre-eminently by the  $\beta$ -hydroxy- $\gamma$ -amino acid, statine and its analogues, for which several new syntheses<sup>207-213</sup> have been reported. A further route<sup>207</sup> to (3*R*,4*S*)-*N*-Boc-statine starting with *N*-Boc-L-leucinal employs the boron enolate of (47), related to the chiral synthon that has been used in an analogous synthesis of (3*S*,4*S*)-(+)-statine



Reagents: i,  $\text{H}_2\text{O}_2$ ; ii,  $\text{CH}_2=\text{CHOR}^3$ ; iii,  $\text{E-X}$ , then base

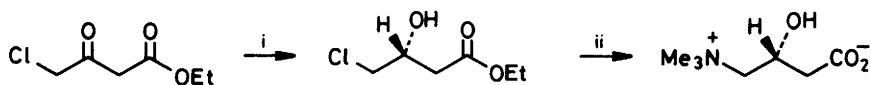
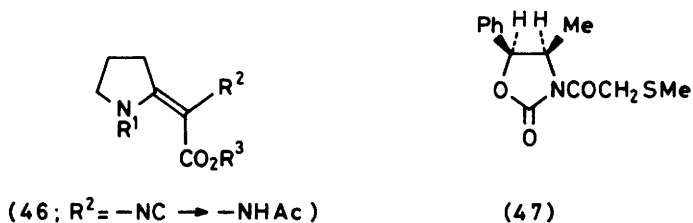
**Scheme 34**



Reagents: i,  $\text{CH}_2=\text{C}(\text{CO}_2\text{Et})_2$ ; ii, pig liver esterase; iii,  $\text{ClCO}_2\text{Et}$ ; iv,  $\text{NaN}_3$ ; v,  $\text{Pd-C/H}_2$

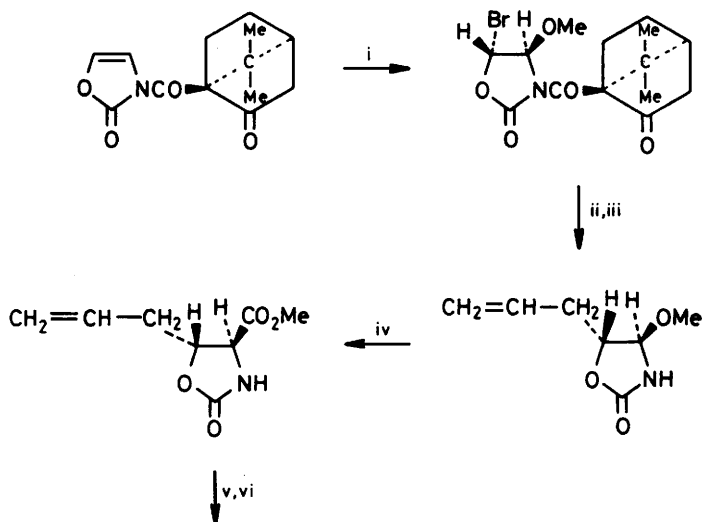
**Scheme 35**





Reagents : i, (S)- BINAP - Ru (II) ,  $H_2$ , 100 atm ; ii,  $Me_3N$

**Scheme 36**



(2*S*, 3*R*) - 3 - hydroxyglutamic acid

Reagents: i,  $Br_2 - MeC(OMe)_3 - TMSOTf$ ; ii,  $CH_2=CHCH_2SnBu_3$ ; iii,  $Bu_2CuLi$ ; iv,  $TMSCN$ , then  $MeOH$ ; v,  $KMnO_4 + NaIO_4$ ; vi, established methodology

**Scheme 37**

(Scheme 37),<sup>208</sup> and applied also to the synthesis of (2S,3R)-3-hydroxyglutamic acid.<sup>208</sup> Addition of the chiral acetate enolate  $\text{CH}_2=\text{C}(\text{OLi})\text{CHPhCPh}_2\text{OLi}$ ,<sup>209</sup> and of anions of malonate half-esters,<sup>210</sup> to  $\alpha$ -(Boc-amino)aldehydes, and condensation of malonic esters with *N*-protected amino acids<sup>211,212</sup> followed by reduction (including yeast-catalyzed reduction<sup>211</sup>), provide alternative routes to statines. A nine-step route to (+)-statine starts with (R)-2,3-O-isopropylidene glyceraldehyde.<sup>213</sup>

An extended example of the demands of syntheses of the 1,2-amino-hydroxy grouping on an alkanolic acid is provided by galantinamic acid (7 in Scheme 38), which together with seven 6R-diastereoisomers, has been synthesized starting from D- or L-lysine.<sup>214</sup>

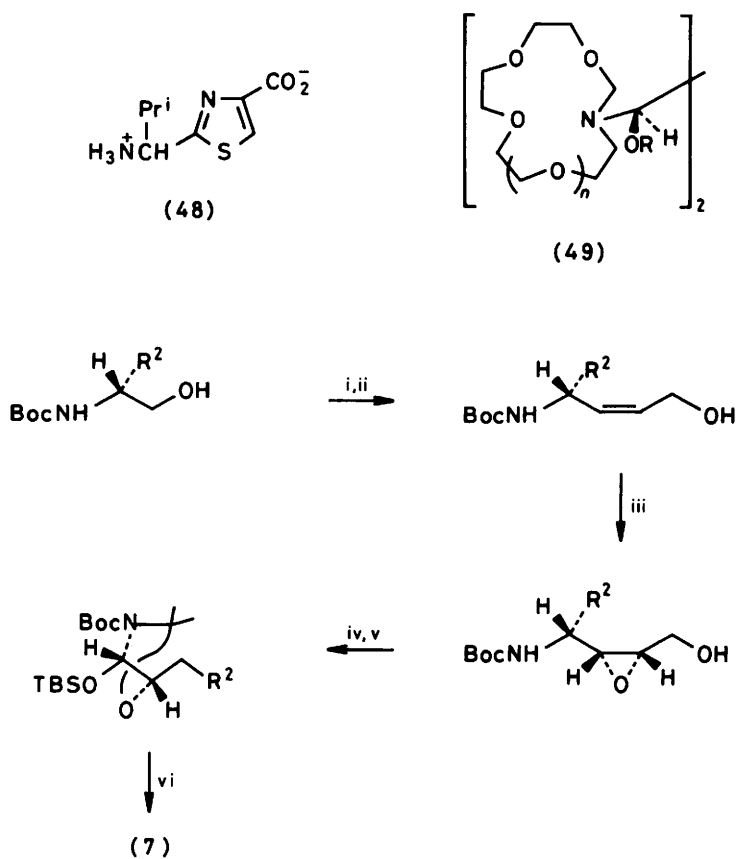
Less demanding methodology leads to 5-amino-4-oxopentanoic acid (4-oxo-pentanoic acid  $\rightarrow$  5-bromo; Gabriel synthesis)<sup>215</sup> and to the condensed dipeptide (48) from natural sources, which could be classified as an  $\omega$ -amino acid derivative [*Z*-Val-C(=NH)OEt + cysteine ethyl ester].<sup>216</sup>

**4.17 Labelled Amino Acids.** - Most of the labelling patterns represented for amino acids in preceding Volumes of this Report are featured again this year, with ever-increasing medical interest in short-lived <sup>11</sup>C and <sup>18</sup>F isotopomers. Coverage in this Section is in order of ascending atomic mass.

Large-scale synthesis of (R)- and (S)-[1-<sup>2</sup>H]glycine from [1-<sup>2</sup>H]furfural or 4-methoxybenzaldehyde[1-<sup>2</sup>H] can be achieved through condensation, respectively, with (+)- or (-)-*R*-isopinocampheyl-9-borabicyclo[3,3,1]nonane followed by amination and oxidation of the initial aldehyde substituent to CO<sub>2</sub>H using O<sub>3</sub> or RuO<sub>4</sub>.<sup>217</sup> The same chiral auxiliary has been used in a route to (2S,3S)-phenylalanine-[3-<sup>2</sup>H].<sup>218</sup> (R)- and (S)- $\alpha$ -methyl-[3,3,2-<sup>2</sup>H<sub>3</sub>]alanines have been prepared by the bis-lactim ether method.<sup>219</sup> (2S,4R)- and (2S,4S)-[4-<sup>2</sup>H]homoserine lactone hydrochlorides and (2S,3R)-[3-<sup>2</sup>H]-, (2S,3S)-[2,3-<sup>2</sup>H<sub>2</sub>]-, and (2S,3R,4R)-[3,4-<sup>2</sup>H<sub>2</sub>]-analogues, have been synthesized from corresponding aspartates, some of these being prepared through long-established routes from fumarates.<sup>220</sup> The last-mentioned labelled homoserine has been secured [stereochemistry (2RS,3S,4S)] by alkylation of (S,S)-[2,3-<sup>2</sup>H<sub>2</sub>]-oxirane by the dianion of hippuric acid.<sup>221</sup> Standard routes have been applied to the synthesis of per-<sup>2</sup>H-DL-leucine from acetone-<sup>2</sup>H<sub>6</sub>.<sup>222</sup> [3,3-<sup>2</sup>H<sub>2</sub>]- and [2,3,3,3-<sup>2</sup>H<sub>4</sub>]- $\beta$ -Alanines have been prepared from NCCH<sub>2</sub>CO<sub>2</sub>Et through treatment of the LiAlH<sub>4</sub> reduction product by oxidation or refluxing <sup>2</sup>HCl, respectively.<sup>223</sup>

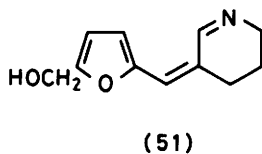
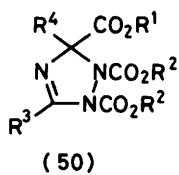
[3-<sup>3</sup>H]-Phenylalanines are accessible through subjecting *N*-Boc phenylalanines to Pd-catalyzed exchange with <sup>3</sup>H<sub>2</sub>.<sup>224</sup> A labelled version of the novel thyromimetic, SK & F L-94901 (40) with <sup>3</sup>H in both *ortho*-positions, and  $\beta$ -<sup>14</sup>C in the tyrosine moiety, has been reported.<sup>225</sup>

[3-<sup>11</sup>C]-DL-Alanine,  $\alpha$ -aminoisobutyric acid, norvaline, norleucine and phenylalanine have been synthesized by phase-transfer alkylation of *N*-(diphenylmethylene)glycine tert-butyl ester, and L-enantiomers of alanine and



Reagents: i,  $\text{SO}_3\text{-py}$ , DMSO, then  $(\text{CF}_3\text{CH}_2\text{O})_2\text{P}(\text{O})\text{CH}_2\text{CO}_2\text{Me}$ ; ii, DIBAL; iii, MCPBA;  
iv,  $\text{LiAlH}_4$ ; v,  $\text{TBMSCl}$ ; vi,  $\text{OsO}_4$

**Scheme 38**



phenylalanine have been obtained from their labelled racemates using D-amino acid oxidase.<sup>226</sup> <sup>11</sup>C-Labelled L-valine, and L-enantiomers of those amino acids in this list that are not substrates for this enzyme, were synthesized by asymmetric alkylation of [(+)-2-hydroxypinanyl-3-idene]glycine tert-butyl ester, in 80-82% enantiomeric excesses; but the 50-55 minutes' overall reaction time meant that radiochemical yields were only 9-25%.<sup>227</sup> L- and D-[Methyl-<sup>14</sup>C]methionines can be prepared by methylation of S-benzylhomocysteines<sup>228</sup> (and correspondingly labelled selenomethionines<sup>229</sup>), and from L-homocysteine thiolactone.<sup>230</sup> These routes start with <sup>11</sup>CO<sub>2</sub>, and the use of this and of <sup>11</sup>CO have been reviewed.<sup>231</sup>

L-[2-<sup>13</sup>C]Serine formation from [2-<sup>13</sup>C]glycine and HCHO, catalyzed by serine hydroxymethylase, can be followed by <sup>13</sup>C-n.m.r.<sup>232</sup> N-Tosyl-[2-<sup>13</sup>C]glycine has been used for the synthesis of 2-amino-1,5-dihydro-1-[N-methyl-<sup>13</sup>C]-4H-imidazol-4-one-[5-<sup>13</sup>C], alias creatinine-<sup>13</sup>C<sub>2</sub>.<sup>233</sup> [2-<sup>14</sup>C]-α-Aminoisobutyric acid and -2-amino-2-methylbutanoic acid, intriguingly described in the abstract as "potential tumour-seeking agents", have been synthesized by modified Bucherer reaction.<sup>234</sup>

S-Adenosyl L-[1-<sup>14</sup>C]homocysteine can be prepared from L-methionine-[1-<sup>14</sup>C] via S-benylation followed by reaction with 5'-Q-tosyladenosine.<sup>235</sup>

Isotopically-enriched indoles condensed with L-serine catalyzed by E.coli tryptophan synthetase provide [1-<sup>15</sup>N]-, [indole C-2-<sup>13</sup>C]-, or [indole C-3-<sup>13</sup>C]-labelled L-tryptophans.<sup>236</sup> Microbial syntheses employing (<<sup>15</sup>NH<sub>4</sub>>)<sub>2</sub>SO<sub>4</sub> have provided <sup>15</sup>N-labelled leucine and isoleucine, and their [<sup>15</sup>N, 3-<sup>13</sup>C]-analogues.<sup>237</sup>

Direct aromatic substitution of phenylalanine and tyrosine by AcO<sup>18</sup>F gives 2-[<sup>18</sup>F]- and 3-[<sup>18</sup>F]- analogues, respectively,<sup>238</sup> the use of <sup>18</sup>F<sub>2</sub> for the purpose being less efficient.<sup>239</sup> A conventional synthesis route from p-nitrobenzaldehyde that involves <sup>18</sup>F- displacement of the nitro-group and resolution by chiral chromatography, has been used for the synthesis of L-4-[<sup>18</sup>F]-phenylalanine (cf. Ref. 539).<sup>240</sup> 4- and 6-[<sup>18</sup>F]-5-Hydroxytryptophans have been prepared by <sup>18</sup>F<sub>2</sub> substitution.<sup>241</sup>

Aromatic bromination provides radiobromine-labelled 6-bromo-m-tyrosine.<sup>242</sup>

**4.18 Resolution of Amino Acids.**— Enzymic resolution continues to develop into new and perhaps unexpected variants of well-established applications, which have been reviewed.<sup>243</sup> Thus, α-nitro-α-methylalkanoate esters can be kinetically resolved using α-chymotrypsin<sup>244</sup> as a component of a general amino acid synthesis, and the same enzyme yields methyl L-phenylalaninate through catalyzing the asymmetric transesterification of n-propyl DL-phenylalaninate in MeOH.<sup>244</sup> The more conventional approach is represented in yeast-catalyzed asymmetric hydrolysis of ethyl N-acetyl-DL-fluorophenylalaninates,<sup>245</sup> Aspergillus niger lipase with Z-amino acid 2-chloroethyl esters,<sup>246</sup> and Mycobacterium neoaurum cells acting on α-alkyl-α-amino acid amides.<sup>247</sup> The principle has been adapted for assessing the optical purity of amino acids using L- or D-amino acid oxidase-coated electrodes.<sup>248</sup>

Diastereoisomer-forming derivatization also continues to serve the need for enantiomerically-pure  $\alpha$ -amino acids, illustrated by (S)-2-chloropropionylation of  $\alpha$ -alkyl- $\alpha$ -amino acids.<sup>247</sup> Further development of the use of chiral binaphthyl hosts that show high chiral recognition for phenylglycinates is reported,<sup>248</sup> and conventional uses of resolving agents [(+)-1-phenylethanesulphonic acid and DL-p-hydroxyglycine;<sup>250</sup> (+)-tartaric acid and *N*-salicylylidene-DL-thiazolidine-4-carboxylic acid<sup>251</sup>] have the added virtue that the L-enantiomer that remains in solution in these cases is epimerized. Diastereoisomeric salt formation with (+)-dibenzyltartaric acid has been used for the resolution of *cis*- and *trans*-3-ethyl prolines.<sup>153</sup>

Preferential crystallization of one enantiomer from supersaturated solutions is attracting a wider circle of users [DL-valine hydrochloride;<sup>252</sup> DL-thiazolidine-4-carboxylic acid;<sup>253</sup> *N*-acetyl DL- $\alpha$ -aminoisobutyric acid and DL-norvaline ammonium and alkylammonium salts<sup>254</sup>], with an extraordinary result<sup>255</sup> for [1-<sup>14</sup>C]-DL-leucine, resolved by seeding with unlabelled D- or L-leucine.

Chromatographic resolution (discussed further in Section 7.5) is also developing rapidly, with promising results for new chiral polymers<sup>256</sup> and detailed information on use of the chiral medium formed by bonding L-phenylalanine to polyacrylamide (Biogel P4) through Mannich condensation with formaldehyde, loaded with copper(II) ions, for ligand exchange resolution of DL-[1-<sup>3</sup>H]-amino acids.<sup>257</sup>

Explanations for prebiotic resolution of amino acids developed in the recent literature deal with amplification of small local enantiomeric excesses (the Frank model; see Vol.20, p.34) through the slightly different rate constants shown by enantiomers in a given reaction,<sup>258</sup> and with the slightly different energy of one kaolinite enantiomer compared with the other, resulting in preferential adsorption and consequent activation of one enantiomer of a DL-amino acid (see also Ref.158).<sup>259</sup> The underlying 'weak interaction' principle that assigns microscopically-different energies to each of a pair of enantiomers underlies these reports, and the general topic has been exhaustively reviewed.<sup>260</sup>

## 5 Physical and Stereochemical Studies of Amino Acids

### 5.1 X-Ray Crystal Analysis.-

This Section does not, as a rule, need an introductory paragraph of general information, but it seems an appropriate way of drawing attention to a review of the crystallization behaviour of amino acids.<sup>261</sup>

Crystal structures of familiar amino acids and simple derivatives are featured in the recent literature: glycine cyclo-tetraphosphate,<sup>262</sup> DL-alanine,  $\beta$ -alanine, and sarcosine,<sup>263</sup> L-histidinium dihydrogen phosphate,<sup>264</sup> DL-lysine monohydrochloride monohydrate and D-lysine monohydrochloride dihydrate,<sup>265</sup> L- $\alpha$ -aminoadipic acid,<sup>266</sup> *N*-carbamoyl-L-asparagine,<sup>267</sup> *N*-Z-L-arginine hemihydrate,<sup>268</sup> the  $\alpha$ -methylDOPA derivative 3,4-dimethoxy- $\alpha$ -methyl-DL-phenylalanine

sesquihydrate,<sup>269</sup>  $\alpha$ -pivaloyl-L-proline  $\alpha$ -methylamide,<sup>270</sup> 1-amino-cyclopentane-1-carboxylic acid derivatives,<sup>271</sup> and amino acid constituents of the ristomycins.<sup>272</sup>

**5.2 Nuclear Magnetic Resonance Spectrometry.**— The continual raising of the level of sophistication of n.m.r. instruments seems to occur in annual increments, as reflected in this section Volume by Volume. Solid state studies, illustrated this year<sup>273</sup> by  $^2\text{H}$ -n.m.r. of polycrystalline L-[3,3- $^2\text{H}_2$ ]methionine, can be expected to become ever more prominent. These data have been interpreted in terms of side-chain conformational changes as a function of temperature over the range -35 to 106°.<sup>273</sup>

400 MHz  $^1\text{H}$ -n.m.r. spectra have been published for 38 amino acids in  $^2\text{H}_2\text{O}$  at p $^2\text{H}$  values 2, 7 and 12 (a total of 114 spectra).<sup>274</sup>  $^1\text{H}$ -n.m.r. data for  $\alpha$ -trityl-(2S,3R)-[3- $^2\text{H}$ ]- and -(2S,3S)-[2,3- $^2\text{H}_2$ ]homoserine lactone have been reconsidered, explaining a previous mis-assignment of C-4 proton resonances through unique shielding and deshielding effects of the trityl group.<sup>275</sup> Further  $^1\text{H}$ -n.m.r. studies of a more routine nature deal with  $\alpha$ -acetylvaline, norvaline, and  $\alpha$ -aminoisobutyric acid methylamides<sup>276</sup> and (3R,4S)- and (3S,4S)-statines.<sup>277</sup> The conformational information agrees well with that inferred from molecular orbital calculations in the former case, while the  $^1\text{H}$ -n.m.r. of statines, backed up with i.r. and X-ray data, give insights into conformational mobility and the propensity of these  $\gamma$ -amino acids towards self-association. Shift reagent -  $^1\text{H}$ -n.m.r. estimation of enantiomeric purity of  $\alpha$ -acetylamino acids has been elevated to an accurate operation.<sup>278</sup> Prototropic equilibria  $\text{HO}\cdots\text{N}^+ \rightleftharpoons \text{O}^-\cdots\text{HN}^+$  for  $\alpha$ -(1H-2-oxopyrimidin-4-yl)amino acids in DMSO have been quantified through a combined  $^1\text{H}$ -n.m.r. - FT-i.r. study.<sup>279</sup> A detailed  $^1\text{H}$ - $^{13}\text{C}$ -n.m.r. investigation of ethyl  $\alpha$ -( $\beta$ -benzoyl-ethyl)- $\beta$ -alaninate has been reported.<sup>280</sup>

Solid-state  $^{17}\text{O}$ -n.m.r. of a single crystal of  $^{17}\text{O}$ -enriched glycine reveals five  $^{17}\text{O}$  transitions, each comprising two lines, each caused by a dipolar interaction between a  $^{17}\text{O}$  atom and nearest protons in the unit cell.<sup>281</sup>

**5.3 Optical Rotatory Dispersion and Circular Dichroism.**— Fundamental studies of vibrational c.d. (v.c.d.; differential absorption of left- and right-circularly polarized light in the infrared wavelength region) continue for alanine, observing the general topography of the spectra with much still to be understood.<sup>282</sup>  $\alpha$ -Boc- and  $\gamma$ -amino acids have been studied by  $^{13}\text{C}$ -n.m.r., i.r., and v.c.d. in chloroform or DMSO;<sup>283</sup> these show a strong v.c.d. couplet in the carbonyl stretch wavelength region while  $^{13}\text{C}$ -n.m.r. spectra indicate that the amide configuration is predominantly *cis* at room temperature.

A folded conformation is indicated by intense c.d. for DMSO solutions of the Markownikov adducts formed between  $\alpha$ -acetyl-L-cysteine and the  $\alpha,\alpha$ -vinyl group of bilirubin-IX $\alpha$ .<sup>284</sup> This study, and a use of c.d. to investigate binding of 6-

nitro-L-tryptophan to human serum albumin,<sup>285</sup> are representative of major applications of the technique over many years.

#### 5.4 Mass Spectrometry of Amino Acids.-

Like the n.m.r. field, considerable changes in instrumentation are taking place in m.s. Multiphoton ionization m.s. of amino acids and derived N-phenylthiohydantoins have been reported, including the observation of the molecular ion of L-arginine for the first time.<sup>286</sup>

FAB M.s. of 24 amino acids, noting metastable ions and collisional activation spectra of  $[M + H]^+$  and  $[M - H]^-$  ions,<sup>287</sup> illustrate substantial progress in the operation of this m.s. variant, which is sufficiently mild in molecular structural terms to study easily decarboxylated species such as 5-substituted proline 4,4-dicarboxylic acids formed between  $\gamma$ -carboxyglutamic acid and aldehydes,<sup>288</sup> yet can generate 'precursor ions', e.g.  $[Leu + metal\ atom + glycerol]^+$  and  $[Leu + metal\ atom]^-$  through ionization of amino acids and alkali metal halides in a glycerol matrix.<sup>289</sup>

<sup>2</sup>H-Labeling has been resorted to, to aid identification of ions formed from amino acids by high-energy collisional activated fragmentation.<sup>290</sup> These spectra show characteristic fragmentation patterns, promising to assist m.s. identification of those  $\beta$ -branched amino acids that are difficult to distinguish from isomeric and  $\beta$ -unbranched amino acids by classical m.s. methods.

Desorption of valine through bombardment with fast alkane ions ( $C_nH_n^+$ ) has been achieved,<sup>291</sup> offering a secondary molecular ion approach to the difficult problem of obtaining m.s. of underivatized amino acids. The sputtering yield, of valine negative molecular ions per incident carbon atom in these bombarding alkane ions, increases with increasing numbers of carbon atoms.<sup>292</sup>

#### 5.5 Other Spectroscopic Studies.-

A non-routine, but preliminary, study of amino acids by near-i.r. reflectance spectrometry (1100-2500 nm) has been reported.<sup>293</sup>

#### 5.6 Other Physical Studies.-

Reports of complex formation involving amino acids are often the prelude to novel chromatographic separations, and the preferential binding of one enantiomer of an amino acid ester by chiral porphyrin - Rh(III)Cl complexes may be exploitable.<sup>294</sup> The binding of amino acids to benzo crown ethers occurs between the amine group and the ether oxygen atom;<sup>295</sup> 1:1- and 1:2-complexes of the crown ether ( 49 ) with L- or D-valine methyl ester hydrochloride or with  $NH_4Cl$  are probably of the sandwich type;<sup>296</sup> no enantioselectivity is shown.

Thermal data have been collected,<sup>297-300</sup> including a novel method for determining the optical purity of an amino acid based on differential scanning calorimetric measurement of enthalpy of solid-solid phase transitions,<sup>297</sup> and a use of the same instrument to quantify the interaction of amino acids with phospholipids.<sup>298</sup>

Apparent molal volumes,<sup>301</sup> partial molar heat capacities and volumes,<sup>302</sup> and viscosity coefficients, heats of solution and surface activity measurements<sup>303</sup> are representative of a vast number of routine papers.

Results based on somewhat similar laboratory methods, but with a wider significance, have been reported for polarities of amino acids; side chain distribution coefficients between vapour and cyclohexane, 1-octanol, and neutral water are closely related to 'inside-outside' distribution of side-chains seen in globular proteins.<sup>304</sup> The acidities in DMSO of a series of Schiff bases (six of the type  $\text{Ph}_2\text{C}=\text{NCHRCO}_2\text{Et}$ , five  $\text{ArCH}=\text{NCHRCO}_2\text{Et}$ ) has special relevance to practical procedures for optimizing mono-alkylation at the expense of di-alkylation (or *vice versa*).<sup>305</sup>

Sooner or later, improved understanding of fundamental thermodynamic and other physical properties will feed in to the understanding of biological transport processes of amino acids, e.g. L-alanine transport in rabbit kidney luminal membrane vesicles,<sup>306</sup> and 1-aminocyclohexanecarboxylic acid transport across the blood-brain barrier.<sup>307</sup>

**5.7 Molecular Orbital Calculations.**— Calculations have been outlined for charge densities and dipole moments of amino acids<sup>308</sup> and N-acetylasparyl methylamide ion and similar systems,<sup>309</sup> including GABA, GABA imine, and aminoxyacetic acid.<sup>310</sup> The influence of hydration on rotation barriers of glycine has been computed.<sup>311</sup>

The validity of an m.o. approach (AM1) has been tested with calculations of equilibrium structures of the non-ionic tautomer of representative amino acids.<sup>312</sup>

## 6 Chemical Studies

**6.1 Racemization.**— Racemization rates in aqueous acidic media are very slow with some well-known exceptions among natural amino acids,<sup>313</sup> and even slower in normal environmental conditions, thus permitting age estimations for relatively young fossils based on degree of racemization of amino acids extracted from them. The criticisms have been eloquently stated, and reviewed in these pages, as far as free amino acids are concerned, and recent criticisms<sup>314</sup> have been extended to include protein-bound amino acids. Fragmentation of a protein into dioxopiperazines has been established to occur rapidly enough, so that it must be a major degradation pathway to free amino acids;<sup>315</sup> however, the general validity of age estimation based on racemization survives the criticisms because the dioxopiperazines are not unduly prone to racemization.<sup>315</sup>

The use of L-isoleucine epimerization measurements has been described from the geologist's viewpoint.<sup>316</sup>

**6.2 General Reactions of Amino Acids.**— This Section collects papers describing



current interests in reactions of amino or carboxy groups separately, or reactions involving the  $\text{NH}_2\text{---CO}_2\text{H}$  moiety as a whole.

Classic  $\alpha$ -amino acid reactions, such as ninhydrin oxidation and other colour-forming processes, and Maillard condensations and Amadori rearrangements with carbohydrates, seem to gain a new lease of life on an annual basis. Ninhydrin conversion into  $\text{CO}_2$ , of amino acids separated by preparative g.l.c. from tissue hydrolysates, allows  $^{13}\text{C}:^{12}\text{C}$  ratios to be determined.<sup>317</sup> Ten  $\text{Q}$ -acylated benzene derivatives have been compared with ninhydrin for their colour-forming reactions with amino acids.<sup>318</sup>  $\alpha$ -Alkyl- $\alpha$ -amino acids produce a very low colour yield with ninhydrin, and low relative fluorescence yields with the  $\alpha$ -phthalaldialdehyde (OPA) - 2-mercaptoethanol reagent, due to incomplete derivatization.<sup>319</sup> The use of OPA with simple thiols is reliable for amino acid quantitative analysis in experienced hands, when exact protocols are followed that take account of the decay of the fluorophore, and  $\text{N,N}$ -dimethyl-2-mercaptoethylammonium chloride is advocated to yield an iso-indole showing more stable  $\epsilon$  values.<sup>320</sup>

Diethyl azodicarboxylate and  $\text{Ph}_3\text{P}$  accomplish oxidation of amino acid esters to  $\alpha$ -keto-esters.<sup>321</sup>  $\text{N}$ -Acylamino acid esters yield adducts ( 50 ) with this system,<sup>321</sup> but give  $\alpha$ -methoxy derivatives through anodic oxidation in methanol.<sup>322</sup>

Hypochlorous acid oxidation of amino acids is not what it might be surmised to be; chloramine originating in this reaction involves the nitrogen atom of the amino acid, even if  $\text{NH}_4^+$  salts are present, and it ends up as cyanogen chloride (conversion yields 3-4% for valine, leucine, isoleucine, 11.2% for serine, 13.7% for threonine).<sup>323</sup> Aqueous chlorine degradation of amino acids via  $\text{N}$ -chlorination and subsequent decomposition is featured in other papers;<sup>324,325</sup> in one of these studies,<sup>324</sup>  $\text{ClO}_2$  was surprisingly found to be unreactive towards most of the familiar amino acids.

Careful studies (see also Ref.398) of radicals formed from aliphatic  $\alpha$ -amino acid derivatives continue to explore the factors discriminating between various sites for deprotonation by  $\text{SO}_2\text{Cl}_2$  or by  $\text{N}$ -bromosuccinimide,<sup>326</sup> which lead to the  $\alpha$ -carbon radical (surprisingly, more stable than the tertiary  $\beta$ -carbon radical in the case of valine). At the other end of the spectrum of amino acid chemistry, but closely related to some of the foregoing papers in mechanistic terms, is  $\alpha$ -carbanion formation by proton abstraction by a nucleophilic site of D-amino acid oxidase coupled with electron transfer from the amino- $\text{N}$  of the substrate to the oxidized flavin cofactor.<sup>327</sup> Routine (repetitive, even) mechanistic studies of amino acid oxidation employing familiar inorganic oxidants [manganese-based;<sup>328-330</sup> chromium(VI) - methionine;<sup>331</sup> silver(I) and cerium(IV)<sup>332</sup>] continue to be reported. One citation (use of  $\text{N}$ -bromobenzamide to form aldehydes from amino acids<sup>333</sup>) is representative of many such studies employing organic oxidants.

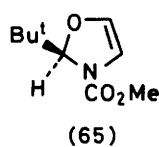
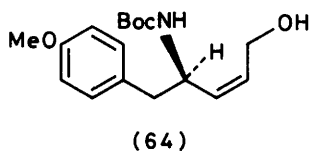
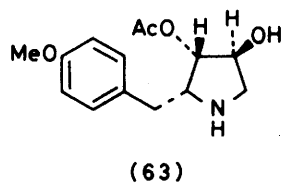
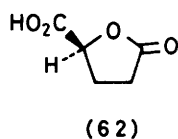
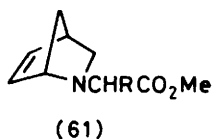
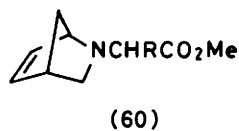
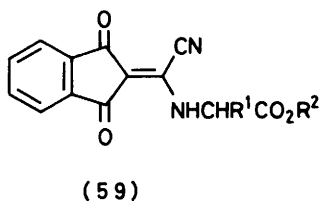
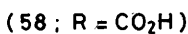
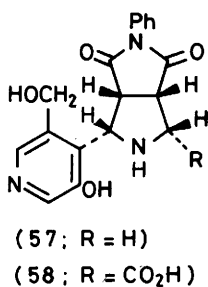
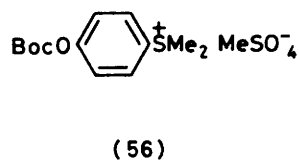
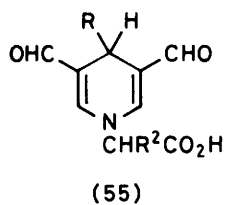
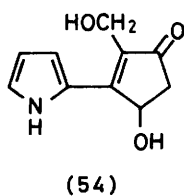
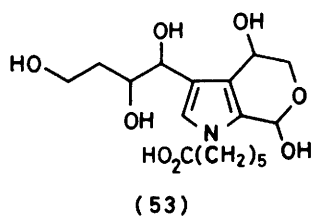
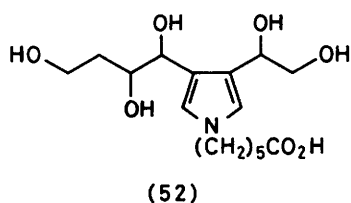
Heterocyclic compounds formed by reaction at both amino and carboxy groups of the amino acid include  $\text{N}$ -carboxyanhydrides formed using crystalline (but very

expensive) 'triphosgene' [bis(trichloromethyl)carbonate],<sup>334</sup> and corresponding derivatives of  $\alpha\beta$ -dehydroamino acids.<sup>335</sup> Boroxazolidinones are formed between amino acids and borinic and boronic esters.<sup>336</sup> Conversion of  $\beta$ -amino acids into  $\beta$ -lactams employing diphenylphosphinic chloride<sup>337</sup> or tris( $N$ -2-oxazolinonyl)phosphine oxide<sup>338</sup> offers improved methodology.  $\beta$ -Amino acids are formed by stereospecific ring-opening of aziridinecarboxylic acids by thiols.<sup>339</sup> Reclosure of the resulting  $\alpha$ -arenethio- $\beta$ -amino acids using  $\text{Ph}_3\text{P}$  and bis(pyrid-2-yl)disulphide gives  $\beta$ -lactams.<sup>339</sup>

Betaines  $\text{Me}_3\text{N}^+(\text{CH}_2)_n\text{CO}_2^-$  are effective phase-transfer catalysts for reactions involving dichlorocarbene.<sup>340</sup>

Reports of reactions at the amino group of amino acids include some of interest in their own right, as well as the interest in products formed in this way, which include novel  $N$ -protecting groups and improved routes to known derivatives. The extraordinary range of products from the Maillard reaction (more than one hundred volatile compounds - furans, pyrroles, pyrazines, etc - from the condensation of valine with D-glucose<sup>341</sup>) is only now being appreciated, the result of improved analytical separation procedures in organic chemistry.  $^{13}\text{C}$ -N.m.r. identification of four products ( $\alpha$ -,  $\beta$ -pyranose and -furanose forms of D-fructosylglycine) from a D-glucose - glycine reaction mixture (initial Schiff base formation followed by Amadori rearrangement) has been reported.<sup>342</sup> The early phase ( $130^\circ$ ), developed phase ( $130$ - $150^\circ$ ), and final phase ( $>150^\circ$ , leading to insoluble polymers) in the 1 : 1 - D-glucose : DL-phenylalanine Maillard reaction have been defined.<sup>343</sup> More control of the process is possible, with (E)-5-(3,4,5,6-tetrahydropyrid-3-ylidenemethyl)-2-hydroxymethylfuran ( 51 ) shown (by X-ray analysis) to be formed from D-glucose and L-lysine,<sup>344</sup> 1-alkyl-2-formyl-3,4-diglycosylpyrroles ( 52 ) and ( 53 ) from glucose or xylose with 6-aminoheptanoic acid in the presence of sodium sulphite (which inhibits the formation of brown fluorescent melanoidins in the later stages of the Maillard reaction,<sup>345</sup> and xylose - lysine condensation yielding the 3-(pyrrol-2-yl)cyclopentenone ( 54 )<sup>346</sup> and a second crop of products [(3- $N^{\epsilon}$ -lysino)lactic acid and D-glyceric acid; the first set was established to be  $N^{\epsilon}$ -carboxymethyl-lysine and D-erythronic acid] identified among products from oxidative cleavage of the Amadori rearrangement product,  $N^{\epsilon}$ -formyl- $N^{\epsilon}$ -fructolysine.<sup>347</sup> At a simpler level as far as products are concerned, malondialdehyde reacts with amino acids to give highly fluorescent 1,4-dihydropyridines ( 55 ) via Michael addition of alkylidene-malondialdehydes with enaminals.<sup>348</sup> Unidentified strongly-coloured green products formed between ethyl caffeate with tyrosine, phenylalanine, and histidine (weaker colour depth was seen with aliphatic amino acids; no colour with proline) may have some connection with the preceding processes.<sup>349</sup> The reaction may have some diagnostic usefulness since it is more sensitive than the biuret reaction.

Attention continues to be given to reactions of the  $\text{NH}_2$  group that preserve



other structural features intact. Some of the recent papers under this heading deal with reactions broadly applicable to amines in general, for example, that *N*-nitrosation of sarcosine and proline, is subject to nucleophilic catalysis by thioureas,<sup>350</sup> and that these imino acids react with 4-methyl-*q*-benzoquinone to give 4-methylcatechol and *N*-(5-methyl-*q*-benzoquinon-4-yl)imino acids in a type of redox process.<sup>351</sup> Degradation of labelled amino acids by hydroxylamine *Q*-sulphonic acid in aqueous EtOH has been used for formation of <sup>14</sup>C-labelled acids.<sup>352</sup>

Many other papers are specifically concerned with *N*-protection strategies for amino acids, including *N*- $\alpha$ -thiasuccinoylation starting with a polymeric xanthate PEG-OCS<sub>2</sub>CH<sub>2</sub>CONH<sub>2</sub>,<sup>353</sup> tritylation of *N*,*Q*-di(trimethylsilyl)amino acids using trityl chloride in refluxing CHCl<sub>3</sub>,<sup>354</sup> preparation of Boc-amino acids using the water-soluble reagent ( 56; see also ref.402);<sup>355</sup> introduction of two Boc groups at *N*,<sup>356</sup> and formation of *N*-Boc-*N*-*Q*-amino acids.<sup>356</sup> Replacement of *Q* by Boc under neutral conditions involves either hydrogenolysis (Pd-C) in the presence of (Boc)<sub>2</sub>O,<sup>356</sup> or Et<sub>3</sub>SiH/Pd(OAc)<sub>2</sub>/(Boc)<sub>2</sub>O.<sup>357</sup> *N*-Arenesulphonyl groups can be removed from  $\alpha$ -amino acids carrying hydroxyalkyl side-chains by electrolysis using phenol as proton source;<sup>358</sup> the earlier-reported alkaline hydrolysis regime<sup>359</sup> was tried, without success.<sup>358</sup> Results of preliminary screening in a search for micro-organisms capable of stereospecific cleavage of *N*-methoxycarbonyl-DL-amino acids, have been reported.<sup>360</sup>

Further novel *N*-protection strategies include *N*-(3-cyano-4,6-dimethylpyridyl)-sulphenylation,<sup>361</sup> *N*-(2-*p*-biphenyl)-2-propyloxycarbonylation,<sup>362</sup> the latter group cleavable under mild conditions (CH<sub>2</sub>Cl<sub>2</sub> - 0.5% TFA), and *N*-[bis(4-nitrophenyl)-ethoxycarbonylation (base-labile).<sup>363</sup> *N*-Acetyl-*N*-(benzoyloxy)lation of amino acid esters employs acetyl chloride and benzoyl peroxide.<sup>364</sup>

The volume of work reported by Grigg's group, on amino acid-derived imines, can only be described as substantial, with several full papers appearing in the year under review.  $\alpha$ -Amino acids and  $\alpha$ -disubstituted amino acids react with pyridoxal and *N*-phenylmaleimide to give two series of cycloadducts, one ( 57 ) from azomethine ylides from decarboxylated pyridoxal imines, the other ( 58 ) from azomethine ylides formed by 1,2-prototropy.<sup>365</sup> These results are relevant to the mechanism of Strecker degradation and will also assist progress in establishing the mode of action of decarboxylases. Further results, based on cycloadditions to maleimides, deal with kinetics<sup>366</sup> and anionic cycloaddition to imines ( 59 ) formed between amino acid esters and (1,3-dioxo-indan-2-ylidene)malononitrile.<sup>367</sup>

The Schiff base formed *in situ* between an amino acid ester and formaldehyde can be trapped by *aza*-Diels-Alder addition to cyclopentadiene;<sup>368</sup> an extraordinary sensitivity to amino acid structure is indicated in the distribution of isomers in this reaction ( 60:61 = 93:7 for L-isoleucine, but 20:80 for D-phenylglycine).

Papain-catalyzed esterification of *Z*-DL-amino acids<sup>369,370</sup> and other *N*-protected amino acids<sup>370</sup> give corresponding *N*-protected-L-amino acid esters, while variation

of the reaction medium allows serine protease-catalyzed esterification of *N*-acyl amino acid 2-chloroethyl esters to be directed either to D- or L-ester.<sup>371</sup> DMAP-Catalyzed formation of tert-butyl esters of *N*-protected amino acids can be achieved using either tert-BuOH - DCCl<sup>372</sup> or (Boc)<sub>2</sub>O - pyridine.<sup>373</sup> Valid recipes for *N*-Boc-L- $\alpha$ -ethyltyrosine pentafluorophenyl, pentachlorophenyl, and *N*-hydroxysuccinimidyl esters have been published,<sup>374</sup> and a careful assessment of the optimum specification for active esters accompanies the description of the preparation and use of representative 2,3,5,6-tetrafluorophenyl esters.<sup>375</sup> Full details of the highly reactive tetrahydrothiophen 1,1-dioxide-based active esters (TDO-esters)<sup>376</sup> and base-labile bis(4-nitrophenyl)ethyl esters<sup>383</sup> have been published.

Pd(0)-Catalyzed rearrangement of allyl esters of amino acid Schiff bases yields isomeric  $\alpha$ -allyl- $\alpha$ -amino acid derivatives.<sup>377</sup>

Friedel-Crafts acylation using *N*-methoxycarbonyl-L-aspartic  $\alpha$ -acid chloride is the first step in a synthesis of enantiomerically-pure  $\beta$ -amino- $\gamma$ -aryl- $\gamma$ -butyrolactones.<sup>378</sup> Similar acylation of primary organometallic reagents gives  $\alpha$ -Fmoc-aminoalkyl ketones, elaboration of which gives enantiomerically-pure  $\alpha$ -substituted alkanolic acids.<sup>379</sup>

Anodic  $\alpha$ -substitution of *N*-protected amino acids leading essentially to solvent incorporation has been mentioned a number of times in preceding sections. The curious fact that this 'non-Kolbe' behaviour is the opposite of that of analogous hydroxy acid derivatives, has been pointed out.<sup>380</sup>

A full account is available<sup>381</sup> of *N*-Boc amino acid symmetrical anhydrides, some of which have already been characterized, but reports are scattered through the literature of the last ten years or so. Fmoc-Amino acid mixed anhydrides have been used to acylate cyclopentadienyl iron carbonyls, Cp(CO)<sub>2</sub>FeNa to give Fmoc-NHCHRCOFe(CO)<sub>2</sub>Cp.<sup>382</sup>

A mechanistic study shows hydrolysis of 4-(*N*-methylalanyl)morpholine at 50° in aqueous HCHO to proceed *via* the 5-oxazolidinone, implicating *N*-hydroxymethylation at an initial stage.<sup>383</sup> Much is being made, in terms of potential rewards, of the chances of improving the slender chiral discrimination seen in the hydrolysis of racemic esters in the presence of D- or L-amino acid derivatives, and the converse equivalent process. Many papers cited in recent Volumes are supplemented this year by accounts of stereoselective hydrolysis of amino acid *p*-nitrophenyl esters<sup>384-387</sup> in the presence of L-histidine derivatives<sup>384-387</sup> covalently linked to poly(ethyleneimine)<sup>384</sup> or contained in surfactant co-aggregates formed by cholesterol-containing amphiphiles.<sup>385</sup> The last-mentioned system<sup>386</sup> and an analogous chiral copper(II)-chelating micelles<sup>387</sup> show the highest enantioselectivity yet reported for such processes. The (S)-1-benzyl-2-[(2-hydroxyethylamino)methyl]pyrrolidine - copper(II) complex shows modest chiral discrimination in catalyzing the hydrolysis of DL-valine methyl ester.<sup>388</sup>

### 6.3 Specific Reactions of Amino Acids.-

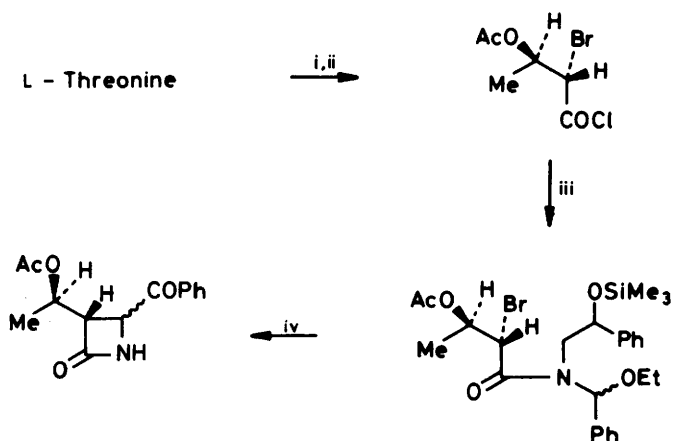
These are reactions associated specifically with amino acid side chains, though also involving the  $\text{NH}_2\text{---CO}_2\text{H}$  moiety in some cases.

There is an ever-increasing number of applications of natural amino acids in asymmetric synthesis of natural products, though these often employ straightforward methodology until a point has been reached in a synthetic scheme where the structural link with the amino acids has been extinguished. Thus, no attempt at complete coverage is offered in this Section; representative syntheses incorporating less routine reactions of amino acids include 2,3-deoxy-D-ribose from L-glutamic acid *via* (62),<sup>399</sup> (-)-anisomycin (63) from D-tyrosine *via* (64),<sup>390</sup> (+)- and (-)-N-methylpseudoconhydrine by anodic  $\alpha$ -methoxylation of protected lysine enantiomers followed by replacement of the methoxy group using allylTMS/TiCl<sub>4</sub>,<sup>391</sup> and diastereoisomers of the near relative, 5-hydroxypipelicolic acid, from L-glutamic acid.<sup>392</sup>

L-Threonine is the starting point for (3R,4R)-3-[[1-(R)-hydroxyethyl]-4-(benzyloxy)azetidin-2-one (Scheme 39), revealing a use of the novel phenyl alkoxymethyl N-protecting group.<sup>393</sup> The uses in asymmetric synthesis, of enantiomers of N-methoxycarbonyl 2-tert-butyloxazoline (65; from L-serine) and its 4-methyl homologue (from threonine) have been surveyed.<sup>394</sup> Methyl L-pyroglutamate has been employed in a route to (5S)-2-[(3',4'-methylenedioxy)phenylethyl]-5-( $\alpha$ -hydroxybenzyl)pyrrolidines as potential hypotensive agents.<sup>395</sup> Oxidative decarboxylation is a useful synthetic operation at or near the end of asymmetric syntheses such as these, and iodosobenzene is capable of effecting the conversion of cyclic imino acids (proline, pyroglutamic and pipelicolic acids) into lactams under neutral conditions.<sup>396</sup>

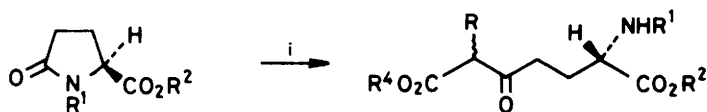
Conversions of easily-available enantiomers of amino acids continue to provide alternative access to other amino acids that are only available with difficulty. The preparation of  $\beta$ -aryl- $\alpha$ -alkyl amino acids from different stereoisomers of N-phthaloylthreonine methyl ester  $\beta$ -Q-methanesulphonate and arenes proceeds without racemization except for C-3 in allothreonines (where it is extensive).<sup>397</sup> Full details of mechanistic studies that have been featured in this section in recent years describe the formation of  $\beta$ -chloro- and (to a lesser extent)  $\gamma$ -chloro-valine through reactions of  $\text{SO}_2\text{Cl}_2$  with N-benzoylvaline methyl ester (cf. Ref.326).<sup>398</sup>

Adaptation of the L-lysine side-chain in suitably-protected derivatives gives corresponding amides through  $\text{Ru}_2\text{O}_4$  oxidation<sup>399,400</sup> (L-2,4-diaminobutyric acid and L-ornithine behave similarly<sup>399</sup>). N-Benzyloxycarbonyl lysine is converted into (2S)-amino-6-hydroxyhexanoic acid through treatment with aqueous sodium nitroprusside at pH 9.5, thence into N'-Z-L-aminoadipic acid through  $\text{RuO}_2\text{---NaIO}_4$  oxidation.<sup>401</sup> N'-Benzyloxycarbonyl lysine has been prepared, to demonstrate the use of the novel water-soluble acylating agent (56; Z in place of Boc; cf. Ref. 355).<sup>402</sup>



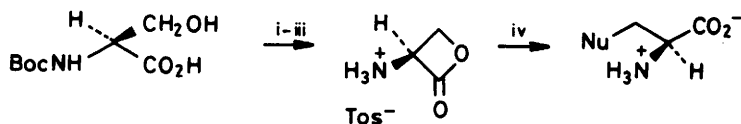
Reagents: i,  $\text{Ac}_2\text{O}$ ; ii, oxalyl chloride; iii,  $\text{PhCH=NCH}_2\text{CHPhOSiMe}_3$ ; iv,  $\text{K}_2\text{CO}_3$ ,  $\text{MeOH}$  (some reagent details lacking in ref. 393)

Scheme 39



Reagents: i,  $(E)\text{-R}^3\text{CH=C(OLi)CO}_2\text{R}^4$

Scheme 40



Reagents: i,  $\text{Ph}_3\text{P}$ ,  $\text{DEAD}$ ,  $-78^\circ\text{C}$ ; ii,  $\text{TFA}$ ; iii,  $\text{TsOH}$ ; iv,  $\text{Nu-X}$

Scheme 41

Cleavage of  $\alpha$ -alkoxycarbonyl-L-pyroglutamates occurs through mono-addition of lithium enolates, to give (2S)-amino-5-oxoalkanoic acids, useful as starting materials for routes to carbapenams (Scheme 40).<sup>403</sup>  $\alpha$ -t-Butyl- $\gamma$ -methyl- $\alpha$ -trityl-L-glutamate reveals itself as capable of acting as a ' $\gamma$ -amino acid anion equivalent' through conversion into the  $\gamma$ -ester enolate through use of lithium isopropyl cyclohexylamide; the anion adds stereospecifically to electrophiles (aldehydes in this study).<sup>404</sup>

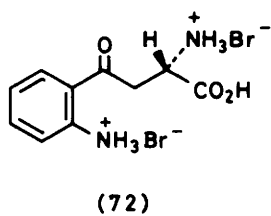
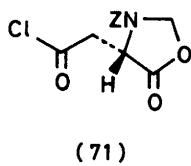
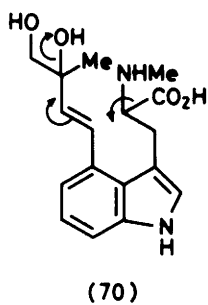
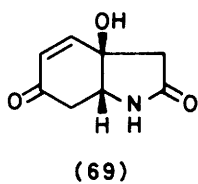
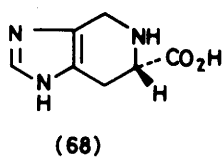
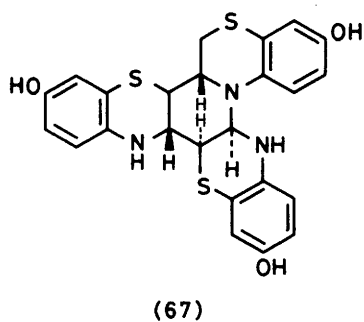
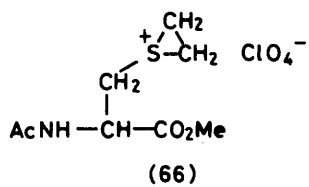
Uses of serine enantiomers for the stereospecific synthesis of other amino acids are perhaps more varied and extensive than those of other protein amino acids. A further application stems from the first preparation of 3-amino-2-oxetanone as its stable toluene-*p*-sulphonate salt, through Mitsunobu cyclization of  $\alpha$ -Boc-L-serine followed by deprotection with TFA (Scheme 41); nucleophilic ring-opening to give  $\beta$ -substituted L-alanines has been explored.<sup>405</sup>

Photo-exchange between  $\alpha$ -acetylsine and cytosine (or 5-methylcytosine) occurs at pH 7.5 to give 2-acetyl-amino-6-(1'-cytosinyl)hexanoic acid (or the 5'-methyl-1'-cytosinyl analogue).<sup>406</sup> A study of the condensation of arginine with phenyl and substituted-phenyl glyoxals<sup>407</sup> is distantly related in the sense that it illustrates mechanistic interest in amino acid reactions of potential biochemical significance. Other papers concerning arginine deal with protection of the side-chain (for a review, see ref. 408), the  $\alpha$ -9-anthracenesulphonyl group being removeable by mild reduction (dissolving metals).<sup>409</sup> A one-pot preparation of mono- and di-Z-histidines has been reported,<sup>410</sup> and studies of  $\alpha$ -trityl histidines and distribution (14%, 70%) of the Boc group on side-chain nitrogen atoms through reacting (Boc)<sub>2</sub>O with  $\alpha$ -Z-histidine methyl ester.<sup>411</sup>

Reactions of cysteine and its derivatives [rate constants for S-nitrosation,<sup>412</sup> and formation of  $\alpha$ -acetylcysteic acid via  $\alpha$ -acetyl-S-nitrosocysteine through use of excess NaNO<sub>2</sub> or NO;<sup>413</sup> synthesis of the episulphonium salt ( 66 ) from  $\alpha$ -acetylcysteine via its S-(2-trifluoroacetoxyethyl) derivative;<sup>414</sup> and estimation of ratios of singly-charged cysteine tautomers in solution by u.v.spectrometry<sup>415</sup>] include important revisions of earlier work. Condensation of L-cysteine with aromatic aldehydes to give thiazolines is not stereoselective;<sup>416</sup> and cysteine reacts with 1,4-benzoquinone to give more complex adducts ( 67 ) than previously reported.<sup>417</sup>

Modifications of aromatic and heteroaromatic side-chains include chlorination of L-tyrosine [Cl<sub>2</sub>-(MeO)<sub>2</sub>CMe<sub>2</sub>] to give the 3',5'-dichloro homologue.<sup>418</sup> Iodination is faster at higher pH, in marked contrast with peroxidase-catalyzed iodination, which operates at maximum rate at low pH.<sup>419</sup> Substitution in the phenolic moiety of tyrosine by laser-irradiated (308 nm) 5-bromouracil,<sup>420</sup> and analogous substitution of tryptophan and histidine, gives highly fluorescent products. Highly stereoselective threo- $\beta$ -hydroxylation of Boc-tyrosine by K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> with 2 equivalents of CuSO<sub>4</sub> (50° - 70° during 1.5-4h) is explained by the formation of a





cyclic carbamate involving the degraded Boc group.<sup>421</sup> A similar strapping of the amino group to the side chain is seen in the condensation of L-histidine with trifluoroacetaldehyde ethyl hemiacetal to give two diastereoisomers (but with low bias, 68:32) of 4-(trifluoromethyl)-L-spinacine ( 68 ),<sup>422</sup> and in Rose Bengal-sensitized photo-oxidation of L-tyrosine to give ( 69 ) *via* the corresponding hydroperoxide, in 10-20% yields (proline was decarboxylated through the same treatment).<sup>423</sup> Cyclisation of ( 70 ), synthesised from 4-formylindole as a likely biosynthetic precursor to chanoclavine I, occurs under mild conditions.<sup>424</sup>

Pd(0)-Catalyzed cross-coupling of *p*-Boc-aminophenyl trimethylstannane with the L-aspartic acid derivative ( 71 ) using Pd<sub>2</sub>(DBA)<sub>3</sub> gives the homophenylalanine derivative ( 72 ), which was converted through routine further steps into L-kynurenine.<sup>425</sup> Overall, this represents a convenient route that will allow access to analogues of this tryptophan degradation product. 5-Hydroxylation of N<sup>α</sup>-trifluoroacetyltryptophan methyl ester can be achieved using H<sub>2</sub>O<sub>2</sub> - SbF<sub>5</sub> - HF.<sup>426</sup>

Simpler structures are involved in reports of four different routes to α- and β-benzyl esters of N-benzylaspartic acid,<sup>427</sup> and reductive esterification of α-acetamido-acrylic acid [CH<sub>2</sub>=C(NHAc)CO<sub>2</sub>H → N-acetylalanine methyl ester] using H<sub>2</sub>/MeOH catalyzed by RhCl<sub>3</sub> or Rh(I)chloro(1,5-hexadiene dimer).<sup>428</sup> Apple tissue is employed in the conversion of 2,3-dimethyl-1-aminocyclopropane-1-carboxylic acids into mixtures of *cis*- and *trans*-butenes (results are consistent with a stepwise enzymatic radical mechanism).<sup>429</sup> The dehalogenating enzyme present in *Proteus mirabilis* IFO 3849 can act upon L-2-amino-4-chloropent-4-enoic acid to degrade it to 2-ketopent-4-enoic acid.<sup>430</sup> Ovocithiol C (alias N<sup>α</sup>N<sup>β</sup>N<sup>γ</sup>-trimethyl histidine-5'-thiol; Vol.20, p.3) acts as a H<sub>2</sub>O<sub>2</sub> scavenger in sea urchin eggs, i.e. as a glutathione peroxidase substitute, preventing oxidative damage to the eggs at fertilization.<sup>431</sup>

Higher amino acids are represented by conversion of appropriate Boc-statine stereoisomers into (3S,4S)- and (3R,4S)-3-aminodeoxystatines (in Scheme 37; 3-OH replaced by NH<sub>2</sub>) through subjecting the carboxy derivative [-CO<sub>2</sub>H → -CONHOMe] to Mitsunobu treatment.<sup>432</sup>

**6.4 Effects of Electromagnetic Radiation on Amino Acids.** - Excitation effects of radiation, as opposed to radiation-induced chemical changes, provide this section with its *raison d'être*, and papers cited here apply, almost exclusively, to phenylalanine, tyrosine or (particularly) to tryptophan.

During sonolysis, a rapidly-growing field of study, radiation acts on the solvent rather than solute, to generate H and OH radicals in Ar-saturated neutral aqueous amino acid solutions; products are readily accounted for on this basis.<sup>433</sup> Single crystal pulse radiolysis of tryptophan yields a transient absorption at 450 nm.<sup>434</sup>

Developing techniques are also featured in u.v.-Raman excitation profiles (217 -

240 nm),<sup>438</sup> and circularly-polarized laser (266 nm) photolysis of aqueous DL-tyrosine,<sup>439</sup> the result of the latter treatment being faster destruction of the D-enantiomer. Although enantioselective photodegradation has been claimed before, it has also been disputed before; another result that is relevant to this general topic is the 15% more efficient formation of the intermolecular excimer involving N-acetyl-L-pyrenylalanine methyl ester, compared with its D-isomer, in a chiral medium [(+)-octan-2-ol and (+)-methyl 2-chloropropionate].<sup>437</sup>

Some protection against He-Ne laser degradation of L-tryptophan in solutions containing haematoporphyrin is observed, but rather less protection is offered by meso-tetra(p-sulphophenyl)porphin.<sup>438</sup> Fluorescence-quenching of tryptophan in 90% methanol, in the presence and absence of 18-crown-6,<sup>439</sup> laser-induced fluorescence following supersonic jet-induced solvation of tryptophan derivatives,<sup>440</sup> and problematical long-wavelength fluorescence of tryptophan and tyrosine solutions,<sup>441</sup> represent less-routine fluorescence studies. There is no longer a need to rationalize the long-wavelength fluorescence data since optical artifacts (second order diffraction) are responsible (rather than molecular excitation).<sup>441</sup>

## 7 Analytical Methods

**7.1 General.** - Routine amino acid analysis in the clinical laboratory has been reviewed.<sup>442</sup>

**7.2 Gas-Liquid Chromatography.** - Finer details are now being dealt with for g.l.c. analysis of amino acids, since the main principles of methodology are well established. One of these established principles is derivatization of amino acids in order to achieve adequate volatility, although pyrolysis-g.l.c. has its uses, e.g. in estimation of cysteine and methionine in proteins; complex mixtures are formed, of course, but estimates of these amino acids based on H<sub>2</sub>S and MeSH peaks show 10-12% and 5-6% standard deviations, respectively.<sup>443</sup>

N-Trifluoroacetyl amino acid n-butyl esters,<sup>444</sup> isobutyl esters,<sup>445</sup> and n-butylamides<sup>446</sup> continue to have their champions; in one of these papers, the simple change of solvent from CH<sub>2</sub>Cl<sub>2</sub> to CHCl<sub>3</sub> for the acylation step is shown to be beneficial.<sup>446</sup> Equally widely used are N-heptafluorobutyryl n-butyl esters,<sup>447-449</sup> these papers illustrating the analysis of amino acids in streptococcal peptidoglycan polysaccharide complexes,<sup>449</sup> and the equality of g.l.c. with ion-exchange analysis, for reliable quantitation of proline, threonine and serine in mixtures.<sup>447</sup> N-Pentafluoropropionyl  $\alpha$ -alkyl- $\alpha$ -amino acid n-propyl esters and heptafluorobutyryl analogues are formed somewhat incompletely, and low results are therefore obtained for these sterically-hindered  $\alpha$ -amino acid analogues.<sup>319</sup> Continuing advances are being made on behalf of tert-butyltrimethylsilyl derivatives,<sup>21</sup> including one-step derivatization of amino acids

by *N*-methyl-*N*-(tert-butyldimethylsilyl)trifluoroacetamide.<sup>450</sup>

G.l.c.-m.s. studies are becoming more prevalent in the literature,<sup>444,448-451</sup> with equally good results, on the basis of selected-ion monitoring,<sup>451</sup> for analyte levels at the lowest limits applying to h.p.l.c. and ion-exchange methods.

Derivatization also underpins the use of g.l.c. for standard approaches to the determination of enantiomer ratios, either based on separating *N*-trifluoroacetyl,<sup>452</sup> *N*-heptafluorobutyryl,<sup>12,455</sup> or *N*-trimethylsilyl<sup>453</sup> amides<sup>453</sup> and esters<sup>452,454</sup> over chiral stationary phases (*n*-pentyl- or -acetyl- $\alpha$ - or  $\beta$ -cyclodextrins,<sup>452</sup> Chirasil-Val<sup>453-455</sup>), or by separating enantiomer mixtures which have been derivatized so as to form diastereoisomer mixtures (e.g. by acylation with *N*-trifluoroacetyl-L-prolyl chloride<sup>318</sup>), over achiral stationary phases.

Experienced practitioners in g.l.c. of amino acids have reported differences between results obtained with wide bore glass capillary columns, in comparison with packed columns.<sup>456</sup>

**7.3 Ion-Exchange Chromatography.**- Useful modifications of the amino acid analyzer and adjustments to the classic chemistry involved, allow acidic amino acids such as phosphoserine, phosphothreonine, phosphotyrosine, cysteic and homocysteic acids, to be accommodated,<sup>457</sup> and allow easier methodology through use of a set of sodium citrate buffers.<sup>458</sup> The substantial series of one-man papers on amino acid analyzer techniques, started two or three years ago, is lengthening with assessments of buffer preparation<sup>459</sup> and integrator reliability.<sup>460</sup>

Difficulties experienced with ion-exchange analysis of  $\alpha$ -alkyl- $\alpha$ -amino acids (as with other standard analytical methods for these sterically-hindered  $\alpha$ -amino acid analogues) have been discussed.<sup>319</sup>

An accolade to the reliability of this classic method for amino acid analysis, has emerged as a recommendation that concentrations of solutions of reference standard proteins should be determined on the basis of the ion-exchange analysis of their hydrolysates, but estimates of protein concentration obtained in this way tend to be low.<sup>461</sup> Routine use of the amino acid analyzer has been reported for mixtures,<sup>462</sup> 3-hydroxyproline,<sup>463</sup> and diaminopimelic acid in proteins,<sup>464</sup> the latter di-amino acid being accessible after performic acid oxidation prior to hydrochloric acid hydrolysis of proteins containing it (methionine is analysed in such hydrolysates as its sulphone). The numerous protein precipitation techniques introduce sources of error when applied for the preparation of standard samples for hydrolysis and determination of cysteine content by ion exchange analysis, but h.p.l.c. of *o*-phthalaldialdehyde-derivatized hydrolysates is satisfactory.<sup>465</sup>

**7.4 Thin-Layer Chromatography.**- Before arriving at h.p.l.c. (next Section), this Chapter navigates through ever-more-routine methods. All relevant aspects of t.l.c. are, surely, almost second nature, perhaps genetically-imprinted into

organic chemists and biochemists. However, there are those who continue to publish on the technique, covering t.l.c. analysis of amino acid *N*-phenylthiohydantoins (for a review, see ref. 466) on silica impregnated with simple metal salts.<sup>467</sup> The unlikely suggestion is made<sup>467</sup> that reported differences are explained by ion-pairing phenomena. Improved solvent systems have been proposed for t.l.c. of amino acids and their derivatives.<sup>468</sup> A modified spray regime has been proposed, claimed to reveal most amino acids on t.l.c. plates, involving successive treatment with fluorescein isothiocyanate and ninhydrin, with intermediate heating and air-drying and finally heating at 90° for 10 minutes.<sup>469</sup> This generates distinctive colours for 0.5 - 1 microgram amounts of amino acids; finally, observation in 280 nm u.v. light reveals certain amino acids that have not been revealed by the foregoing regime.

T.l.c. data in seven solvent systems are included with molecular weight and van der Waals volume data, in a scheme for parametrization applied to forty eight amino acids (see also Ref. 507).<sup>470</sup>

**7.5 High Performance Liquid Chromatography.**- Those operating h.p.l.c. analytical methods for amino acids, in preference to g.l.c. or ion-exchange, are being offered new options on a regular basis, such as supercritical liquids as mobile phase. For those with long-established roots in analysis of *N*-phenylthiohydantoins (PTHs), a paper describing rapid, efficient h.p.l.c. employing supercritical CO<sub>2</sub> will be a tempting introduction to the technique.<sup>471</sup> A timely review of electrochemical detection methods in h.p.l.c.<sup>472</sup> consolidates the experience of several years of increasing numbers of practitioners. Completing this paragraph of citations of work of general significance, an extraordinary observation that is perhaps obvious when thought through, has been published; a non-racemic mixture will chromatograph as two peaks in some cases on an achiral stationary phase, one being the enantiomer in excess and the other being the racemate.<sup>473</sup> It will be interesting to see whether re-evaluations of problematical h.p.l.c. traces will start to appear in the literature, embodying this intriguing principle.

As far as the reliable analysis of amino acids in physiological fluids is concerned, a warning has been published, that unless immediate deproteinization is carried out, levels of certain amino acids can increase. Thus, in a check on a routine h.p.l.c. analysis of glutamic acid and glutamine in serum,<sup>474</sup> it was found that the glutamic acid level had doubled, two hours after sampling.

The main preoccupation of this Section in recent years has been to report the search for the derivatization protocol shown to be 'best' for a particular purpose. Reagents come and go, but there is no doubting the pre-eminence of the  $\alpha$ -phthalaldehyde + alkanethiol approach (OPA), or that it is being caught, or even overtaken, by phenyl isothiocyanate (PITC) derivatization. A reasoned

comparison of these two methods<sup>475</sup> concludes that the PITC method has several advantages, in the stability of the products under the conditions of the reaction itself and under the analytical separation conditions, and in the fact that proline and hydroxyproline (and other imino acids) give PTC derivatives but imino acids do not react with OPA without processing. A comparison of the OPA method, not only with PITC but with dansylation, dansylation and PTH formation, offers similar support for the PITC technique.<sup>476</sup> A notable feature of this study is the use of the same h.p.l.c. column for the comparison, tending to reduce the variables inherent in such a comparison. OPA Derivatization has been compared (very favourably) with a further standard method (but one which is used less, year by year), viz., fluoresceamine.<sup>477</sup>

There are several papers, among a large group of papers describing applications of the OPA method, that explore possible improvements. Efficient ion-pair separation of amino acids, and post-column reaction with OPA - EtSH, seems to offer a good compromise, minimising the time taken between derivatization and absorbance measurement.<sup>478</sup> Low 'colour yields' are achieved with (sterically-hindered)  $\alpha$ -alkyl- $\alpha$ -amino acids in the OPA - 2-mercaptoethanol reaction, as with all other derivatization methods, but more drastic reaction conditions are in some ways self-defeating due to the easily-degraded fluorophore formed in the process.<sup>313</sup> Nowadays, the OPA technique is generally a matter of pre-column derivatization, and recent specific applications<sup>479-485</sup> include leucine and other branched aliphatic amino acids,<sup>479</sup> analysis of  $\gamma$ -aminobutyric acid, glutamic acid, and glycine using 5-amino-n-valeric acid as internal standard,<sup>480</sup> acidic amino acids including N- and Q-phosphorylated compounds, lombricine and N-phosphoryl-lombricine.<sup>481</sup> A combination of the OPA - 3-mercaptopropionic acid method (for primary amino acids) and Fmoc chloride (for imino acids) has been carefully evaluated.<sup>482</sup> The sensitivity of post-column electrochemical detection of OPA - 2-mercaptoethanol derivatives has been stressed,<sup>483</sup> as for the OPA - t-BuSH reagent used for rat brain neurotransmitter amino acids,<sup>486</sup> and in recent developments, employing naphthalene-2,3-dicarboxaldehyde in place of OPA, and electrochemical detection, an extraordinary 36 attomole lower limit for the detection of asparagine has been claimed.<sup>487</sup>

PITC Derivatization of amino acids, giving N-phenylthiocarbamoylamino acids (PTC-amino acids) suitable for h.p.l.c. separation, has been reviewed<sup>488,489</sup> and a lower limit of 1 pmol,<sup>490</sup> or a rather higher figure,<sup>491</sup> established for it. The chemistry involved is identical with the Edman 'coupling' step for sequence analysis of peptides, which, applied to amino acids, will lead easily to PTH derivatives; this may be the reason for variable yields that have been shown to be the result of derivatization in the presence of salts.<sup>492</sup> Yields also depend on the way in which the vacuum drying stage is conducted.<sup>492</sup> Analysis of glutamic and aspartic acids as PTC-derivatives gives low yields, particularly in analysis

of protein hydrolysates that have been conducted rapidly (i.e. acid hydrolysis at 160° for short times).<sup>493</sup> These losses are, surprisingly, associated with the presence of materials extracted from glass surfaces.<sup>493</sup> An improved buffer for conducting the amino acid - PITC reaction is water:MeCN:pyridine:triethylamine = 35:30:25:10.<sup>493</sup> Comments at the start of this Section, quote practitioners' experience with the high stability of PTC-amino acids, and the method has been used confidently<sup>494-499</sup> for assays of branched chain amino acids (norleucine as standard),<sup>494</sup> assay of basic amino acids ( $\text{H}^+$ -trimethyl-lysine and  $\text{H}^+$ -mono- and dimethyl-arginines),<sup>495</sup> and assay of acidic amino acids [glutamic and aspartic acids;<sup>493</sup>  $\gamma$ -carboxy-glutamic acid;<sup>496</sup> and opines (alanopine, strombine, tauropine,  $\beta$ -alanopine)<sup>497</sup>]. An interesting application is the derivatization of amino acids cleaved from peptide C-termini by carboxypeptidase P to allow C-terminal identification.<sup>498</sup>

PTH Derivatives have been found to be unstable in various widely-used h.p.l.c. solvents; for example, PTHs of amino acids with aliphatic side chains (alanine and leucine) have half-lives of 15-20 h in mixtures containing tetrahydrofuran,<sup>500</sup> so it is likely that PTHs of protein amino acids with side-chain functional groups will be even more rapidly degraded. Thiazol-5-ones from aliphatic amino acids were shown earlier to undergo oxidative dimerization in solvents prone to peroxide formation (such as dioxan and tetrahydrofuran),<sup>501</sup> and it is conceivable that this PTH 'degradation' may be exploited as a pathway to more stable characteristic derivatives that are amenable to h.p.l.c. identification. The h.p.l.c - thermospray m.s. combination gives spectra showing strong  $(\text{M} + \text{H})^+$  ions for 19 PTHs.<sup>502</sup>

DNP-Amino acids are still favoured,<sup>503,504</sup> allowing identification at low picomole levels,<sup>503</sup> and establishing cellulose as a useful h.p.l.c. phase that tolerates iso-amyl alcohol - MeCN - aqueous buffers at various pH and ionic strengths.<sup>504</sup> H.p.l.c. of dansylamino acids<sup>505-507</sup> also offers sensitive analysis of canavanine and canaline,<sup>505</sup> and has been used as a test-bed for comparison of laser polarimetry, refractive index, and u.v. absorption as detection methods,<sup>506</sup> and as part of a multivariate characterisation process for amino acids (see also ref.470).<sup>507</sup>

Fluoresceamine has been applied to  $\alpha$ -amino nitriles<sup>508</sup> as well as to amino acids,<sup>477,508</sup> and 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-F) continues to show its merits as a highly fluorescent tagging agent, for  $\underline{\text{S}}$ -sulphocysteine<sup>509</sup> and for tryptophan.<sup>510,511</sup> The case of tryptophan is interesting, since its NBD-derivative lacks the intense green fluorescence shown by other NBD-amino acids,<sup>511</sup> but electrochemical oxidation converts it into fluorescent NBD-dioxindolylalanine.<sup>510</sup> The careful NBD-tryptophan study has established detection limits down to 10 fmole for NBD-amino acids.<sup>510</sup> Femtomole levels are also routine for Fmoc derivatives formed using Fmoc-Cl and using 1-aminoadamantane to remove excess reagent.<sup>512</sup> The

h.p.l.c. of dansylamino acids has been developed further.<sup>513</sup>

Among numerous h.p.l.c. studies employing routinely some of these derivatization methods, or relying on the inherent absorption or fluorescence properties of the amino acids themselves in some cases (aromatic and heteroaromatic amino acids), the following references complete what is intended to be a representative review: glutamic acid released from perfused cerebellar slices;<sup>514</sup> cysteine and *N*-acetylcysteine;<sup>515</sup> 45 common cysteine-based mixed-disulphides;<sup>516</sup> *S*-adenosyl-L-methionine;<sup>517</sup> *O*-galactosyl-hydroxylysine;<sup>518</sup> 3-hydroxypyridinium crosslinking amino acids (pyridinolone and deoxypyridinolone);<sup>519</sup> phenylalanine<sup>520,521</sup> tyrosine<sup>521</sup> and its *O*- and *m*-isomers;<sup>522</sup> tryptophan;<sup>523</sup> and 3-methylhistidine.<sup>524</sup> The h.p.l.c. - FAB-m.s. combination has been explored for derivatized amino acids.<sup>525</sup>

H.p.l.c. flourishes as the method of choice for determining the enantiomeric composition of amino acids. The topic has been reviewed.<sup>526</sup> Analytical resolution is accomplished either by conversion of the sample into diastereoisomeric derivatives using a chiral derivatizing agent, or through using derivatives such as those portrayed above, in a chiral h.p.l.c. environment (chiral mobile phase or chiral stationary phase). While the latter approach is more widely used now, there are many convenient aspects associated with diastereoisomeric derivatives, since some derivatization techniques follow well established protocols; e.g. OPA with *N*-acetyl-L-cysteine as the thiol component, used for  $\alpha$ -substituted glutamic acids<sup>527</sup> and for other acidic amino acids (aspartic, glutamic, and lombricine).<sup>528</sup> Other derivatization procedures have been used,<sup>529,530</sup> notably Marfey's reagent [*N*-5-(1-fluoro-2,4-dinitrophenyl)alaninamide];<sup>529</sup> in one of these applications<sup>530</sup> [to Baclofen;  $\beta$ -(*p*-fluoro- or -chloro-phenyl)- $\gamma$ -aminobutyric acid] the greater distance apart of chiral centres in the derivative does not hinder the resolution. A novel application of established principles arises in the use of a post-column reactor packed with immobilized L-amino acid oxidase, with quantitation of products based on peroxyoxalate chemiluminescence.<sup>531</sup> Chiral stationary phases [2-carboxymethylamino-1,2-diphenylethanol<sup>532</sup> or an analogue (2-methyl in place of 2-phenyl),<sup>533</sup> (-)-*trans*-1,2-cyclohexanediamine,<sup>534</sup> *N*-(2-naphthoyl)-L-leucine,<sup>535</sup> or *N*-(3,5-dinitrobenzyl)-D-phenylglycine<sup>536</sup> bonded to silica, or analogous tris(3,5-dimethylphenyl carbamate)s of cellulose or amylose<sup>537</sup>] effect resolution, in some cases<sup>532-534</sup> through the ligand-exchange principle with underivatized amino acids in a copper(II)-containing mobile phase. One of these studies<sup>535</sup> requires *N*-acylamino acid butyl esters, another<sup>536</sup> is based on *N*-protected amino acids. Dansylamino acids<sup>537,538</sup> have been resolved by the ligand-exchange h.p.l.c. technique, and determination of the enantiomer composition of amino acids labelled with short-lived isotopes ([methyl-<sup>11</sup>C]methionine,<sup>539</sup> [<sup>18</sup>F]-5-fluoroDOPA<sup>540</sup>) has benefited from rapid chiral-h.p.l.c.



**7.6 Other Analytical Methods.** - Attomole levels of dansylamino acids ( $5 \times 10^{-8}$  M for methionine  $\approx 37$  amole, and  $5 \times 10^{-7}$  M for aspartic acid  $\approx 450$  amole, are the amounts actually injected) can be assayed by capillary zone electrophoresis with thermo-optical absorbance detection.<sup>540</sup> Similar studies of OPA-derivatives have claimed attomole levels.<sup>541</sup> Levels at an astonishing 4 orders of magnitude smaller can be attained by derivatization by fluorescein isothiocyanate and laser-induced fluorescence quantitation;  $9 \times 10^{-21}$  M alanine ( $\approx 6000$  molecules) are actually assayable.<sup>542</sup> Differences in fluorescence lifetimes of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -tyrosines imply that analytical exploitation might be worthwhile as a topic of study.<sup>543</sup>

Extension of earlier studies of amino acid analysis based on isotachophoresis has been reported.<sup>544</sup>

Potentiometric titration of all common amino acids except tyrosine, cysteine, and cystine, is feasible using  $\text{HClO}_4$  in  $\text{AcOH}$  or in 90%  $\text{MeCN}$ - $\text{AcOH}$ .<sup>545</sup>

**7.7 Assay of Specific Amino Acids.** - The term 'specific' in this context conjures up the topic of enzymatic analysis with biosensor connotations, and, indeed, most of (but not, by any means, all) the relevant recent literature is enzyme-based. Electrodes carrying immobilized L-lysine decarboxylase<sup>546</sup> or L-glutamic decarboxylase<sup>547</sup> respond to the corresponding L-amino acids, while an electrode carrying immobilized *Proteus mirabilis* whole cells combined with an ammonia sensor has been advocated for L-asparagine analysis.<sup>548</sup> L-Glutamic acid synthetase is the crucial part of an ion-selective field effect transistor-type L-glutamate sensor.<sup>549</sup> Branched chain L-amino acids are amenable to analysis by a continuous flow bioluminescence method based on immobilized L-leucine dehydrogenase.<sup>550</sup>

A review has appeared of the analysis of glutamic acid in foods by enzymatic assays.<sup>551</sup> Assays of the respective L-amino acids based on NAD-dependent L-phenylalanine dehydrogenase<sup>552</sup> and L-arginine kinase,<sup>553</sup> and of S-adenosyl-L-methionine (DNA - cytosine methyltransferase) employing [methyl- $^3\text{H}$ ]-S-adenosyl-L-methionine,<sup>554</sup> have been published.

Other 'wet' methods, but based on classical chemical and instrumental techniques, involve molybdenum blue formation from cysteine and ammonium molybdate (concentration linear with absorbance at 780 nm),<sup>555</sup> cysteine-cystine ratios based on the inhibitory effect of  $\text{Hg(II)}$  salts on catalyzed photo-oxidation of thiosemicarbazones,<sup>556</sup> and exploitation of a 'potential jump' that occurs at the point of formation of the insoluble L-cysteine - copper(II) acetate 2:1-complex.<sup>557</sup> More sophisticated polarographic and cathode-stripping voltammetric methods applied to S-adenosylmethionine are claimed to operate successfully with very low levels of analyte.<sup>558</sup> Arginine levels in hair can be measured by catalytic polarography after  $\text{NiNO}_3$  - borax treatment of hydrolysates.<sup>559</sup> Nopaline and octopine can be revealed on electrophoretograms by the Sakaguchi reagent.<sup>560</sup>

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# 2

## Peptide Synthesis

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BY D. T. ELMORE

### 1. Introduction

The triumvirate of reporters assembled last year in some haste following the tragic death of Ian Galpin has been amicably reduced to one. Fortunately, the ready availability of the Senior Reporter has helped to maintain a recognizable pattern to the review, but personal preferences have precipitated some changes. For example, Appendix II listing useful amino acid derivatives is discontinued. It was felt to be incomplete and its concentration on recording m.p. and specific rotation had an archaic if nostalgic flavour which suggested that a cost-benefit analysis would recommend its demise. Lack of time precluded the former and so the latter is un fait accompli. Likewise Appendix III (renamed Appendix II) has been trimmed to include only those papers that seemed likely to the reporter to be applied in laboratories other than those of the author(s). Doubtless the importance of some papers describing the analysis or purification of peptides by hplc has been overestimated whereas other deserving additions to the literature have been omitted. While this may be unfortunate, it is probably not too serious, since a review of peptide synthesis methodology is not likely to be regarded as the most probable repository for the latest critical review of an important but different methodology. Some new sections to Appendix I have been included. There is a great interest in designing peptides and analogues with specific secondary structures especially in the search for new peptide-based drugs. Likewise there is a surge of interest in the production of antibodies, especially in the mapping of linear epitopes. These topics now have separate sections (4.4 and 4.6) in Appendix I.

The locations of the bulk of the literature on peptide synthesis are much as they were last year<sup>1</sup>. Nevertheless, the contribution made by chemists and biochemists in the U.K. to peptide synthesis is shrinking steadily. Although two R.S.C.



journals are amongst the more important vehicles for reporting new successes in peptide synthesis, the authors are frequently not U.K. scientists. The prevalence of automatic peptide synthesizers has led to the propagation of the uninformed view that peptide synthesis involves only pushing a button and this has perhaps deterred young scientists from becoming involved. In addition, old age and death have taken their toll in recent years, but this should not inhibit research effort:

"Old age hath yet his honour and his toil;  
Death closes all: but something ere the end,  
Some work of noble note, may yet be done,  
Not unbecoming men that strove with Gods<sup>2</sup>."

If this hope is to be realized, the underfunding of relevant research councils and the contraction of British universities will have to be halted and hopefully reversed. Indeed, if this Specialist Report survives for another decade in the present climate, the mother tongue of the majority of its readers may well not be English or its derivatives. Since only a very small number of British libraries will be able to afford to subscribe to the journals that will have supplanted some of those that are presently near the top of the reading list for any informed peptide chemist, not only will it be difficult to obtain funds to work on peptide synthesis, but it may even be burdensome to locate the journals to read of the successes of others.

The literature reviewed here has been culled directly from the major journals listed last year as well as from *Peptide Information* (Peptide Institute, Protein Research Foundation, Osaka) and *Chemical Abstracts*. Most of the papers cited in this report were published during 1988 but a few that escaped mention in 1986 and 1987 are included. As in previous years, individual contributions to symposium proceedings have usually been excluded and the explanation advanced last year still obtains. Patent applications are also excluded. Again this report concentrates on "ordinary" peptides and close analogues of these. Efforts are multiplying to produce agonists and antagonists of peptide hormones and inhibitors of proteinases to combat tissue and

skeletal damage caused in certain diseases. The development of numerous surrogate peptide bonds to confer a particular shape or to resist proteolysis is making it more difficult by the year to adhere to this comfortable division of labour. More radical changes in the format of this Specialist Report may be needed in the not too distant future.

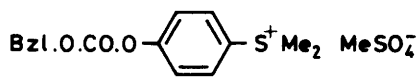
A considerable number of reviews have appeared during the year. Some cover the whole or most of the methodology and special mention must be made of the symposium edited by Hruby and Schwyzler.<sup>3</sup> Some of the individual papers in this symposium are cited elsewhere in this review. There have been a few other comprehensive but shorter reviews.<sup>4-7</sup> Most review articles published<sup>8-23</sup> during this period, however, are slanted towards particular methodologies (e.g. protecting groups, coupling methods) or peptides with special biological properties or structural features.

## 2 Methods

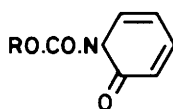
The arrangement of the main body of the review broadly follows the pattern of recent years, although some sections have been renumbered.

### 2.1.1 Amino-group protection

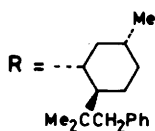
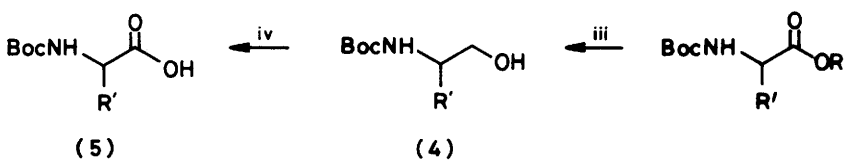
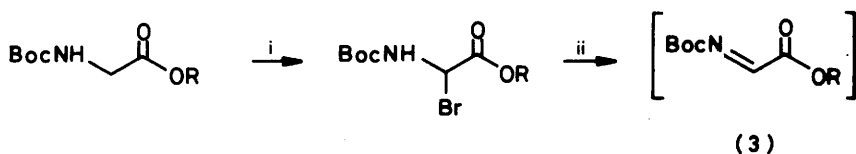
New reagents (1,2) have been proposed for the introduction of the *N*-benzyloxycarbonyl (Z) group.<sup>24-26</sup> The popularity of protecting groups is dependent in the main on chemical considerations, but the choice of reagent for introducing a particular group is partly dependent on the commercial availability and stability of suitable reagents. Since (1) is crystalline and soluble in water,<sup>25</sup> it may soon be commercially available. The synthesis of 4-methoxybenzyloxycarbonylamino acids has been reported.<sup>27</sup> Support for the use of the 2-nitrobenzyloxycarbonyl group has been advanced<sup>28</sup> because of its sensitivity to photolytic cleavage on irradiation at 350 nm. Enthusiasm for photolabile protecting groups, however, remains tempered by the sensitivity of some amino acids to photo-oxidation. The cinnamyloxycarbonyl group introduced in 1985<sup>29</sup> has been reused,<sup>30</sup> but its disadvantages do not encourage its



(1)



(2) R = Bzl or Bu<sup>t</sup>



Reagents: i, *N*-Bromosuccinimide in CCl<sub>4</sub>; ii, R'MgX; iii, LiAlH<sub>4</sub>; iv, RuCl<sub>3</sub>/NaIO<sub>4</sub>/MeCN

**Scheme 1**

general acceptance. The 4-nitrocinnamyloxycarbonyl (Noc) group, however, offers some interesting possibilities.<sup>31</sup> The Noc group favours the formation of crystalline derivatives, is stable to acid but can be cleaved by Pd(0)-catalysed allyl transfer to a suitable acceptor such as *NN'*-dimethylbarbituric acid without affecting, for example, *t*-butyl ester groups. Conversely, allyl esters can be converted into the corresponding acids by reaction with (Ph<sub>3</sub>P)<sub>3</sub>RhCl in aqueous ethanol at 70°C for 1 h without affecting Noc groups. The 3,5-dimethoxy- $\alpha$ -dimethylbenzyloxycarbonyl (Ddz) group, previously proposed for solution synthesis of peptides<sup>32</sup> has been used for the synthesis of peptides containing Trp on a polyethyleneglycol support<sup>33</sup> because of its ease of cleavage by acids.

New reagents comparable to those for introducing the Z-group have been proposed for the Boc-group.<sup>26,34</sup> A one-pot method for replacing the Z-group by Boc has been described.<sup>35</sup> A stereoselective synthesis of Boc amino acids involves the addition of Grignard reagents to the chiral electrophilic glycine equivalent,  $\alpha$ -bromo-*N*-Boc-glycine (-)8-phenylmenthyl ester (3) (Scheme 1).<sup>36</sup> Unfortunately, the phenylmenthyl ester group could not be removed by hydrolysis or transesterification without accompanying racemization. Consequently, the esters were reduced with LiAlH<sub>4</sub> and the Boc aminoalcohols (4) were oxidized to the acids (5) with RuCl<sub>3</sub>/NaIO<sub>4</sub> in aqueous MeCN. It might be worth trying to hydrolyse the esters enzymically which should avoid racemization. Although the Bpoc group is not particularly popular nowadays, a useful paper details the preparation of many of these derivatives including novelties such as Bpoc-Arg(Mtr)-OH.<sup>37</sup> During deprotection in 0.5% CF<sub>3</sub>CO<sub>2</sub>H in CH<sub>2</sub>Cl<sub>2</sub>, both 2-(4'-biphenyl)-propene and the dimer, 2,4-bis(4'-biphenyl)-4-methyl-1-pentene were formed. PhCH<sub>2</sub>SH and PhSH were the most effective scavengers. The novel *NN*-(Boc)<sub>2</sub>-amino acids and *N*-Boc-*N*-Z-amino acids have been synthesized and used to make a few dipeptides,<sup>38</sup> but no searching tests were applied to determine if such derivatives offer enhanced resistance to racemization. A new protecting group, *N*-1-isopropylallyloxycarbonyl, has been briefly examined.<sup>39</sup> The protecting group is removed by a boiling solution of Pd<sub>2</sub>(PhCH:CH.CO.CH:CHPh)<sub>3</sub>.CHCl<sub>3</sub> - PPh<sub>3</sub> in dioxan. A

one-pot synthesis of dipeptides was devised in which the protecting group was removed concurrently with condensation with a reactive ester.

Two further groups, bis(4-nitrophenyl)ethoxycarbonyl-<sup>40</sup> and (4-chlorophenyl)sulphonylethoxycarbonyl-,<sup>41</sup> which are removable by exposure to base, have been proposed. Treatment of persilylated amino acids with trityl chloride in  $\text{CHCl}_3$  under reflux provides a useful new route to *N*-trityl derivatives.<sup>42</sup> Although hydroxyl groups are unaffected, the  $\epsilon$ - and  $\delta$ -amino groups of Lys and Orn and the thiol group of Cys are simultaneously blocked. The same workers have used 4-polystyryltriphenylmethyl chloride to produce insoluble trityl amino acids for peptide synthesis.<sup>43</sup> The complexes derived from amino acids and  $\text{Br}(\text{CO})_3\text{Re}$  can be coupled with amino acids and the dipeptide ester can be released by treatment with pyridine or  $\text{Ph}_3\text{P}$ .<sup>44</sup> More information about racemization risks and application of the technique to polyfunctional amino acids would be of interest. Incorporation of the  $\alpha$ -amino group into a pyrrole ring has novelty,<sup>45</sup> but deprotection requires ozonolysis followed by reduction with  $\text{NaBH}_4$  so this is not likely to be widely used. Conversion of di-isopropyl phosphite into the phosphorochloridate by the classical Todd method provides a route to *N*-(di-isopropoxyphosphoryl)-amino acids and -peptides.<sup>46</sup>

### 2.1.2 Carboxyl-group protection

*t*-Butyl esters have been made by treating *N*-protected amino acids with either *t*-BuOH and  $\text{DCCI}$ <sup>47</sup> or di-*t*-butylpyrocarbonate<sup>48</sup> in the presence of pyridine and 4-(dimethylamino)pyridine. The 4-(methylsulphonyl)benzyl ester group, which can be introduced using either the corresponding alcohol or halide, has been recommended.<sup>49</sup> The esters are stable to  $\text{CF}_3\text{CO}_2\text{H}$  but are easily reduced to the corresponding 4-(methylthio)benzyl esters which are labile to acid.  $4\text{-MeS.C}_6\text{H}_4\text{.CH}_2^+$  ions generated during acidolytic cleavage are trapped with  $\text{Me}_2\text{S}$ . This idea would be worth extending to solid-phase peptide synthesis (SPPS). Some small peptides have been synthesized using esters of *N*-benz-hydrylglycolamide for carboxyl group protection.<sup>50</sup> The

requirement for treatment with aqueous alkali, however, is unlikely to popularise the method in view of the problems caused by possible formation of aspartimide peptides under these conditions. A similar comment may be applied to the proposal to protect carboxyl groups as bis-(4-nitrophenyl)ethyl esters.<sup>40</sup> The novel idea of incorporating the C-terminal amino acid as an aminoalcohol and then oxidizing it to the corresponding acid at the end of the peptide assembly<sup>51</sup> is also unlikely to be widely used. Apart from the obvious point that one group (CO.OH) requiring protection has simply been replaced by another (CH<sub>2</sub>.OH), the use of CrO<sub>3</sub>/H<sub>2</sub>SO<sub>4</sub> for the oxidation step introduces further hazards for the side chains of those amino acids that are sensitive to oxidation.

β(γ)3-Picolyl esters of Asp(Glu) have been synthesized<sup>52</sup> by a method analogous to that used for preparing the 4-picolyl esters. The 3-picolyl esters are preferred because of their greater stability to nucleophilic attack. The factors affecting aspartimide formation have been studied.<sup>53</sup> β-1- and β-2-adamantyl aspartates have been synthesized<sup>54</sup> and subjected to searching appraisal and were found to resist aspartimide formation under acidic and basic conditions. Both groups are stable to piperidine under the conditions used to cleave Fmoc groups. The β-1-adamantyl ester is cleaved by CF<sub>3</sub>.CO.OH, but the 2-isomer requires CH<sub>3</sub>.SO<sub>3</sub>H. These derivatives of Asp appear to be very suitable for SPPS using either Boc or Fmoc for protection of the amino group. 2-Cyanoethyl<sup>55</sup> and 2-bromoethyl<sup>56</sup> esters have also been briefly examined but their lability to base would probably preclude their use with the Fmoc group.

### 2.1.3 Side-chain protection

The search for better protecting groups for the guanidino group of Arg,<sup>18,57</sup> which was discussed in some detail in last year's report, has continued. There are three problems which have to be addressed. Firstly, the protecting group has to be removable under mild conditions and in most cases this means treatment with acid. Secondly, the product(s) of deprotection should not react with other parts of the peptide under construction. Finally, the protecting group should suppress as

completely as possible the nucleophilicity of the guanidino group which could otherwise lead to lactam formation during the coupling reaction. The *N*<sup>C</sup>-(2,2,5,7,8-pentamethylchroman-6-sulphonyl) group reported last year has been used in the SPSS from Fmoc amino acids of 6 peptides containing 10-34 residues including one or two Arg residues.<sup>58</sup> This group is very promising and deserves wider evaluation by other researchers. The successful search for protecting groups for Arg that can be removed by very mild acid treatment means, of course, that the  $\alpha$ -amino group can not be protected with Boc. Another acid-labile group has been reported.<sup>59</sup> The 9-anthracenesulphonamido group is easily introduced using the sulphonyl chloride and removed by  $\text{CF}_3\text{CO}_2\text{H}$  in the presence of anisole and thioanisole as scavengers. It is also cleaved under a variety of reducing conditions including the photo-induced reduction with 1-benzyl-4-dihydronicotinamide under catalysis with tris-(2,2'-dipyridyl)ruthenium dichloride. Fmoc and Bpoc can be used in conjunction with the 9-anthracenesulphonamido group. The 2,4,6-tri-isopropylbenzenesulphonyl group has been proposed for protecting the Arg side chain but no peptides were synthesized.<sup>60</sup> Moreover, since the group is stable to  $\text{CF}_3\text{CO}_2\text{H}$  and requires  $\text{CH}_3\text{SO}_3\text{H}$  for its removal, it appears to offer no advantages over other groups which require strong acids for their removal.

The diphenylphosphinothioyl group, which had earlier been proposed for the protection of the side chains of Tyr and Cys as well as  $\alpha$ -amino groups, has been used to protect the indole ring of Trp in the synthesis of bradykinin-potentiating peptide.<sup>61</sup> Deprotection is effected by either 0.25 M  $\text{CH}_3\text{SO}_3\text{H}$  - 0.25 M PhSMe in  $\text{CF}_3\text{CO}_2\text{H}$  or, more interestingly, by 0.5 M KF - 0.5 M 18-crown-6 in MeCN. The hydroxyl group of Ser and Thr can be protected by reaction with 4-chloromethylpyridine.<sup>52</sup> The Q-4-picolyl group is stable to acids and bases but is removed by hydrogenolysis in 80%  $\text{CH}_3\text{CO}_2\text{H}$  over 10% Pd on charcoal or by galvanostatic electrolysis in 0.025 M  $\text{H}_2\text{SO}_4$  at 40 mA during 1 h. Addition of pentamethylbenzene during the acidolytic deprotection of Tyr(Bzl) and Lys(Z) residues is a useful ploy to prevent alkylation of a synthetic peptide by carbocations.<sup>62</sup>

Although protection of peptide bonds is not normally considered an essential procedure in peptide synthesis, the  $[\text{Fe}(\text{CO})_3(\text{C}_6\text{H}_7)]^+$  group is easily introduced and then removed with  $\text{CF}_3\text{CO.OH}^{63}$  and may be of use to some workers in the field.

## 2.2 General deprotection

Apart from some specialized procedures mentioned above, this year has been one of consolidation rather than revolutionary discovery in the field of deprotection. The widely used technique involving a hard acid and soft base has been further studied<sup>64</sup> and the combination of  $\text{CF}_3\text{SO}_3\text{SiMe}_3$  and  $\text{PhSMe}$  is preferred. Thioanisole is both a good accelerator of the cleavage reaction(s) and a good scavenger of carbocations produced. A combination of  $\text{Me}_3\text{SiBr}$  and  $\text{PhSMe}$  in  $\text{CF}_3\text{CO.OH}$  is recommended for the more limited objective of removing Bzl and Z groups.<sup>64,65</sup> In a related fine-tuning exercise, a combination of  $\text{Me}_3\text{SiCl}$  and  $\text{PhOH}$  in  $\text{CH}_2\text{Cl}_2$  removed t-Bu and Boc groups selectively in presence of Bzl and Z groups.<sup>66</sup> The catalytic effect of phenol was attributed to complexation with  $\text{Me}_3\text{SiCl}$  and the concomitant labilization of the phenolic proton. A Pd catalyst bound to polystyrene in the presence of formic acid was found to be convenient for removing Z groups,<sup>67</sup> the most complex substrate being Z-His(Z)-Trp-Ser-Tyr-OH. It should be noted, however, that removal of Bzl and related protecting groups by catalytic transfer hydrogenation using palladium acetate - ammonium formate or palladium charcoal - formic acid mixtures can cause reduction of Trp and the former reagent can also racemize His.<sup>68</sup> In a paper not specifically concerned with peptide synthesis,<sup>69</sup> three methods for the removal of *N*-arylsulphonyl groups were compared. In general,  $\text{HBr}/\text{CH}_3\text{CO.OH}$  and electrolytic reduction afforded better yields than Na in liquid  $\text{NH}_3$ . This last reagent has virtually disappeared from the peptide chemist's repertoire, but electrolytic reduction as a means of removing arylsulphonyl groups from guanidino groups may be worthy of more detailed examination. These workers preferred the 2,4-dimethylbenzenesulphonyl group. The observation<sup>70</sup> that a penicillin acylase from *E. coli* (EC 3.5.1.11) can remove the *N*-terminal protecting group from *N*-phenylacetyl dipeptide esters will be of interest to workers in the penicillin field. Finally, the



reported lability of C-terminal imino acids<sup>71</sup> under acidic conditions is rather disconcerting since the installation of a C-terminal *N*-methylamino acid is an effective method for protecting synthetic peptides designed as drugs against carboxypeptidases. The mechanism proposed for this "back-biting" degradation is not completely convincing. For example, it does not explain why *N*-acylamino acids such as *N*-trifluoroacetyl-*N*-methyl-L-phenylalanine are much more stable than peptides containing the same imino acid as the C-terminal residue. It seems reasonable to suggest that the carbonyl oxygen of the penultimate peptide bond participates in an acid-catalysed cyclization and degradation analogous to the Edman sequential degradation of peptides.

### 2.3 Peptide bond formation

Avoidance of chlorides of *N*-protected amino acids has been almost a golden rule in peptide synthesis since the days of Emil Fischer. A report<sup>72</sup> that they can be made from the reaction of *Z*-amino acids and 1-chloro-*N,N*,2-trimethyl-1-propene-1-amine<sup>73</sup> and used without undue delay to obtain stereochemically pure dipeptides will doubtless prompt a wider study of this route. Complexes of amino acid chlorides with Pt(II) and Pd(II) can be prepared by treatment of the corresponding amino acid complexes with a trialkylsilyl chloride to give  $\text{Cl}_2\text{M}(\text{NH}_2.\text{CHR}'.\text{CO}_2\text{SiR}_3)_2$  which is converted into  $\text{Cl}_2\text{M}(\text{NH}_2.\text{CHR}'.\text{COCl})_2$  by reaction with  $\text{COCl}_2$  in  $\text{CHO.NMe}_2$ . These last complexes acylate esters of amino acids to give metal complexes of dipeptide esters.<sup>74</sup>

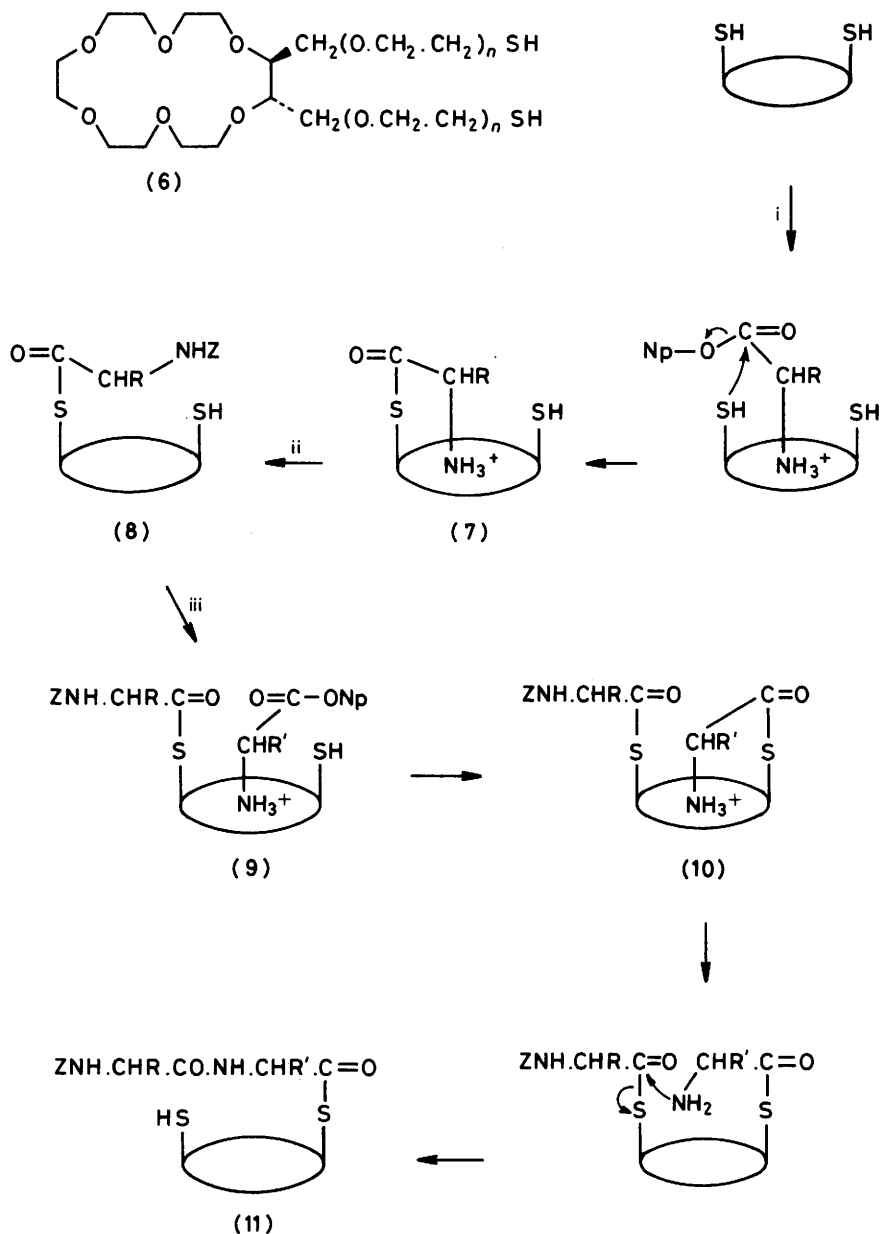
*N*-Carboxyanhydrides (oxazolid-2,5-diones) also have a long and somewhat chequered history. A new synthesis using bis(trichloromethyl)carbonate in place of  $\text{COCl}_2$ <sup>75</sup> and the use of 4-methyloxazolid-2,5-dione in a rapid synthesis of enalapril<sup>76</sup> in high yield should revive interest in these intermediates. This preparation is also notable for the stereoselective hydrogenation of the enamines obtained by the condensation of H-Ala-Pro-OH or *N*-Lys(Tfa)-Pro-OH with ethyl 2-oxo-4-phenylbutyrate.

A number of water-soluble carbodiimides have been synthesized,<sup>77</sup> but they were examined only for their suitability as reagents for peptide synthesis in the coupling of *N*-phthaloyl-glycine and H-Gly-OEt which is hardly a searching test. Further

experiments have been reported revealing the efficacy of  $\text{CuCl}_2$  and 1-hydroxybenztriazole for suppressing racemization in couplings mediated by carbodiimides.<sup>78</sup> For example, the coupling of Boc-L-Tyr(Bzl)-Gly-Gly-L-Phe-OH and H-L-Leu-OMe effected by 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide in  $\text{HCO.NMe}_2$  routinely yielded  $\geq 84\%$  of product with  $< 0.1\%$  racemization. The same workers examined the influence of the configuration of amino-acid residues on the extent of racemization during the coupling of Z-Gly-Xxx-OH or Bz-Xxx-OH with amino-acid esters but did not observe a consistent pattern.<sup>79</sup> An improvement to the classical Anderson test has been proposed;<sup>80</sup> after the initial coupling of Z-Gly-L-Phe-OH and Gly-OEt, the Z-group is removed by hydrogenolysis and Z-Ala-OSu is coupled in  $\text{HCO.NMe}_2$  to give a diastereoisomeric mixture if racemization occurs in the first step. The product is analysed by reversed-phase hplc. The report<sup>81</sup> that addition of various liquid crystals during couplings involving DCCI or mixed anhydrides suppressed racemization, unfortunately could not be repeated.<sup>82</sup> Benoiton<sup>83,84</sup> has continued his studies of racemization during aminolysis of symmetrical and mixed anhydrides in aqueous  $\text{HCO.NMe}_2$ . Racemization was less extensive when a symmetrical anhydride was used, especially in presence of  $\text{Na}_2\text{CO}_3$  as base, although mixed anhydrides from chloroformates of a secondary alcohol also gave reasonably satisfactory results. It was also shown that mixed anhydrides of *N*-acetyl-, *N*-benzoyl- and *N*-Z-glycyl-amino acids were racemized by base in tetrahydrofuran by formation of 2,4-dialkyl-5(4H)-oxazolones.

There has been considerable activity with reactive esters. 2,3,5,6-Tetrafluorophenyl esters have been prepared in high yield and some have higher m.p.s than the pentafluorophenyl esters.<sup>85</sup> Methyl pentafluorophenyl carbonate has been used for the preparation of the pentafluorophenyl esters.<sup>86</sup> Kinetic studies of the reaction of *N*-acyloxysuccinimides with amino acids gave results that could have safely been predicted.<sup>87</sup> Since 2-nitrophenyl esters are considerably more reactive than the 4-nitrophenyl derivatives, the structure of Boc-L-alanine 2-nitrophenyl ester was determined by X-ray crystallography.<sup>88</sup> This ester was found to have a rather rigid structure with very restricted

rotation about the O-C(aryl) bond as a result of an oxygen atom of the nitro group being wedged between the two ester oxygen atoms. Accordingly, solvation due to the polarity of the nitro group is probably inhibited and one side of the carbonyl group is exposed to nucleophilic attack. The kinetics of reaction of 4-nitrophenyl esters with amino acids and derivatives under phase-transfer conditions in 1-BuOH - aqueous buffers were studied.<sup>89,90</sup> Reaction rates could be correlated with the nature of the quaternary ammonium ions used to catalyse reaction.  $\alpha$ -Cyano- $\beta$ -styryl esters with or without a 4'-substituent have been used for peptide synthesis.<sup>91</sup> The derivatives without a 4'-substituent and NSu- esters have comparable reactivity. Isopropenyl esters of *N*-protected amino acids can be made by direct interaction of the latter with propyne under the influence of catalysis by Ru(II) complexes such as  $\text{RuCl}_2(\text{PPh}_3)(p\text{-cymene})$  at 100°C for 4 h.<sup>92</sup> Reactive esters derived from 2-chloro-4,6-dimethoxy-1,3,5-triazine are of interest for several reasons.<sup>93</sup> The weak basic properties of reagent and byproducts facilitate removal by washing with acid. Peptide synthesis is claimed to proceed in good yield without racemization; in addition, protection of the Ser hydroxyl group is unnecessary. Long-chain thiol esters of *Z*-amino acids couple with similar esters of amino acids in a micelle-forming system in  $\text{CHCl}_3$  to give the corresponding dipeptides.<sup>94</sup> An interesting application<sup>95</sup> of thiol esters to peptide bond formation involves several steps (Scheme 2). A key intermediate is a chiral crown ether having two thiol groups (6). The crown ether is a ligand for the  $\text{NH}_3^+$ -group of a salt of an amino-acid 4-nitrophenyl ester. A monothiolester (7) is formed by an intramolecular nucleophilic reaction. Reaction with *Z*-Cl blocks the amino group (7-8) and clears the crown ether for the formation of a dithiolester by reaction with a second amino acid 4-nitrophenyl ester (9-10). Peptide bond formation occurs in presence of base giving a monothiol ester of a *Z*-dipeptide (11). Racemization is mostly unacceptably high but is strongly dependent on structure. Since this is a method for chain propagation in the natural direction rather than by acylation of the *N*-terminus, it is to be hoped that the method will be investigated in depth. Thionesters undergo aminolysis

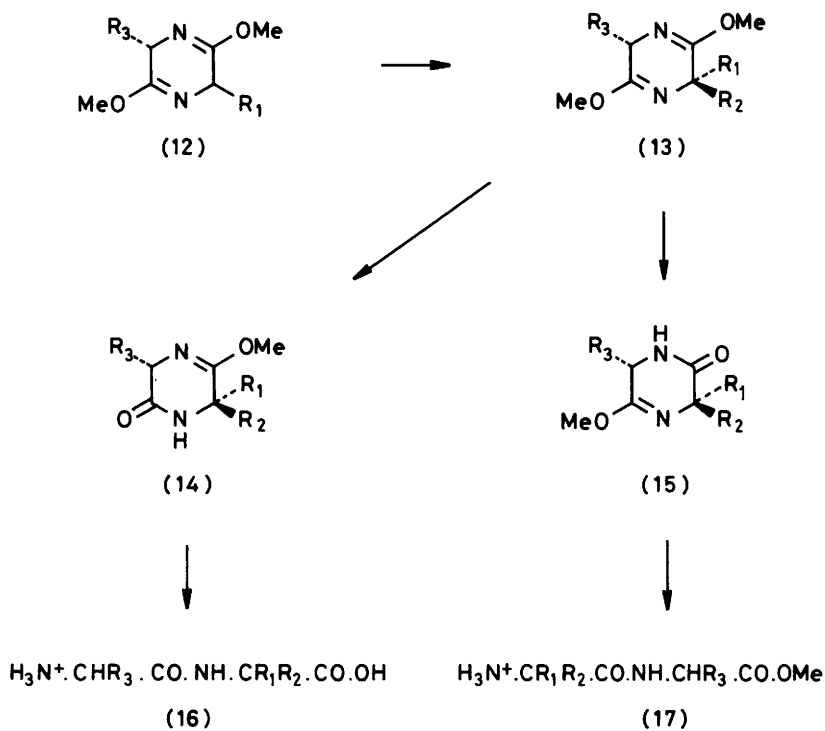


Scheme 2

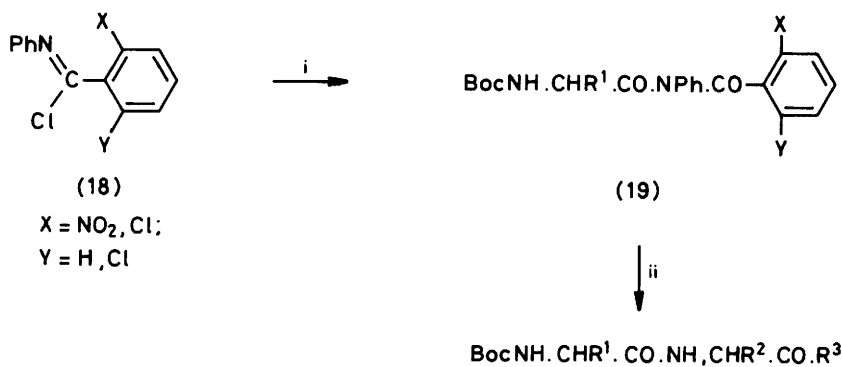
more readily than oxoesters and this has provided a method with defined limitations for the synthesis of thiono-peptides.<sup>96</sup> A stereoselective synthesis of dipeptides containing an  $\alpha$ -disubstituted amino acid has been described.<sup>97</sup> The method (Scheme 3), which avoids the difficulty in coupling amino acids with bulky side chains, involves the synthesis of a bislactim ether (12) followed by its stereoselective alkylation to give (13) which is a masked dipeptide. Regioselective conversion of (13) into (14) or (15) was not generally possible, but acidic hydrolysis of (14) gave (16) whereas (15) gave (17). Several dipeptides containing either  $\alpha$ -methyl-proline or -phenylalanine were synthesized. The synthesis of derivatives of  $\alpha$ -methyl-proline involved an additional alkylation step.

Mixed anhydrides of Boc-, Z- and Fmoc-amino acids derived from  $\text{ClCO.OCHMe}_2$  are reported to be fairly stable and to undergo aminolysis with less racemization than related anhydrides.<sup>98</sup> *N*-Acylamides display a formal resemblance to acid anhydrides and the reaction between sterically hindered benzimino chlorides (18) and *N*-protected amino acids gives isolatable *N*-acylamides (19). These give stereochemically pure peptide derivatives in reasonably good yield both by classical and solid-phase methodologies.<sup>99</sup> Significantly, coupling of Boc-L-Phe-L-Phe-OH or Boc-L-Phe-D-Phe-OH with H-L-Phe-OH proceeded without detectable racemization. *N*-Protected amino acid amides have been prepared with the EEDQ reagent.<sup>100</sup>

The use of various phosphorus derivatives in peptide synthesis has continued. Stable mixed anhydrides were obtained from *N*-protected amino acids and  $\text{Me}_2\text{P}(\text{:S})\text{Cl}$  in presence of base as reported earlier.<sup>101,102</sup> Consequently, coupling can be carried out in alcoholic solvents. Fragments of VIP were synthesized by this method. The mixed anhydride procedure using diphenylphosphinic chloride has afforded good yields of stereochemically pure peptides of *N*-methylamino acids when the amino group was protected as a urethane derivative.<sup>103</sup> BOP-Cl has been used satisfactorily in conjunction with Fmoc or Boc amino group protection.<sup>104</sup> Benzotriazol-1-yl diethyl and diphenyl phosphates have been prepared in high yield and shown to be useful in peptide coupling.<sup>105</sup> No racemization was detected in the Young



Scheme 3



Reagents: i,  $\text{BocNH} \cdot \text{CHR}^1 \cdot \text{CO} \cdot \text{OH}$ ; ii,  $\text{NH}_2 \cdot \text{CHR}^2 \cdot \text{CO} \cdot \text{R}^3$

and Anderson tests.  $NN'$ -Bis(2-oxo-3-oxazolidinyl)phosphorodiamidic azide has been used in the synthesis of some simple peptides but no rigorous tests for racemization were applied.<sup>106</sup>

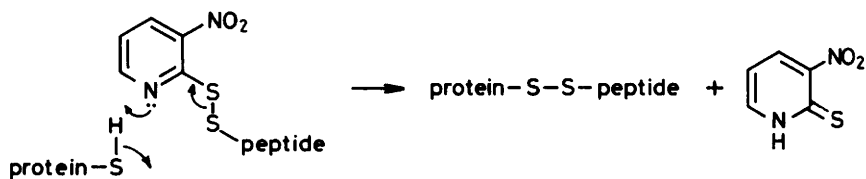
Each year brings to light a few side reactions that raise peptide synthesis above the button-pushing level of action. Some of these have been reviewed.<sup>107</sup> Only minor dehydration of the amide group of Z-homoGln was observed in couplings mediated with DCCI and this was eliminated by adding HOBT.<sup>108</sup> Finally,  $N$ -protected amino acids ( $R.CO.NH.CHR'.CO.OH$ ) couple with 2,2-dialkyl-3-amino-2H-azirines to give quantitative yields of  $R.CO.NH.CHR'.CO.NH.CR^1R^2.CO.NR^3R^4$ .<sup>109</sup> The dialkylamide is readily replaced by other groups such as amino acids.

#### 2.4 Disulphide bond formation

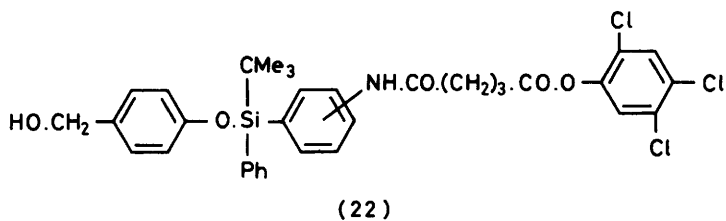
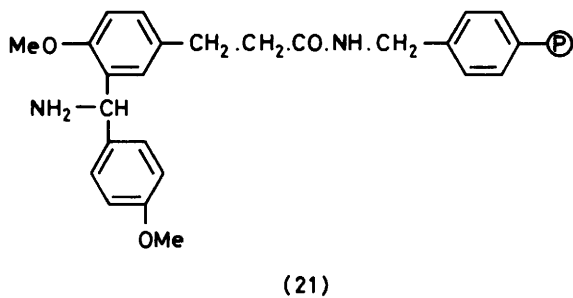
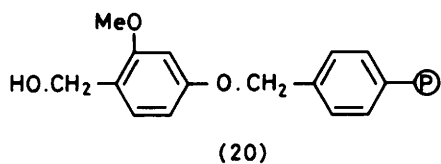
The use of thallium (III) trifluoroacetate for the simultaneous cleavage of some  $S$ -protecting groups and intra- or inter-molecular formation of a disulphide bond, which was reported last year, has been reviewed.<sup>64</sup> The other new method for synthesizing cystine peptides reported by the same group<sup>64</sup> has been further examined and exploited. Although peptides containing  $-Cys(Acm)(O)-$  and  $-Cys(MeO.Bzl)-$  form disulphide bonds on treatment with  $CF_3.CO.OH$  as catalyst, other combinations of protecting groups may require the use of a stronger acid such as  $CF_3.SO_3H$ .<sup>110,111</sup>

If a peptide containing an  $N$ -terminal  $S$ -3-nitro-2-pyridine-sulphenyl group is synthesized, the latter can be cleaved by interaction with a thiol group in a protein to yield a peptide-protein conjugate linked by a disulphide bond<sup>112</sup> (Scheme 4). The other product, 3-nitropyrid-2-thione, can be used to monitor the progress of the reaction spectrophotometrically.

Some 30 years ago, Rydon studied the aerial oxidation of synthetic peptides containing two cysteinyl residues. There are two new reports that parallel cyclic dimers are favoured<sup>113,114</sup> and when the antiparallel cyclic dimer,  $(H-Thr-Cys-Pro-Pro-Cys-Pro-Ala-Pro-OH)_2$ , was subjected to thiol-disulphide exchange, the parallel cyclic dimer was formed in >90% yield; it is suggested that protein biosynthesis does not require disulphide isomerase. This is a topic of fundamental interest and merits systematic



Scheme 4





study on both chemical and biochemical fronts.

The feasibility of assembling peptides containing asymmetric disulphide bonds by SPPS has been examined as follows.<sup>115</sup> A peptide containing a Cys(Acm) residue was constructed on the resin. This residue was converted into Cys(S.CO.OMe) by reaction with MeCO.S.Cl in MeOH - CH<sub>2</sub>Cl<sub>2</sub> (1:1). Subsequent exposure to cysteine caused an exchange reaction in which the unprotected cysteine residue was linked to the immobilized peptide through a disulphide bond.

## 2.5 Solid-phase peptide synthesis

Several new supports have been described, some of them being intended for special purposes. The use of crosslinked poly[N-[2-(4-hydroxyphenyl)ethyl]acrylamide] for ultra-high loading in SPPS has been reviewed<sup>116</sup> and the use of a selectively labile C-terminal linker group and a method for monitoring product homogeneity have been reported.<sup>117,118</sup> A graft copolymer has been prepared by anionic polymerization of ethylene oxide on a crosslinked polystyrene.<sup>119</sup> The polymer was converted into an amino-copolymer and used in the continuous-flow mode under pressure to synthesize the C-terminal octapeptide of porcine insulin B chain. The same group synthesized peptides which were left on the resin for use in the affinity chromatography of oligodeoxyribonucleotides.<sup>120</sup> The use of a similar graft copolymer has been reported independently.<sup>121</sup> A synthetic resin with oxime functional groups has been prepared and used for the synthesis of peptide amide derivatives by cleaving the peptide-resin ester bond with an amine.<sup>122</sup> Side-chain ester groups were unaffected in the time required for cleavage. Treatment of Merrifield resin with tetrachlorohydroquinone gave a derivative with free phenolic hydroxylic groups which could be used to make insoluble aryl esters of N-protected amino acids.<sup>123</sup> An intriguing development permits the production of a peptide attached to a soluble polymer as carrier in order to evoke antibody formation.<sup>124</sup> A copolymer of NN-dimethylacrylamide, a ketal crosslinking agent, (CH<sub>2</sub>:CH.CO.NH.CH<sub>2</sub>.CH<sub>2</sub>.O)<sub>2</sub>CR<sub>2</sub>, and a functionalizing agent, CH<sub>2</sub>:CH.CO.NH.(CH<sub>2</sub>)<sub>6</sub>.OAc, was produced within the pores of macroporous kieselguhr particles. After

removal of the *O*-acetyl group, the peptide was assembled on the resin using Fmoc amino acids in the continuous flow mode. The polymeric ketal was hydrolysed with 95%  $\text{CF}_3\text{CO.OH}$ , the soluble peptide-polyacrylamide conjugate was deprotected and used directly and successfully to raise antibodies to the peptide in rabbits.

A new linker, 4-hydroxy-2-methoxybenzyl alcohol, similar to that designed by Sheppard, has been incorporated<sup>125,126</sup> into a polystyrene resin (20). Peptide cleavage is achieved with 0.5-1.0%  $\text{CF}_3\text{CO.OH}$  in  $\text{CH}_2\text{Cl}_2$  and consequently Fmoc and *t*-Bu groups can be safely used for protection. Scavengers are unnecessary unless the peptide being synthesized contains Trp. More photolabile anchoring groups have been reported,<sup>127-130</sup> but moderate yields at the cleavage stage and the risk of photo-oxidation of the peptide leave the Reporter unconvinced that this is a fruitful development. Design and development<sup>131</sup> of a new benzhydrylamine linker (21) was rapidly followed by the report<sup>132</sup> of the synthesis of human pancreastatin (52 residues) and a C-terminal amide fragment (29 residues). The overall yield of the former (9%), its hplc elution pattern and other characterization details make this an important development. Several other peptide amides have been synthesized by the same method.<sup>133</sup> Deprotection was achieved with  $\text{Me}_3\text{SiBr} - \text{PhSMe}$  which is a further recommendation for that technique (see section 2.2). Two further examples of peptide cleavage from the resin by fluoride have been described. In one case, peptide assembly was accomplished on a conventional Pam resin and the peptide was obtained by treatment with  $\text{Bu}_4\text{N}^+\text{F}^- \cdot 3\text{H}_2\text{O}$  in  $\text{HCO.NMe}_2$  at 25°C.<sup>134</sup> In the other case, a Si-containing preformed handle (22) was synthesized (10 steps), the C-terminal Fmoc amino acid was attached to the hydroxyl group and the whole was coupled to an amino-resin by virtue of the 2,4,5-trichlorophenyl ester.<sup>135</sup> Although this method may appear complex, cleavage with  $\text{Bu}_4\text{N}^+\text{F}^-$  in  $\text{HCO.NMe}_2$  gave >90% yield of peptide with no evidence of racemization and without formation of aspartimide when the peptide contained C-terminal Asn. A glycolamide linker has been used to synthesize a short peptide amide by using  $\text{NH}_3$  in  $\text{CF}_3\text{CH}_2\text{OH}$  for cleaving the peptide from the resin.<sup>136</sup> In an attempt to develop new linker groups, it was found that a

polyacrylamide resin with free amino groups reacted with  $\alpha$ -bromopropionic anhydride, but rather surprisingly the resin was found by  $^{13}\text{C}$ -nmr spectroscopy and tandem mass spectroscopy to contain some  $\beta$ -alanine residues.<sup>137</sup> A linker designed for attachment to a soluble polymer, polyethylene glycol, could be cleaved by sodium dithionite,<sup>138</sup> but detection of failure sequences after synthesis of a tetrapeptide provides an unfavourable prognosis. An improved method for attaching the C-terminal amino acid to aminopolyethylene glycol for soluble phase peptide synthesis has been described.<sup>139</sup>

A detailed account of the use of pentafluorophenyl esters of Fmoc amino acids in SPPS has appeared.<sup>140</sup> The use of esters of Fmoc amino acids with 4-hydroxy-3-oxo-2,5-diphenyl-2,3-dihydrothiophene-1,1-dioxide, which was reported last year, has also been described in greater detail.<sup>141</sup> Coupling rates achieved with the BOP reagent rival those obtained with DCCI with or without HOBT and symmetrical anhydrides.<sup>142</sup> In the synthesis of [Ala<sup>15</sup>]-GRF(1-29)NH<sub>2</sub>, a single BOP cycle was found to give a better yield than repeated use of DCCI. Some problems were encountered with Asn and these were attributed to formation of aspartimide. It is claimed that hydroxyl groups need not be protected provided that  $\leq 10$  additional cycles are required after introduction of the unprotected hydroxyamino acid.

Methods for monitoring progress of coupling reactions can be broadly divided into two groups, discontinuous and continuous. Each step in SPPS can be monitored by simultaneous use of Fab and tandem mass spectrometry.<sup>143</sup> Free amino groups can be assayed by tritylation followed by acid hydrolysis of the trityl derivative and spectrophotometric determination of the trityl cation.<sup>144</sup> Addition of bromophenol blue is reported to be a sensitive, internal continuous indicator of the progress of coupling reactions.<sup>145,146</sup> Although it is claimed that the presence of the indicator does not affect coupling, it is specified that it is important not to use too much indicator. One would like to know if the phenolic hydroxyl group of bromophenol blue is acylated, even transiently, by an *N*-protected amino acid during coupling. Automation of peptide synthesis which clearly requires monitoring and appropriate feedback action has been reviewed.<sup>147</sup> The use of

3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl (Dhbt) esters of Fmoc amino acids as self-monitoring acylating agents was reported last year. Full publications have now appeared,<sup>148,149</sup> one of which describes the design of a fully automatic peptide synthesizer. Hard-copy output recording the progress of acylation is obtained. The successful synthesis of a nonadecapeptide related to endoplasmin provided a searching test of the system.

It is important to distinguish between methods of peptide synthesis which have adequate feedback control or monitoring together with analytical quality control at the end and those methods that have neither. Each has their place and their limitations. For the production of antibodies, it matters little that the required immunogenic peptide constitutes only a small fraction of the product, but in the study of structure/activity relationships of peptide hormones or in the synthesis of peptide-based drugs, it is essential to produce a pure product in high yield. On the other hand, production of antibodies requires the synthesis of many peptides and esoteric peptide synthesizers, even when completely automated, are too slow unless computer control is included to carry out multiple syntheses simultaneously. There is, however, a trend now towards performing multiple peptide syntheses with suitable quality control. The resulting technique may use conventional chemistry in a manual mode<sup>150</sup> or may use functionalized cellulose discs in a machine which can control several syntheses simultaneously.<sup>151,152</sup> For example, the synthesis of 13 related heptapeptides required only 16 cycles compared to 78 cycles which would have been required in individual syntheses.<sup>152</sup> Continuous-flow methodology is becoming more common<sup>153,154</sup> and another trend is towards large-scale hardware.<sup>155</sup>

An interesting development of SPPS has been reported from two laboratories.<sup>156,157</sup> After assembling linear peptides analogous to either growth-hormone releasing factor (GRF)<sup>156</sup> or dynorphin A,<sup>157</sup> cyclic peptides were generated before general deprotection and cleavage from the resin by forming amide bonds between the  $\delta$ -NH<sub>2</sub> of Orn or the  $\epsilon$ -NH<sub>2</sub> of Lys and the  $\beta$ -CO.OH of Asp. A comparative study<sup>156</sup> indicated that the cyclization was effected more efficiently by the BOP reagent than by DCCI/HOBt.

There are a few specialized points about SPPS to report. Difficulties in synthesizing hydrophobic peptides are well known. In the synthesis of C-peptide fragments of human proinsulin, it was shown by ir spectroscopic methods that peptide chains interacted with each other even at low loading to form  $\beta$ -sheets.<sup>158</sup> Synthesis of a large fragment of the  $\beta$ -subunit of baboon chorionic gonadotropin was successfully achieved<sup>159</sup> with the Fmoc - polyacrylamide method but failed with the Boc - polystyrene approach; two Asp-Pro bonds were particularly sensitive to acidic cleavage by HF. The attachment of the C-terminal Gln residue as its Fmoc derivative caused no problem with the 44'-dimethoxybenzhydryl linker. Synthesis of an analogue of gramicidin A which is rich in Trp was successful by the Fmoc procedure;<sup>160</sup> avoidance of acidic conditions prevented oxidation of Trp residues and decreased the amount of peptide truncation. The production of  $-\text{CH}_2\text{NH}-$  pseudopeptide bonds by reductive alkylation during SPPS has been reviewed.<sup>161</sup>

## 2.6 Enzyme-mediated synthesis and semi-synthesis

A very mixed bag of publications is included under this heading, some research is very basic with small substrates, some work involves semi-synthesis of much larger molecules; there is a substantial dose of kinetics and better syntheses of commercially important products are still being sought. Most of the common proteinases are represented together with a few esterases. There is no sign of the emergence of a theory to cover all applications and there are several reasons for this: (1) the possibility of consecutive or concurrent hydrolytic, peptide forming, transesterification and transpeptidation reactions, (2) strong dependence of reaction rates on the structure of substrate(s), solvents, apparent pH and the use of free or conjugated enzymes. Perhaps the best way of dealing with such a heterogeneous collection of research is simply to list relevant headings with attached references: amino-acid resolution,<sup>162-165</sup> 'Aspartame' and related derivatives,<sup>166-170</sup> larger peptides,<sup>171-173</sup> semi-synthesis,<sup>174-176</sup> kinetics/mechanism,<sup>177-182</sup> modification of enzyme specificity,<sup>177,183,184</sup> enzyme immobilization,<sup>167,169,170,184-187</sup> papain,<sup>164-166,168,171-173,183,188-190</sup> thermolysin,<sup>166,167,169,170,172,184,187,191-193</sup> carboxypeptidase Y,<sup>194,195</sup>

chymotrypsin,<sup>166,171,173,178-180,183,185,186,191,194</sup> trypsin,<sup>174,176,183,194</sup>  
 pepsin,<sup>182</sup> subtilisin,<sup>178,183,196</sup> and esterases/lipases  
 /acylases.<sup>162,163,196,197</sup>

A few papers deserve individual comment because of their novelty, general importance or commercial relevance. Thus, several serine and cysteine proteinases retain esterase but lose amidase activity in presence of water-miscible solvents.<sup>183</sup> These conditions permit satisfactory peptide synthesis. A mixture of Z-Tyr-OMe and Arg-OMe in presence of  $\alpha$ -chymotrypsin in  $\text{Na}_2\text{CO}_3$  buffer containing 50% MeCN quickly gives 75-80% of Z-Tyr-Arg-OMe. Conversion of  $\alpha$ -chymotrypsin into an esterase with no trace of amidase activity<sup>177</sup> is permanent after selective methylation of the imidazole ring of His<sup>57</sup>. Thermolysin entrapped in cross-linked polyurethane achieved 94% coupling of Z-Asp-OH and H-Phe-OMe in Me.CO.OEt saturated with water.<sup>170</sup> Finally, a 2,6-dichlorophenyl ester of an amino acid can be coupled enzymically to the C-terminus of a peptide fragment of a protein and the resulting peptide ester can be coupled to an appropriate peptide to provide a semi-synthesis of a protein.<sup>176</sup>

### 3. Synthesis: selected examples

Following earlier precedents, no attempt is made here to describe any synthesis in detail. The examples selected for particular mention are not just the biggest molecules synthesized in the year, although this is still a matter for interest, but include molecules of biological interest or, in one case, a modified peptide for assaying the natural peptide. It is noteworthy that although SPPS may be considered to have delivered the coup de grâce to classical methodology, long peptides and proteins are frequently made by this route using fragment coupling.

One of the more spectacular syntheses is the assembly by SPPS of the 99-residue HIV-1 aspartyl proteinase.<sup>198,199</sup> Sidechain protection was accomplished as follows: Arg(Tos), Glu( $\text{OC}_6\text{H}_{11}$ ), Asp( $\text{OC}_6\text{H}_{11}$ ), Lys(4-Cl-Z), Tyr(2-Br-Z), His(Bom), Trp(For), Ser(Bzl), Thr(Bzl).  $\alpha$ -Amino groups were protected by Boc. Deprotection and cleavage was achieved with HF in a mixture of 4-MeC<sub>6</sub>H<sub>4</sub>OH/4-MeC<sub>6</sub>H<sub>4</sub>SH/HS.CH<sub>2</sub>.CH<sub>2</sub>.SH/Me<sub>2</sub>S. The proteinase cleaved the octapeptide, H-Val-Ser-Gln-Asn-Tyr-Pro-Ile-Val-OH, at the

Tyr-Pro bond which is the cleavage site between p17 and p24 in the *gag* protein. It also splits HIV *gag* p55 into p17 and p24. The synthesis of  $\alpha$ -inhibin 92 was carried out by a combination of SPPS for the production of fragments with C-terminal Gly and the coupling of these to produce the complete molecule.<sup>200</sup> This achievement is perhaps a fitting memorial to the late C.H. Li who scaled many mountains in a lifetime of protein research, but few tougher than this. The 88-residue, cardiodilatin, has been synthesized by SPPS using Boc amino acids on Pam resin.<sup>201</sup> The synthesis was interrupted after 27 cycles to yield some  $\alpha$ -hANP. Porcine C5a anaphylotoxin (74 residues) has been synthesized by classical methodology by coupling 9 fragments.<sup>202</sup> The natural and synthetic peptides differed at residue 65. The synthetic molecule had Gln<sup>65</sup> whereas the natural compound after storage had Glu<sup>65</sup>. It is suggested that amide hydrolysis may occur on storage. Such a process might receive anchimeric assistance from a neighbouring carboxyl group. Human thymopoietin (48 residues) and deacetyl thymosin  $\beta$ 7 (41 residues) have been synthesized by the same team using classical methodology.<sup>203,204</sup> Both peptides restore activity to T-lymphocytes with impaired blastogenic response. A 55-residue peptide from the C-terminal portion of the Shope fibroma virus growth factor, which is homologous to EGF, has been synthesized and fully characterized.<sup>205</sup> Frog-skin sauvagine (44 residues) has been assembled from 8 fragments which were prepared by classical methods.<sup>206</sup> A part of the  $\beta$ -subunit of bovine heart mitochondrial ATP synthase (fragment F1) has been synthesized.<sup>207</sup> It binds ATP and an analogue of ATP without a requirement for  $Mg^{2+}$  ion, behaviour which resembles ATPase. Finally, the introduction of a D-Tyr residue via *N,O*-bis-Fmoc-D-Tyr-ONSu at the *N*-terminus of synthetic peptide analogues of natural peptide hormones serves two purposes. It provides a site for iodination for radioimmunoassay and it secures the peptide against attack by aminopeptidases.<sup>208</sup>

4. Appendix I. A List of Syntheses Reported in 1988

The syntheses are listed under the name of the peptide/protein to which they relate, but no arrangement is attempted under the subheading. In some cases, closely related peptides are listed together (e.g. atrial natriuretic peptide, cardiodilatin).

| <u>Peptide</u>                                                                     | <u>Ref.</u> |
|------------------------------------------------------------------------------------|-------------|
| 4.1 <u>Natural Peptides, Proteins and Partial Sequences</u>                        |             |
| ACTH                                                                               |             |
| fragment (1-4)                                                                     | 209         |
| covalently dimerized fragments                                                     | 210         |
| Adipokinetic peptide                                                               |             |
| Adipokinetic peptide from corpora cardiaca of cricket                              | 211         |
| Adrenoxin precursor                                                                |             |
| synthesis of two extension peptides                                                | 212         |
| Angiotensin(ogen)                                                                  |             |
| D-Amino acid analogues of angiotensin II                                           | 213         |
| Importance of residues 2 and 6 in antagonists                                      | 214         |
| Ala <sup>1</sup> , Ala <sup>2</sup> , Ala <sup>3</sup> analogues of angiotensin II | 215         |
| Analogues with substituents at positions 1 and 2                                   | 216         |
| Metallocene analogues of angiotensin II                                            | 217         |
| <sup>15</sup> N-Angiotensin II analogues                                           | 218         |
| Aspartame                                                                          |             |
| Aspartame and related compounds                                                    | 166-170     |
| ATP Synthase                                                                       |             |
| ATP-binding fragment of bovine heart enzyme                                        | 207         |
| Atrial natriuretic peptide (factor), cardiodilatin                                 |             |
| Partial retro-inverso analogues                                                    | 219         |
| ANP(1-16)                                                                          | 220         |
| ANP(17-28)                                                                         | 221         |
| Des-Ser <sup>5</sup> -ANPII, [Ser <sup>6</sup> ]-ANPII                             | 222         |
| SPPS of hANPIII                                                                    | 223         |
| SPPS of porcine cardiodilatin 88                                                   | 201         |
| fragments of α-hANP                                                                | 224         |
| α-rANP and fragments                                                               | 225         |
| tritiated ANP derivatives                                                          | 226         |
| Bradykinin                                                                         |             |
| cyclic analogues                                                                   | 227         |



|                                                                 |                 |
|-----------------------------------------------------------------|-----------------|
| SPPS of bradykinin                                              | 228             |
| Calcitonin                                                      |                 |
| Salmon calcitonin II and fragments                              | 229-231         |
| Analogues of salmon calcitonin                                  | 232             |
| Calcitonin-gene related peptide (CGRP)                          |                 |
| Analogue of hCGRP                                               | 233             |
| chicken CGRP                                                    | 111             |
| Calcium-binding proteins                                        |                 |
| hypothetical ancestral protein                                  | 234,235         |
| Ca <sup>2+</sup> -binding site of troponin C                    | 236             |
| Chemotactic peptides                                            |                 |
| porcine C5a anaphylatoxin                                       | 202             |
| β-casomorphin-5                                                 | 237             |
| neutrophil chemotactic factor                                   | 238             |
| nonapeptide sequence of elastin                                 | 239             |
| small chemotactic peptides                                      | 240-242         |
| Cholecystokinin and gastrin                                     |                 |
| analogues of CCK in Met <sup>29</sup> -Gly <sup>29</sup> region | 243             |
| analogues of CCK7                                               | 244,245         |
| hCCK33                                                          | 246-248         |
| thiono peptide analogue of gastrin fragment                     | 249             |
| CCK8 and analogues                                              | 172,245,250-252 |
| gastrin fragments                                               | 135             |
| pentagastrin                                                    | 253,254         |
| analogues of little gastrin II and minigastrin                  | 255             |
| analogues of CCK4                                               | 256,257         |
| analogues of CCK C-terminal region                              | 258             |
| chicken antral peptide                                          | 259             |
| Collagen                                                        |                 |
| model of triple helix                                           | 260             |
| Cyclosporin                                                     |                 |
| fragment and precursor analogue                                 | 261,262         |
| Cytochromes                                                     |                 |
| cytochrome c analogues by semi-synthesis                        | 176             |
| rabbit cytochrome b <sub>5</sub> fragment (92-97)               | 263             |
| cytochrome P <sub>450</sub> fragments                           | 264             |
| Delta sleep-inducing peptide                                    |                 |
| enzymic synthesis                                               | 171             |

|                                                                      |             |
|----------------------------------------------------------------------|-------------|
| DNA-cleaving protein                                                 |             |
| model of <i>Hin</i> recombinase                                      | 265         |
| Endothelin                                                           |             |
| disulphide isomers of porcine/human endothelin                       | 266         |
| [D-Trp <sup>21</sup> ]endothelin                                     | 267         |
| Enterotoxins                                                         |             |
| C-terminal fragments of <i>Yersinia enterocolitica</i> toxin         | 268         |
| analogues of enterotoxin (ST <sub>h</sub> ) of <u><i>E. coli</i></u> | 269         |
| Fibrinogen                                                           |             |
| peptide Bβ15-42, inhibitor of platelet aggregation                   | 270         |
| Gastrin-releasing factor                                             |             |
| SPPS of analogue                                                     | 142         |
| SPPS of two canine GRFs                                              | 271         |
| Glucagon                                                             |             |
| analogues with increased C-terminal helicity                         | 272         |
| Glucose-dependent insulinotropic polypeptide (GIP)                   |             |
| SPPS of 31-residue fragment                                          | 273         |
| Glutathione                                                          |             |
| nucleophilicity of glutathione analogues                             | 274         |
| Glycophorin A                                                        |             |
| antimalarial activity of pentapeptide fragments                      | 275         |
| Gonadotropin                                                         |             |
| β-subunit of baboon chorionic gonadotropin                           | 159         |
| GnRH/LHRH                                                            |             |
| Trp reduction and His racemization during synthesis                  | 68          |
| analogues                                                            | 136,276-280 |
| analogues with -CH <sub>2</sub> .NR- peptide bond replacement        | 281         |
| antagonists                                                          | 282-285     |
| Gramicidins                                                          |             |
| analogue of gramicidin A                                             | 160         |
| analogue of gramicidin S                                             | 286         |
| Growth hormone-releasing hormone                                     |             |
| SPPS                                                                 | 287         |
| various analogues                                                    | 156,288-290 |
| Haemoglobin                                                          |             |
| HbA β-chain fragment (57-100)                                        | 291         |
| HbA α-chain fragments                                                | 292         |
| Head activator of <i>Hydra</i>                                       | 293         |

|                                                            |             |
|------------------------------------------------------------|-------------|
| Hirudin                                                    |             |
| fragment (54-65)                                           | 294         |
| fragments delineating thrombin-binding domain              | 295         |
| HIV                                                        |             |
| aspartyl proteinase                                        | 198,199     |
| CD4 peptides which inhibit HIV-induced cell fusion         | 296         |
| IgG                                                        |             |
| bis-cystinyl fragment                                      | 113         |
| Inhibin                                                    |             |
| segment synthesis of $\alpha$ -inhibin-92                  | 200         |
| Insulin                                                    |             |
| A chain                                                    | 297         |
| B chain fragments                                          | 119,298,299 |
| insulin analogues                                          | 175,300-302 |
| [ $^3\text{H-Tyr}^{226}$ ] human insulin by semi-synthesis | 303         |
| enzyme conversion of rabbit into pig insulin               | 174         |
| modified C-peptide and fragments                           | 304         |
| insulin-like growth factor                                 | 305         |
| Interleukin $1\beta$ antagonists                           |             |
| peptides antagonizing hyperalgesia evoked by interleukin   | 306         |
| Lepidopterin                                               |             |
| structure/activity studies                                 | 307         |
| Magainins                                                  |             |
| analogues                                                  | 308,309     |
| Melanin concentrating hormone                              |             |
| analogues                                                  | 310         |
| Metallothionein                                            |             |
| N-terminal heptapeptide                                    | 311         |
| Myelin                                                     |             |
| inducer of experimental allergic encephalomyelitis         | 312         |
| Myosin                                                     |             |
| peptides based on calmodulin-binding region                | 313,314     |
| MSH                                                        |             |
| cyclic lactam analogues                                    | 315         |
| analogues with sterically constrained amino acids          | 316         |
| Neurofilament protein                                      |             |
| peptides containing phosphorylation site                   | 317         |
| Neuropeptides                                              |             |

|                                                            |                    |
|------------------------------------------------------------|--------------------|
| neuromedin B                                               | 133                |
| cardioexcitatory peptides and analogues                    | 318,319            |
| neuropeptide Y fragments                                   | 320,321            |
| N-methylated analogues of neurokinin A                     | 322                |
| Nisin                                                      |                    |
| total synthesis                                            | 323                |
| Oncoproteins                                               |                    |
| phosphorylation site of <i>ras</i> oncoprotein p21         | 324                |
| BPV1 oncoprotein and C-terminal domain                     | 325                |
| Opioids, antinociceptive peptides and receptors            |                    |
| enkephalins, dermorphins and analogues                     | 33,134,135,326-353 |
| affinity labels for opioid receptors                       | 354,355            |
| dynorphin analogues                                        | 157,356            |
| endorphins                                                 | 357                |
| Pancreastatin                                              |                    |
| SPPS of human pancreastatin                                | 132                |
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# 3

## Analogue and Conformational Studies on Peptide Hormones and Other Biologically Active Peptides

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BY J. S. DAVIES

### 1. Introduction

Activity in this field has reached a state of maturity with many papers reporting very similar approaches and strategies. Once the structure of a biologically active peptide has been elucidated and a reasonable bioassay developed, solid phase synthesis can now furnish a large number of analogues (agonists and antagonists) to study structure-activity relationships; X-ray crystallography, n.m.r., c.d. and related techniques provide detailed information on conformation which can be correlated with theoretical calculations and molecular dynamics. The scene is then set for improving stability and transport of the active peptide by making isosteres of key peptide bonds or by synthesising conformationally-restricted mimetics, to achieve substrate-receptor interactions which hopefully can be developed into a successful pharmaceutical product.

The better understanding of the mechanism of enzyme action has also matured into a fruitful field of endeavour in designing enzyme inhibitors for controlling key metabolic steps of clinical interest. While studies on angiotensin converting enzyme (A.C.E.) have yielded many successful inhibitors there is evidence this year that a wider range of enzymes are now being investigated. The structure of the inhibitor molecules often requires synthetic expertise well outside the usual amide bond-making pursuits of the traditional peptide chemist.

Many contemporary exponents working in the area covered by this Chapter have been influenced greatly by the work of the late Prof. C.H. Li, who published over 1000 papers in this field. His influence has been reflected in two issues (December 1988/January 1989) of the Int.Journal for Peptide and Protein Research being dedicated to his memory, and most of the contents were of significance to this current review.

International Peptide Symposia also cover subjects of real interest to this Chapter but again to prevent overlap with the full papers when they eventually appear in primary journals, no attempt has been made to review in detail the Proceedings of the 10th American Peptide Symposium<sup>1</sup> at St. Louis, Missouri and the Peptide Symposium<sup>2</sup> held in Japan in 1987. The main source of papers has again been abstraction *via* Chemical Abstracts (up to the May 1989 issue) and mainstream primary journals.

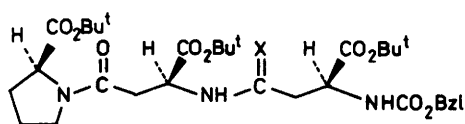
## 2. Peptide-backbone Modifications

**2.1  $\psi$ [CSNH]-Analogues.** - Regioselective replacement of one amide bond in peptide (1) to give the endothiopeptide (2) has been achieved<sup>3</sup> by the Lawesson reagent in 84% yield. The same reagent can also selectively thionate<sup>4</sup> an amide group under the conditions of solid phase synthesis on a resin as summarised in Scheme 1. The thioamide bond attached to the resin proved to be stable under the solid phase protocol. Similarly, a C-terminal thioamide survived conventional solution phase synthesis<sup>5</sup> of diastereoisomeric TRH analogues (e.g., PyroGlu-His-Pro $\psi$ [CSNH]H) using DCCI/HOBt in dimethylformamide solution. Model studies<sup>6</sup> on N-substituted glycine thioesters confirm that they can be used for making simple thiopeptides by coupling with the appropriate amino acid or peptide esters, with the best yields resulting from couplings involving the amino group of glycine or the imino group of proline. Crystal structures of the two monothionated analogues of melanostatin, Boc-L-Pro $\psi$ [CSNH]-L-Leu-Gly-NH<sub>2</sub> and L-5-thioxoPro-L-Leu-Gly-NHEt show<sup>7</sup> that both tripeptides are folded in a type II  $\beta$ -bend at the Leu-Gly sequence and confirms that a thioamide sulfur atom can act as an H-bonding acceptor. Mixed intramolecular H-bonds have also been detected<sup>8</sup> from i.r. and n.m.r. studies on model thioamides.

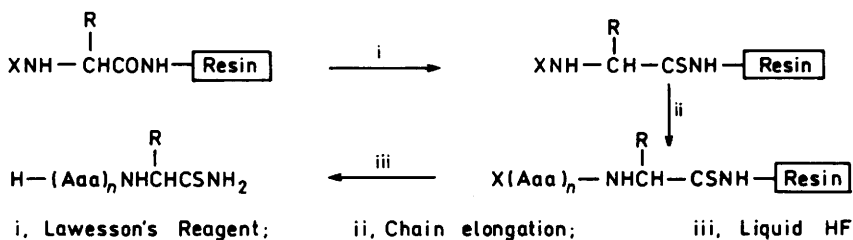
**2.2  $\psi$ [NHCO]-Retro-Inverso Analogues.** The retro-inverso concept has been applied to the design of inhibitors of enkephalin-degrading enzymes, neutral endopeptidase 24-11, aminopeptidase N and a dipeptidyl aminopeptidase. In the compounds RN(OH)CO(CH<sub>2</sub>)<sub>n</sub>CH(CH<sub>2</sub>Ph)NHCOCH(R<sup>1</sup>)CO<sub>2</sub>H (R = H or Me; R<sup>1</sup> = H, Me or CH<sub>2</sub>Ph) only when n = 1 were inhibitory potencies in the nanomolar range achieved<sup>9</sup>. The well-tried iodobenzene (bis)trifluoroacetate route from amide to amine has been used<sup>10</sup> to produce analogues of rat atrial natriuretic factor (rANF<sub>1-28</sub>) *via* solution and solid phase techniques. Insertion of  $\psi$ [NHCO] at the Ser<sup>25</sup>-Phe<sup>26</sup> position gave compounds of comparable potency to the endogenous peptide but none of the peptides showed extended duration of action *in vivo*. An n.m.r. study<sup>11</sup> of [Glp<sup>6</sup>, gPhe<sup>8</sup>, mGly<sup>9</sup>]SP<sub>6-11</sub> and [BocAla<sup>6</sup>, gPhe<sup>7</sup>, D-Phe<sup>8</sup>, mGly<sup>9</sup>]SP<sub>6-11</sub>, derivatives of the bioactive fragment 6-11 of substance P, where gPhe and mGly are the gem diaminoalkyl and malonyl analogues of Phe and Gly, respectively, suggests that the 5-fold lower level of potency of these compounds may be due to the lower accessibility of the Met<sup>11</sup>CONH<sub>2</sub> group.

**2.3  $\psi$ [CONR]-N-Alkylated Analogues.** Cyclosporin *cyclo*-(MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-D-Ala-MeLeu-MeVal) where MeBmt represents N-Me-(4R)-4(E)-2-butenyl-4-methyl-L-Thr is a highly alkylated immunosuppressive peptide. One of the significant contributions made by Ian Galpin's group<sup>12</sup> before

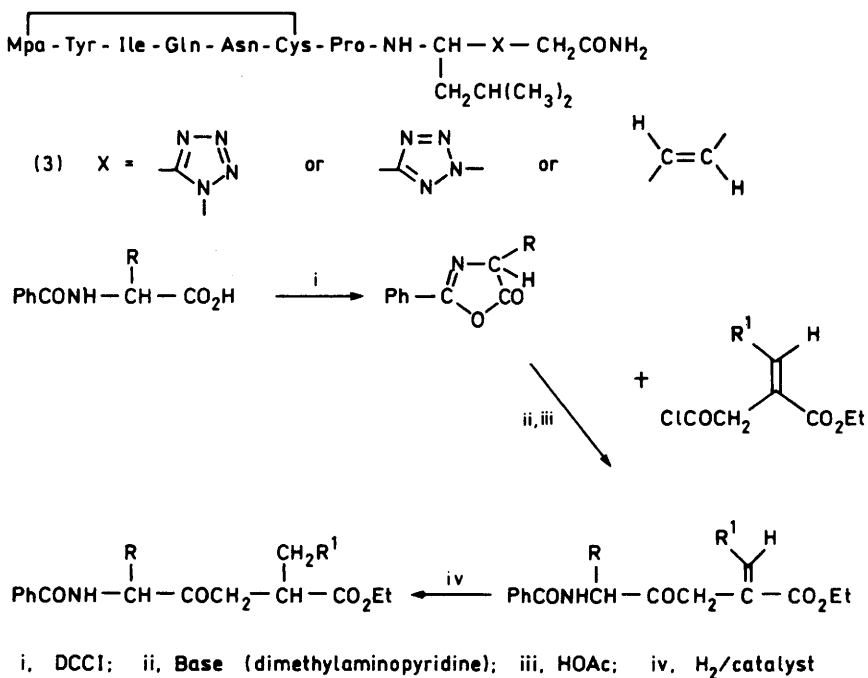




(1) X = O      (2) X = S



**Scheme 1**



**Scheme 2**

his untimely death was to prove the usefulness of the diphenylphosphinic mixed anhydride coupling methodology for N-methylated residues in the synthesis of a number of cyclosporin analogues. Poor solubility has prevented n.m.r. being used for the study of the conformation of cyclosporin, in aqueous media, but the ability of a series of monoclonal antibodies raised<sup>13</sup> against the cyclic peptide to recognise the residue MeBmt has given good evidence to support the good correspondence between the X-ray determined structure and the conformation in aqueous solution. The added sensitivity towards acid of a series of N-alkylated peptides has been investigated<sup>14</sup> in detail and has been explained in terms of a readiness to form dioxopiperazine intermediates.

**2.4  $\psi[\text{CH}_2\text{NH}]$  Amino Methylene Analogues.** Reductive alkylation after coupling of a protected amino-aldehyde in the solid phase offers a rapid route to  $\psi[\text{CH}_2\text{NH}]$  isosteres. Previous work has encountered racemisation problems but new conditions<sup>15</sup> using  $\text{NaBH}_3\text{CN}$  as reducing agent minimises racemisation, and has given maximum potencies for  $\psi[\text{CH}_2\text{NH}]$  insertions in cyclic SRIF analogues. The same methodology<sup>16</sup> was used to insert  $\psi[\text{CH}_2\text{NH}]$  at each of the peptide bonds (except position 8-9) of the LH-RH antagonist  $[\text{NAc-D-Nal}^1, \text{D-Phe}^{2,3}, \text{D-Arg}^6, \text{Phe}^7, \text{D-Ala}^{10}]\text{LH-RH}$  where  $\text{Nal} = 3\text{-(2-naphthyl)Ala}$ . The isosteres caused a loss of antioviulatory activity at the  $50\mu\text{g}$  dose level when inserted into the 1/2, 2/3, 3/4 and 7/8 positions with the remaining insertions showing less marked reduction. Trifluoroacetylated  $\psi[\text{CH}_2\text{NH}]$  dipeptides have been subjected<sup>17</sup> to a chiral purity check using g.c./m.s. techniques. A computer-simulated human renin active site model and systematic variation of Xaa has led<sup>18</sup> to several congeners of the sequence  $\text{Ac-Ftr-Pro-Phe-His-Xaa}\psi[\text{CH}_2\text{NH}]\text{PheNH}_2$  (where Xaa = Leu, Cha or Phe) with  $\text{IC}_{50}$  values in the  $10^{-10}/10^{-9}$  range. One,  $\text{Ac-Ftr-Pro-Phe-MeHis-Phe-}\psi[\text{CH}_2\text{NH}]\text{PheNH}_2$  (Ftr = N-formyltryptophan), was found to be superpotent and very selective against human renin. The conformation of the dipeptides  $\text{t-BuCO-Pro-Gly-}\psi[\text{CH}_2\text{NH}]\text{NRR}^1$  ( $\text{R} = \text{H}$ ,  $\text{R}^1 = \text{Et}$ ;  $\text{R} = \text{R}^1 = \text{Me}$ ) has been shown<sup>19</sup> to depend on the ionic or neutral state of the  $\text{CH}_2\text{NH}$  bond. The flexible molecule becomes highly rigid upon protonation due to strong  $-\text{N}^+-\text{H} \cdots \text{O} = \text{C}-$  interactions closing a 10-membered cycle as in  $\beta$ -turns, which is a feature that is also present at physiological pH.

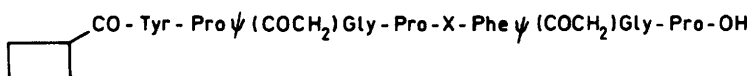
**2.5  $\psi[\text{CH}=\text{CH}]$  - Ethylenic Isosteres.** A synthetic route<sup>20</sup> specifically for the  $\text{Leu}\psi[(\text{E})\text{CH}=\text{CH}]\text{Asp}$  pseudodipeptide unit has been devised as an alternative to methodology reviewed last year. The method was successfully applied to the synthesis of  $\text{CCK}_4$  analogues,  $\text{Boc-Trp-Leu}\psi[(\text{E})\text{CH}=\text{CH}]\text{D-Asp-PheNH}_2$ , and  $\text{Boc-Trp-Leu}\psi[(\text{E})\text{CH}=\text{CH}]\text{L-Asp-PheNH}_2$ . Analogues of deamino-oxytocin (represented by (3)), wherein the Leu-Gly bond has been replaced by a tetrazole or by a *trans* double bond have been synthesised and evaluated<sup>21</sup>. The  $\psi[(\text{E})\text{CH}=\text{CH}]$

isostere was found to be the most appropriate substitution (uterotonic activity 24% of deamino-oxytocin) with a high *in vitro* and *in vivo* activity ratio.

2.6  $\psi$ [NHCONH] - Ureylene Analogues. A full paper<sup>22</sup> has now been published on the synthesis and biological properties of ureylene analogues of Leu-enkephalin.

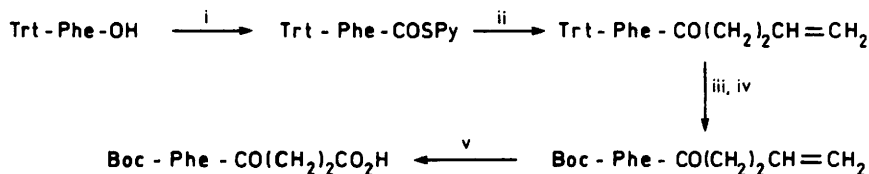
2.7  $\psi$ [COCH<sub>2</sub>] - Ketomethylene Surrogates. This amide bond replacement maintains the H-bond acceptor capacity of the CO group and increases the degrees of freedom. Diastereoisomeric isosteres, represented by Boc(RS)Phe $\psi$ -[COCH<sub>2</sub>](E,Z) $\Delta$ Phe-OH, Boc(RS)Phe[COCH<sub>2</sub>](RS)PheOH and BocGly $\psi$ [COCH<sub>2</sub>](RS)Leu-OH, have been synthesised<sup>23</sup> *via* the route summarised in Scheme 2. Alkylation of malonate esters with appropriately protected amino acid chloro (or iodo) methyl ketones (e.g. ZNHCH(R)COCH<sub>2</sub>Cl) have yielded<sup>24</sup> a diverse series of analogues, exemplified by Z-Phe $\psi$ [COCH<sub>2</sub>](RS)Ala-OH, Z-Ala $\psi$ [COCH<sub>2</sub>](RS)-X-OH (with X = Ala, Asp, Phe-Trp) and H-Trp $\psi$ [COCH<sub>2</sub>]Lys-OH. Nonapeptide analogues (4) and (5) of snake venom ACE inhibitors have also included<sup>25</sup>  $\psi$ (COCH<sub>2</sub>) isosteres, incorporated into a solid phase synthesis protocol using BocPhe $\psi$ (COCH<sub>2</sub>)GlyOBu<sup>t</sup>. The acid precursor of this was synthesised according to Scheme 3. A detailed investigation of the Met<sup>28</sup>-Gly<sup>29</sup> region of the C-terminal heptapeptide of cholecystokinin has involved not only a  $\psi$ [COCH<sub>2</sub>] group at this position but also  $\psi$ [CONMe],  $\psi$ [NHCO] and  $\psi$ [CH<sub>2</sub>CH<sub>2</sub>] insertions as well<sup>26</sup>. Detailed 2D n.m.r. data have been included for each analogue but biological results have been published elsewhere. The key step in the incorporation of the  $\psi$ [COCH<sub>2</sub>] was *via* the synthesis of Z-Nle $\psi$ [COCH<sub>2</sub>]Gly-OH by reaction of BrMgCH<sub>2</sub>CH<sub>2</sub>CH[O-(CH<sub>2</sub>)<sub>3</sub>-O] with Z-Nle-NMeOMe, followed by oxidation with CrO<sub>3</sub>/30% H<sub>2</sub>SO<sub>4</sub>. The retro-inverso unit was introduced *via* well tried methods while the  $\psi$ [CH<sub>2</sub>CH<sub>2</sub>] was inserted using Wakamiya's method involving coupling of Boc- $\beta$ -homo-Nle-CHO and Ph<sub>3</sub>P=CHCO<sub>2</sub>Me followed by hydrogenation.

2.8 Phosphono-Peptides. Synthesis of phosphonodipeptides of general formula Z-NHCH(R)P(O)(OH)OCH(R<sup>1</sup>)CO<sub>2</sub>CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>NO<sub>2</sub>(p) where R = H, R<sup>1</sup> = H, CHMe<sub>2</sub>, CH<sub>2</sub>CHMe<sub>2</sub> or R = Ph, CHMe<sub>2</sub>, R<sup>1</sup> = H has been achieved<sup>27</sup> by esterification of ZNHCH(R)P(O)(OH)<sub>2</sub> with HOCHR<sup>1</sup>CO<sub>2</sub>CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>NO<sub>2</sub>(P) in the presence of SOCl<sub>2</sub> in DMF. A two-step procedure<sup>28</sup> converts H<sub>2</sub>NCH(R)-P(O)(OH)<sub>2</sub> (R = H, Me, CHMe<sub>2</sub> or Ph) in the presence of HC(OEt)<sub>3</sub> to OHCNHCH(R)P(O)(OEt)<sub>2</sub> which on deformylation can function as a C-terminal residue in phosphonopeptide synthesis. <sup>31</sup>P N.m.r. studies on N $\alpha$ -(phenethylphosphono)-L-Ala-L-Pro-OH, a potent inhibitor of ACE and related compounds, have shown<sup>29</sup> that the two exchanging <sup>31</sup>P resonances in the spectra were due to the *s-cis/s-trans* conformational equilibrium about the alanyl-prolyl peptide bond.



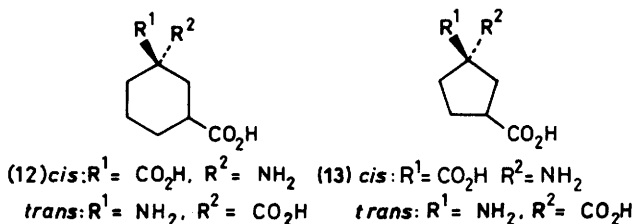
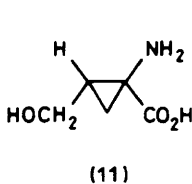
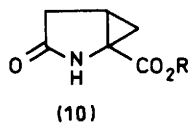
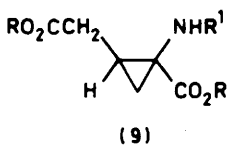
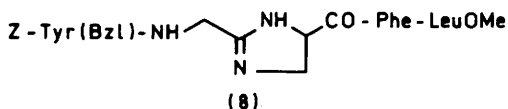
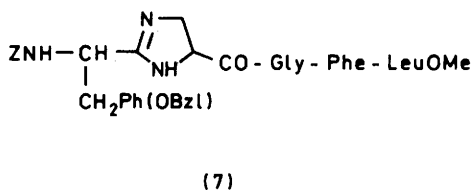
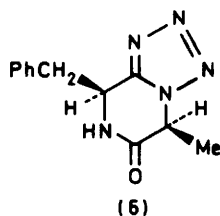
(4) X = Lys

(5) X = Nle



i, DCCl/PySH; ii,  $\text{BrMg(CH}_2)_2\text{CH=CH}_2$ ; iii,  $(\text{CO}_2\text{H})_2$ ; iv,  $(\text{Boc})_2\text{O}$ ; v,  $\text{RuO}_4$

Scheme 3



2.9  $\psi$ [CH<sub>2</sub>S] - Thiomethylene Analogues. Two well established cyclic enkephalins, the  $\mu$ -receptor selective H-Tyr-*cyclo*-[D-Lys-Gly-Phe-Leu] and the  $\delta$ -receptor selective Tyr-D-Pen-Gly-Phe-D-Pen-OH have been modified<sup>30</sup> to include  $\psi$ [CH<sub>2</sub>S] surrogates. Insertion of Boc-Gly $\psi$ [CH<sub>2</sub>S]Phe-OH onto a growing resin-bound peptide gave rise to Tyr-*cyclo*-[D-Lys-Gly $\psi$ [CH<sub>2</sub>S]Phe-Leu] and Tyr-D-Pen-Gly $\psi$ [CH<sub>2</sub>S]Phe-D-Pen-OH. The intermediate pseudodipeptide was prepared either as previously published or the surrogate group was introduced on the resin by condensing a (R)-BrCH(CH<sub>2</sub>Ph)-CO- residue with Boc-NHCH<sub>2</sub>CH<sub>2</sub>SH. Biological activity results suggested that the surrogate produced a drop in potency. The synthesis of *cyclo*-[Gly $\psi$ [CH<sub>2</sub>S]Phe-]<sub>3</sub> has also been described as part of this work. N-Allyl and N,N-diallyl derivatives of H-Tyr-D-Ala-Phe $\psi$ [CH<sub>2</sub>S]-GlyNH<sub>2</sub> and H-Tyr-D-Ala-Phe-Gly $\psi$ [CH<sub>2</sub>S]PheNH<sub>2</sub> have been investigated<sup>31</sup> as potential opioid antagonists. Results showed that whereas substitution by [CH<sub>2</sub>S] was relatively tolerated in g.p.i. and m.v.d. bioassays, N-allylation significantly reduced the relative agonist potency.

2.10 1,5-Disubstituted Tetrazole and Imidazoline Surrogates. The crystal structure of *cyclo*-[Phe $\psi$ [CN<sub>4</sub>]-L-Ala] (6) has shown<sup>32</sup> that the tetrazole ring is suitable for simulating a *cis* amide bond, and could be used as a probe for locking the conformation in that form in molecular recognition studies. Imidazoline residues have been incorporated<sup>33</sup> into the 1,2 pseudo-enkephalin (7) and as a 3,4 bond replacement (8). Key to the synthesis was the coupling of 2,3-diamino propanoic acid residues to imidate salts produced from amino acetonitrile hydrochloride or tetrafluoroborate.

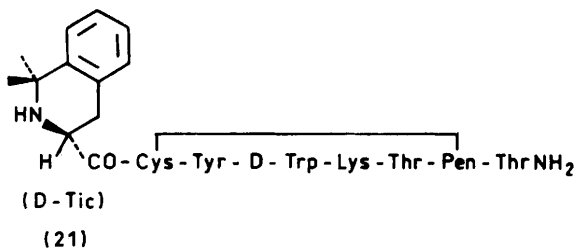
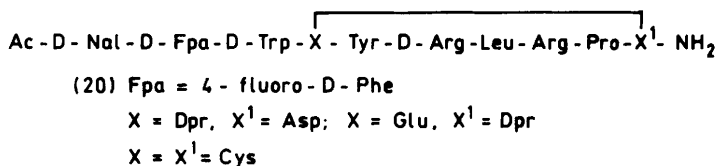
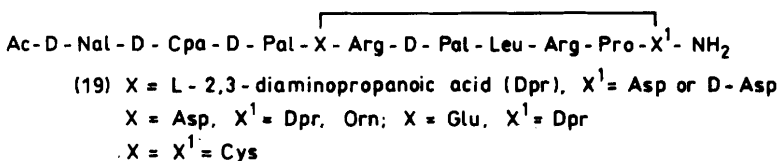
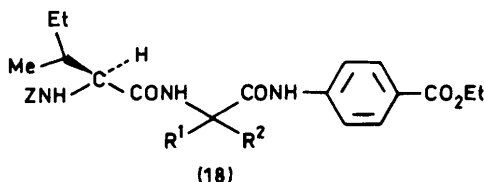
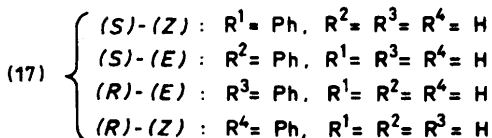
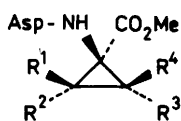
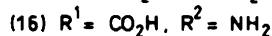
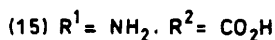
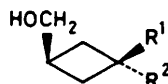
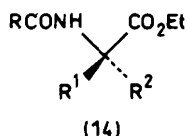
2.11  $\psi$ [COO]-Depsipeptides. To overcome the lack of useful OH-protecting groups for stepwise synthesis, the dichloroacetyl has been developed<sup>34</sup> in conjunction with N-carboxy- $\alpha$ -amino acid anhydrides to form depsipeptide links.

2.12 Replacement of L- by D-residues. In an *in vitro* bioassay Glp-Phe-D-Phe-Gly-D-Leu-MetNH<sub>2</sub>, D-Glp-Phe-D-Phe-Gly-Leu-MetNH<sub>2</sub>, and Glp-Phe-D-Phe-His-Leu-Met-NH<sub>2</sub> have been shown<sup>35</sup> to be antagonists of [Glp<sup>6</sup>] substance P (6-11). In a survey<sup>36</sup> on the effect of various D-substitutions at position 2 and/or ethionine at position 5 of the enkephalins, one of the analogues H-Tyr-D-Nva-Gly-Phe-EthNH<sub>2</sub> (Eth = ethionine) was shown to be 22 times more potent than morphine in the guinea pig ileum assay. Catalytic transfer hydrogenation caused reduction of tryptophan and racemisation of histidine residues during deprotection of LH-RH analogues<sup>37</sup> but a 42% yield of (D-Trp)<sup>6</sup>-LH-RH was possible using this technique.

2.13  $\alpha,\alpha$ -Di-Alkylated Glycine Analogues. Activity in this area has been increasing, highlighted by the synthesis of individual residues, and conformational

studies on both the units and their influence in oligopeptides. 2,3-Methano-analogues of glutamic (9) and pyroglutamic (10) acids have been prepared<sup>38</sup> from dehydro-amino acid precursors *via* dihydropyrazoles. Serendipitous formation of a bromomethylspiro-oxazolone intermediate has afforded<sup>39</sup> a simple method to 2,3-methanomethionine whose configuration was checked by comparison with (E)-2,3-methanohomoserine (11). Microbiological methods used for producing keto-cycloalkyl carboxylic esters represent key steps in the chemical synthesis<sup>40</sup> of all the glutamic acid analogues represented by cyclohexane analogues (12) and (13). Diastereoselective alkylation of lithioenamides of alkylated  $\beta$ -keto esters using L-Val-Bu<sup>t</sup> esters as a chiral auxiliary has provided<sup>41</sup> a range of  $\alpha,\alpha$ -dialkylated  $\beta$ -keto esters which can be converted to amino acid equivalents (14) *via* a Schmidt rearrangement. 2,4-Methanoglutamic acids (15) and (16) have been synthesised stereospecifically<sup>42</sup>, and the well-established bislactim ether methodology has been utilised<sup>43</sup> for the asymmetric synthesis of dipeptides such as, Boc-L-Val-(R) $\alpha$ MeProOMe, Boc-L-Val-(R) $\alpha$ MePheOMe and Ac-L-Val-(R) $\alpha$ MePheOMe. Four stereoisomeric cyclopropane analogues (17) of aspartame have been synthesised<sup>44</sup>, tasted and conformationally analysed. All four isomers were found to be tasteless which was explained in terms of the rigid positioning of the phenyl group. The previously published azirine/oxazolone route has been used<sup>45</sup> for the synthesis of  $\alpha,\alpha$ -disubstituted model tripeptides (18). Application of n.m.r., X-ray, c.d. and energy calculations revealed the presence of a type III  $\beta$ -turn in the molecules but the relative stability of the  $\beta$ -turn was influenced by the nature of the substituents in R<sup>1</sup> and R<sup>2</sup> in (18).

A range of physical methods have been utilised in conformational work on  $\alpha,\alpha$ -dialkyl substituted molecules. X-Ray crystallographic data from Aib-(NH-CMe<sub>2</sub>CO-)-containing peptides reveal, that Boc-(Leu<sub>3</sub>-Aib)<sub>2</sub>OBzl folds<sup>46</sup> into a right-handed  $\alpha$ -helical conformation stabilised by 4 intramolecular (5 $\rightarrow$ 1) H-bonds, and that in the two crystalline forms<sup>47</sup> of Boc-Trp-Ile-Ala-Aib-Ile-Val-Aib-Leu-Aib-ProOMe, the conformation are essentially the same, but small changes in conformational angles are associated with a shift of the helix from predominantly  $\alpha$ - to a  $3_{10}$ -type. Studies<sup>48</sup> on Boc-(Aib-Val-Ala-Leu)<sub>2</sub>-AibOMe as a mimic for natural membrane active peptides showed that it is predominantly an  $\alpha$ -helix and aggregates with the helices parallel to each other in a mode similar to zervamicin IIA analogues. Activation of  $\alpha$ -ethylalanine by 2 equivalents of DCCI/HOBt over 4 days proved successful in the synthesis<sup>49</sup> of C-terminal tetrapeptides of emerimicin, Boc-(R)-EtA-Hyp(OBzl)-Ala-Phe-ol and Boc-(R)-EtA-Hyp(OBzl)-MeA-Pheol. (Note the authors' suggestion of a new convention for  $\alpha$ -substituted acids, i.e. Me or Et + one letter code e.g. MeA =  $\alpha$ -methylalanine, EtA =  $\alpha$ -ethylalanine.) X-Ray data revealed that both peptides assumed a  $3_{10}$ -helical conformation and that alkyl substituents larger than methyl can be accommodated in this conformational form. Similar conclusions have come from i.r. studies<sup>50</sup> on



host-guest peptides of the type Ac-Ala-Xxx-Ala-Ala-Xxx-Ala-Ala-Xxx-Ala-Ala-NH-PEGM where Xxx' can be Aib, (S)-2-ethylalanine or (S) $\alpha$ -methylserine. 2-Ethyl alanine exerts a comparable helix inducing effect to  $\alpha$ -methyl alanine (Aib) but the side-chain of  $\alpha$ -methylserine prevents significant helix promoting effects. A note has appeared<sup>51</sup> describing the absolute configuration of the cyclopropane phenylalanyl moiety in Z(+) $\nabla^E$ Phe-LeuOMe as 2R, 3S, which will assist in rationalising the lack of rat brain binding affinity of an enkephalin analogue containing this moiety. *Ab Initio* and empirical results have been reported<sup>52</sup> for cyclopropyl glycine and X-ray structures have become available for simple derivatives of 1-aminocyclopentane-1-carboxylic acid<sup>53</sup> and 1-aminocyclohexane-1-carboxylic acid (Acc<sup>6</sup>)<sup>54</sup>. Homopeptides of the latter have been subjected to i.r. and <sup>1</sup>H n.m.r.<sup>55</sup> and X-ray diffraction studies<sup>56</sup> and the results are in favour of the onset of an incipient <sub>3</sub>10-helix on reaching the tripeptide stage. The X-ray and computational studies<sup>57,58</sup> on homopeptides CF<sub>3</sub>CO(NHC(Et)<sub>2</sub>CO)<sub>n</sub>OR with n = 1-5 R = Me<sub>3</sub>C have provided data for comparison with  $\alpha,\alpha$ -dimethyl and  $\alpha$ -methyl  $\alpha$ -ethyl analogues. Up to the dipeptide stage the extended conformation is more stable when both the C $\beta$  atoms in the side-chain are substituted, while at the pentapeptide level  $\alpha,\alpha$ -diethylglycine peptides are more like the fully extended  $\alpha,\alpha$ -dipropylglycine peptides and contrast with the  $\alpha,\alpha$ -dimethylglycine analogues (<sub>3</sub>10-helical).

### 3. Conformationally Restricted Cyclic and Bridged Analogues

Distribution of reports in this area demands different subdivisions to what has been used over recent years, so in broad terms the type of conformational restrictor used has become the basis for the sub-themes.

3.1 Rings and Bridges formed via Amide Bonds. The BOP reagent benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate has been shown<sup>59</sup> to be an efficient side-chain lactamisation reagent on solid phase resins, and has been used<sup>60</sup> for the synthesis of a number of cyclic GRF analogues. The analogue *cyclo*-(Asp<sup>8</sup>-Lys<sup>12</sup>)-[Asp<sup>8</sup>-Ala<sup>15</sup>]GRF(1-29)NH<sub>2</sub> was found to retain significant biological activity, and when D-Ala was substituted for Ala<sup>2</sup> and modifications made at position 1, highly potent analogues active *in vivo* were obtained. Conformational analysis revealed a long  $\alpha$ -helical segment in these potent cyclic compounds which also included modifications such as D-Asp<sup>8</sup> and/or D-Lys<sup>12</sup> in the repertoire. The cyclic analogues of dynorphin, *cyclo*-(D-Orn<sup>2</sup>-Asp<sup>5</sup>)-dynorphin A(1-8), *cyclo*-(Orn<sup>5</sup>-Asp<sup>8</sup>)-dynorphin A(1-13), *cyclo*-(Orn<sup>5</sup>-Asp<sup>10</sup>)-dynorphin A(1-13) and *cyclo*-(Orn<sup>5</sup>-Asp<sup>13</sup>) dynorphin A(1-13) have been prepared<sup>61</sup> using solid phase techniques with side-chain lactamisation carried out

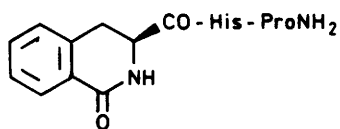


using DCCI/HOBt. Only the first listed analogue showed improved potency relative to its linear counterpart, with the latter three being 460-1350 times less potent than dynorphin A(1-13). It was concluded that cyclisation had given rise to conformations incompatible with the requirement of the  $\kappa$ -receptor. An azide coupling was used<sup>62</sup> to link the side-chains of Asp and Orn in the cyclic gonadotropin releasing hormone (GnRH) antagonist, Ac $\Delta^3$ -Pro-D-4CIPhe-D-Pal-Asp-Tyr-D-Trp-Leu-Arg-Pro-Orn-NH<sub>2</sub> (Pal = 3-(3-pyridyl)alanine). The *in vivo* antioviulatory assay in rats indicated a ED<sub>100</sub> lower than 50 $\mu$ g/rat. Solid phase techniques have been instrumental in the synthesis<sup>63</sup> of a number of GnRH analogues represented by structures (19) and (20). Most potent in the antioviulatory assay in rats was (19) (X = Asp or Glu, X<sup>1</sup> = Dpr) and (20) (X = Asp, X<sup>1</sup> = Dpr). Standard methods utilising pentafluorophenyl esters for coupling have been used in the synthesis of cyclic bradykinin analogues<sup>64</sup> such as H-Lys-Pro-Pro-Gly-Phe-Gly-Pro-Phe-Arg<sup>1</sup>, and cyclic analogues of substance P(5-11)<sup>65</sup>, H-Lys-Gln-Phe-Phe-Gly-Leu-Met<sup>1</sup>. The cyclic enkephalin analogue H-Tyr-*cyclo*-[D-A<sup>2</sup>Bu-Gly-Phe-D-Leu], synthesised by solid phase<sup>66</sup>, showed that though it is active it has little selectivity for the  $\mu$  or  $\delta$ -opioid receptors. Lactamisation across the 3,4 positions of cyclosporin was studied<sup>67</sup> as an aid to understand the interaction between this immunosuppressive *cyclo*-undecapeptide and its receptor. The weak activity of the lactam analogue suggests that the loss of activity is due to steric hindrance with the cyclosporin receptor. Cyclisation under acetic acid catalysis has yielded<sup>68</sup> *cyclo*[Trp(Nps)-Arg]HCl which is 10 times more effective than kyotorphin (Tyr-Arg) in a test for the naloxone reversible antinociceptive effect. The somatostatin analogue *cyclo*(-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba) has been used as a template to study<sup>69</sup> means of inhibiting proteolysis at the Lys<sup>9</sup>-Thr<sup>10</sup> of somatostatin. Stability of compounds containing substituents such as, [D-Lys<sup>9</sup>], [L-Trp<sup>8</sup>, D-Lys<sup>9</sup>], [L-Orn<sup>9</sup>] and [D-aThr<sup>10</sup>] support the premise of a primary initial cleavage site following Lys<sup>9</sup> in somatostatin, since all analogues showed enhancement of stability. The superpotent cytoprotective cyclic somatostatin *cyclo*-[D-Pro-Phe-Thr-Lys(Z)-Trp-Phe] has been the subject<sup>70</sup> of detailed 1D- and 2D-n.m.r. techniques refined by molecular dynamics which support a  $\beta$ I,  $\beta$ II-conformation for the compound.

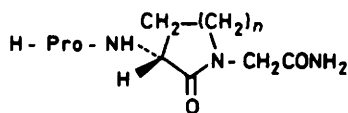
**3.2 Bridges formed by Disulfide Bonds.** Further fine tuning of the probes to study interaction between cyclic enkephalins and their receptors has been made<sup>71</sup> through the synthesis of [(3S)Me-D-Cys<sup>2</sup>, D-Pen<sup>5</sup>]- and [(3R)Me-D-Cys<sup>2</sup>, D-Pen<sup>5</sup>]-enkephalin. The biological activity results backed up by <sup>1</sup>H n.m.r. studies suggest that for [D-Pen<sup>2</sup>, D-Pen<sup>5</sup>]-enkephalin, adverse steric interactions between the D-Pen<sup>2</sup> pro-(R)methyl and the  $\mu$ -receptor binding site might account for the low binding affinity. Spectral properties of the highly selective  $\mu$ -opioid antagonist and somatostatin analogue, D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-ThrNH<sub>2</sub> have been

measured<sup>72</sup> and are consistent with a type II'  $\beta$ -turn for the sequence -Tyr<sup>3</sup>-D-Trp<sup>4</sup>-Lys<sup>5</sup>-Thr<sup>6</sup>-. F.T. <sup>13</sup>C-Relaxation studies confirmed a constrained ring system which led to the design of (21) which has a methylene bridge inserted between the  $\alpha$ -amino and the 2' position of the aromatic ring of D-Phe. This analogue was >9000-fold more  $\mu$ -selective than  $\delta$ -selective with very little somatostatin activity. Disconnection of the methylene bridge by replacement of D-Tic by D-MePhe gave a compound with low affinity for the  $\mu$ -opioid receptor.

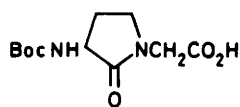
**3.3 Miscellaneous Bridges and  $\beta$ -turn Mimetics.** Stronger CNS action was found in the TRH analogue (22) where pyroglutamic acid was replaced<sup>73</sup> by (3S)-1-oxo-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, but the compound also had 3-4 times the potency of TRH in its TSH releasing activity. Conformationally constrained analogues have been achieved by bridging the peptide chain with methylene groups as, e.g., in the analogues of Pro-Leu-Gly-NH<sub>2</sub> represented by (23). In tests to assess their effect on the binding of the dopamine receptor agonist 2-amino-6,7-dihydroxy 1,2,3,4-tetrahydronaphthalene, only the analogue (23)  $n = 1$  showed significant activity being 10,000 times more active than Pro-Leu-GlyNH<sub>2</sub>. The same bridging principle has been used in the pyrrolidone (24) as a  $\beta$ -bend mimetic<sup>75</sup> for incorporation into the C-terminal gastrin peptide, and the dipeptide isosteres<sup>76</sup> (25) which are capable of use in solution phase synthetic protocols. As part of a study on rigid  $\beta$ -turn analogues, the macrocyclic dilactam (26) has been synthesised<sup>77</sup> but poor solubility and tendency for gel formation have limited its use as a tetrapeptide replacement. The  $\beta$ -turn mimetic (27) has been synthesised<sup>78</sup> and in a model study it has been shown that glycine ester can link to the moiety *via* addition to the N-benzyl amide group (top of molecule). The role of lysine and ornithine side-chains as  $\beta$ -turn stabilisers has been investigated<sup>79</sup>. In Boc-Gly-Lys-Asp-Gly-OMe, the Lys and Asp side-chains interact and contribute to  $\beta$ -turn stabilisation, but ornithinyl side-chains interact with the peptide backbone causing less stabilisation. Other model systems devised for the study of  $\beta$ -turns have been the serine-containing<sup>80</sup> (28) and proline-containing<sup>81</sup> models *cyclo*-[NH(CH<sub>2</sub>)<sub>n</sub>CO-Gly-Pro-X-Gly] where  $n = 2, 4$  and X = Gly or SerBut<sup>+</sup>. In the former series <sup>1</sup>H n.m.r. and FT-i.r. evidence confirm the presence of a type II  $\beta$ -turn in the larger ring compound ( $n = 4$ ), but a lack of this structural pattern when  $n = 2$ . In the proline compounds both of the rings (i.e.,  $n = 2$  and 4) adopt a 1 $\leftarrow$ 4 H-bonded type II  $\beta$ -turn. Conformationally-restricted templates designed to enhance global secondary structure in polypeptides have been reported. The unit (29) ( $\alpha$ -TempOMe) when incorporated<sup>82</sup> into peptide conjugates  $\alpha$ -Temp-(L-Ala)<sub>n</sub>-OR ( $n = 1-4$ ) and  $\alpha$ -Temp-L-Ala-L-Phe-L-Lys(Boc)-L-Lys(Boc)NHMe supports a distorted  $\alpha$ -helix with a bifurcated H-bond, while a  $\beta$ -turn  $\beta$ -sheet structure was confirmed<sup>83</sup> in an n.m.r. study of (30).



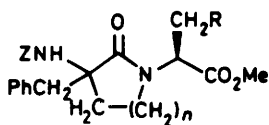
(22)



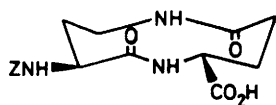
(23)  $n = 1$  or  $2$



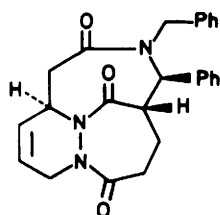
(24)



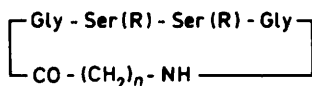
(25)  $n = 1$  or  $2$



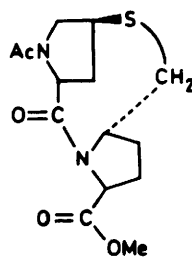
(26)



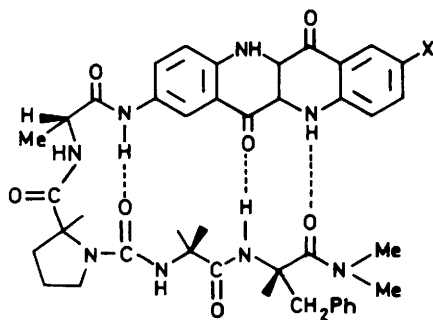
(27)



(28)  $n = 2$  or  $4$ ,  $R = \text{Bu}^t$



(29)

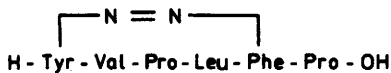


(30)

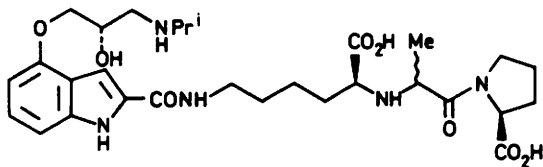
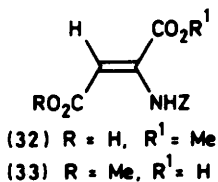
The hexapeptide fragment Tyr-Val-Pro-Leu-Phe-Pro has been shown previously to have the same immunoregulatory potency as native 'Proline Rich Polypeptide' PRP which has a m.w. of 6000. To prove that the location of the aromatic rings is conserved during interaction with its receptor an azo-bridge (previously reported in enkephalin studies) has been introduced<sup>84</sup>. Compound (31) had a higher biological activity than its open chain analogue [4'-aminoPhe<sup>5</sup>]-PRP-hexapeptide, thus suggesting that the active conformation benefits from having the Tyr and Phe rings close together.

#### 4. Dehydroamino Acid Analogues.

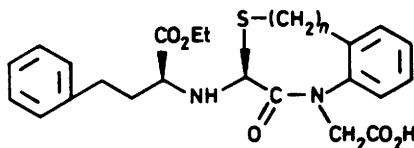
The expanding synthetic interest in these analogues and the existence of these residues in nature are reflected in a comprehensive review<sup>85</sup> of 249 references published between 1982 and 1987. The versatility of the N-carboxyanhydride method has been demonstrated by reports on the synthesis of these intermediates for a wider variety of amino-acids. A number of didehydroamino carboxyanhydrides can be prepared<sup>86</sup> by treating  $RR^1CHCH(N_3)CO_2H$  with phosgene in the presence of catalytic amounts of  $NaReO_4$  in dimethoxyethane, while a series of dehydrotyrosines were obtained<sup>87</sup> by the condensation of appropriately substituted benzaldehydes with  $(EtO)_2PO(CH)(NHZ)CO_2Me$  in a Wittig reaction and after hydrolysis converted to the carboxyanhydrides with  $SOCl_2$ . Condensation of  $HO_2CCH_2CH = C(NHZ)CO_2Bu^t$  with  $RNH_2$  using an N-hydroxysuccinimide ester for activation has given derivatives<sup>88</sup> of dehydroglutamine, e.g.,  $But-NHCOCH_2CH = C(NHZ)CO_2H$  which can be converted to carboxyanhydrides and condensation to dipeptides. Although the isomer (33) will form an N-carboxyanhydride of dehydroaspartic acid, isomer (32) forms an oxazine-2,4-dione which can still be used in the synthesis of dehydropeptides<sup>89</sup>. Base-catalysed  $\beta$ -elimination of  $\beta$ -mesyloxyaspartic acids and derivatives has yielded<sup>90</sup> (E)- and (Z)-isomers which can be identified by n.m.r. data. Synthesis of dehydroalanine and dehydrobutyrine have been developed<sup>91</sup> as an integral part of the total synthesis of the antibiotic nisin. Dehydration of threonine using the water soluble carbodiimide  $Me_2N(CH_2)_3N=C=NEt.HCl/CuCl$  gave dehydrobutyrine and it was found that the  $\Delta Ala$  residue was stable to both HF and TFA, making it possible to use benzyl or t-butyl protecting groups in the synthetic strategy. Synthesis of N-benzoyloxycarbonyl and N-trifluoroacetyl dipeptides of  $\alpha,\beta$ -dehydro-butyrine, -valine, -leucine and -isoleucine has been carried out<sup>92</sup> by condensing the appropriately protected amino acid amides with  $R^1R^2CHCOCO_2H$  in the presence of p-toluenesulphonic acid as catalyst. When the keto acid was (R,S)- $EtCH(Me)COCO_2H$  E/Z mixtures of dipeptides were obtained.  $\beta$ -Bromo- $\alpha$ -isocyanoacrylic acid derivatives have been shown<sup>93</sup> to be useful precursors for the



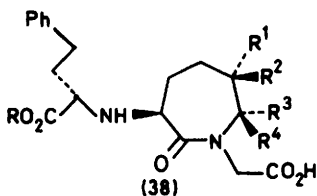
(31)



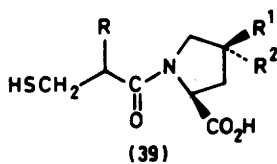
(36) A 575C. R



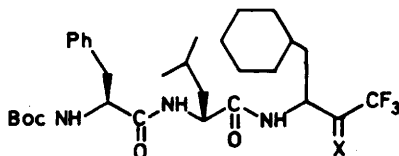
(37)  $n = 1 \text{ or } 2$



(38)

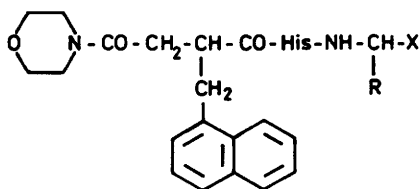


(39)



(40)  $\text{X} = \text{H}, \text{OH}$

(41)  $\text{X} = \text{O}$



(42) 2S, 4S

(43) 2S, 4R

(44) 2R, 4S

(45) 2R, 4R

$\left. \begin{array}{l} \text{(42) 2S, 4S} \\ \text{(43) 2S, 4R} \\ \text{(44) 2R, 4S} \\ \text{(45) 2R, 4R} \end{array} \right\} \begin{array}{l} \text{R} = \text{CH}_2\text{CH}(\text{CH}_3)\text{CF}_3, \\ \text{X} = \text{CO}_2\text{H} \end{array}$

(46)  $\text{R} = \text{CH}_2\text{CHMe}_2, \text{X} = \text{CH}(\text{OH})\text{CO}_2\text{CHMe}_2$

synthesis of  $\beta$ -substituted  $\alpha,\beta$ -unsaturated-cysteine, -serine and - $\alpha,\beta$ -diamino-propanoic acid derivatives. When dehydrotripeptides, incorporating a dehydro-residue in any one of the three positions were subjected<sup>94</sup> to h.p.l.c. a significant relationship was found between separation and conformational structure.

The crystal structure of Boc-L-Pro- $\Delta$ LeuOCH<sub>3</sub> has confirmed<sup>95</sup> a structure similar to a type II  $\beta$ -bend, while more complicated conformational equilibria have been detected<sup>96</sup> from the 270 MHz n.m.r. spectra of the tetrapeptides Boc-Leu-Ala- $\Delta$ ZPhe-LeuOMe (34) and Boc-Leu- $\Delta$ ZPhe-Ala-LeuOMe (35). In (34) a type II  $\beta$ -turn across -Ala- $\Delta$ ZPhe- is in equilibrium with a partially extended conformation, while in (35) an almost completely extended form is in equilibrium with a highly folded  $\beta$ -turn centred on -Leu- $\Delta$ ZPhe-Ala-. In an n.m.r. study<sup>97</sup> on the chemotactic peptide analogue HCO-Met-Leu- $\Delta$ ZPheOMe which shows high activity in stimulating superoxide production by rabbit neutrophils, the data favoured a significant population of extended backbone conformations. The effect of replacement of D-Phe by  $\Delta$ -Phe has been assessed<sup>98</sup> with the availability of [ $\Delta$ Phe<sup>4,4'</sup>]-gramicidin S, which showed strong antimicrobial activities against Gram positive bacteria but not against Gram negative ones. The dehydrophenylalanine residue seems to have reinforced the  $\beta$ -sheet backbone conformation necessary for full biological activity. (Phe<sup>5</sup>,  $\Delta$ Ala<sup>6</sup>)-LHRH and desGly<sup>10</sup>(Phe<sup>5</sup>,  $\Delta$ Ala<sup>6</sup>)-LHRH ethyl amides have been synthesised<sup>99</sup> but both were found to be inactive, suggesting that more flexibility at the 6-position is crucial for biological activity.

An achiral ligand Me<sub>2</sub>N(CH<sub>2</sub>)<sub>2</sub>CH(CH<sub>2</sub>PPh<sub>2</sub>)<sub>2</sub> has induced<sup>100</sup> high selectivity in Rh<sup>I</sup>-catalysed hydrogenation of acetyl and benzyloxycarbonyl dihydrodipeptides.

## 5. Enzyme Inhibitors

The vast majority of reports can again be categorised under two main headings but in general there appears to be a broader range of enzymes being investigated.

**5.1 Angiotensin Converting Enzyme (A.C.E.) Inhibitors.** As part of a programme to develop novel antihypotensive agents a compound (A575C) (36) has been synthesised<sup>101</sup> which shows both A.C.E. inhibition (IC<sub>50</sub> = 10.4 nM) and  $\beta$ -adrenoceptor blockade (pK<sub>B</sub> = 7.2). Benzothiazocine derivative (37) (n = 2) has shown<sup>102a</sup> a comparable A.C.E. inhibitory effect to the seven-membered analogue (37) (n = 1) reported last year. The analogue (37) (n = 2) without its sulfur atom, has been synthesised<sup>102b</sup> in chirally pure form by resolution using L(+)-tartaric salts. Perhydroazepin-2-one derivatives (38) have been synthesised<sup>103</sup> and showed potent inhibition of A.C.E. *in vitro*. The monoester

monoacid forms of (39) orally suppressed the pressor response to angiotensin I administered intravenously, with analogues having the phenyl group at position 6 showing a longer duration of action than the 7-substituted equivalent. A range of substituents at the 4-position of proline in (39) have been synthesised<sup>104</sup> and tested. Amongst the most potent in *in vitro* and *in vivo* assays were analogues of (39) with  $R = (RS)\text{-Me}$ ,  $R^1R^2 = \text{OCH}_2\text{CH}_2\text{O}$ ,  $\text{SCH}_2\text{CH}_2\text{S}$ ,  $\text{S}(\text{CH}_2)_3\text{S}$  and  $R = \text{H}$ ,  $R^1R^2 = \text{SCH}_2\text{CH}_2\text{S}$ , with enhanced activity compared with captopril. Analogues of captopril, enalaprilat, and [hydroxy(4-phenylbutyl)phosphinyl]acetyl-L-proline have been prepared and evaluated<sup>105</sup> in the context of the effect on activity of substituents at the 4-position of proline. In general, hydrophobic substituents were more potent *in vitro*. The S-benzoyl derivative of *cis*-4-(phenylthio)captopril, zofenopril, was one of the most potent compound of this class, and a prodrug of the *trans*-4-cyclohexyl derivative in the phosphinic acid series is being evaluated in a clinical trial. Success as a drug also demands an efficient large scale method of synthesis. For the A.C.E. inhibitors enalapril and lisinopril the dipeptides L-Ala-L-Pro and N $\epsilon$ -trifluoroacetyl-L-Lys-L-Pro have been prepared<sup>106</sup> on a large scale *via* N-carboxyanhydride chemistry. Reductive alkylation with ethyl-2-oxo-4-phenylbutyrate over Raney Nickel takes place with high stereoinduction to afford the direct precursors of enalapril maleate and lisinopril.

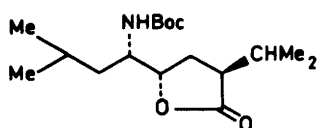
**5.2 Renin Inhibitors.** The design of potent substrate-based inhibitors for the aspartic acid protease renin is still an active field of endeavour. Fluorinated ketones by virtue of their exceptionally high electrophilicity and ease of hydration serve as ideal mimics of the tetrahedral transition state in enzymatic peptide bond cleavage. Coupling<sup>107</sup> of Boc-Phe-Leu-OH with  $\text{NH}_2\text{-CH}(\text{CH}_2\text{-Cyclohexyl})\text{-CH(OTBDMS)CF}_3$  has given rise to the renin inhibitors (40) and (41). Of the two, (41) proved to be a good inhibitor ( $\text{IC}_{50} = 250\text{nM}$ ), ten-fold more potent than the corresponding alcohol (40). Structure-activity relationships utilising a computer model of the active site of renin have been investigated<sup>108</sup> for a series of inhibitors with fluorine-containing leucine analogues (42)-(45). Analogues (42) ( $\text{IC}_{50} = 5.7 \times 10^{-7}\text{M}$ ) and (43) ( $\text{IC}_{50} = 2.6 \times 10^{-6}\text{M}$ ) were more active than (44) and (45) with the inhibition activity of the 4S configuration being 4.5-4.0 times that of 4R. In a related norstatine analogue<sup>109</sup> (46) oral administration to sodium-depleted common marmosets lowered the blood pressure significantly. In the same biological test inhibitor (47) lowered blood pressure for up to 2 hr and had an  $\text{IC}_{50}$  value of  $2 \times 10^{-9}\text{M}$  against human renin<sup>110</sup>. Out of 19 tetrapeptides synthesised<sup>111</sup> to increase aqueous solubility only the four listed under general formula (48) were potent inhibitors ( $\text{IC}_{50} = 10^{-9}\text{M}$ ) for porcine, canine and human plasma renins. Structural modification at the P<sub>3</sub> phenylalanine site has been carried out to evaluate the susceptibility of renin inhibitors towards chymotrypsin<sup>112</sup>. In the two analogues (49) and (50) selected for *in vivo* testing,



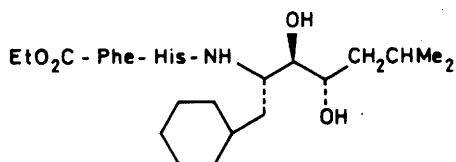


compound (50) showed no clear advantage over (49) although it was not cleaved by chymotrypsin. In a systematic series of replacements at the P<sub>5</sub>, P<sub>3</sub>, P<sub>2</sub>, P<sub>1</sub>, P<sub>1</sub>', P<sub>1</sub>', P<sub>3</sub>' and P<sub>4</sub>' positions of rat plasma renin substrate it was deduced<sup>113</sup> that modification at the C-terminal segment has a marked influence on potency and a secondary-butyl side-chain at the P<sub>2</sub>' position is important for optimal activity. *In vivo* the analogue Ac-His-Pro-Phe-Val-Sta-Leu-Phe-NH<sub>2</sub> (IC<sub>50</sub> = 30nM at pH 7.4) proved to be a potent hypotensive agent. Tripeptide derivatives such as (S)-Me<sub>2</sub>CHCH<sub>2</sub>CH(OH)CH<sub>2</sub>-Gly-Leu-Val-PheOMe have been synthesised<sup>114</sup> by reacting (S)-Me<sub>2</sub>CHCH<sub>2</sub>CH(OBzl)CH<sub>2</sub>SO<sub>3</sub>CF<sub>3</sub> with H-Gly-Leu-Val-PheOMe and deblocking the derivatives by hydrogenolysis. The tripeptide inhibited hog kidney renin with activities comparable to H-Leu-Gly-Leu-Val-PheOMe. A comparison of inhibitory activity between the two series of compounds (51) and (52) has revealed<sup>115</sup> that in general, analogues within the series (51) were more potent than series (52) and within each series of cyclohexylmethyl side-chains gave more potency than the benzyl analogues. Resonance-energy transfer and molecular modelling studies<sup>116</sup> on a previously described potent inhibitor of human renin (IC<sub>50</sub> = 3.0 × 10<sup>-9</sup>M), Ac-Ftr-Pro-Phe-His-Pheψ[CH<sub>2</sub>NH]Phe-Val-Tyr-NH<sub>2</sub> (U-70714E) (where Ftr = For-Trp) have confirmed an extended backbone secondary structure for U-70714E with an average intramolecular donor (Tyr)-acceptor (Ftr) distance of 16.3 Å in aqueous solution. Molecular modelling has also assisted in the design of three conformationally constrained cyclic renin inhibitors<sup>117</sup> with ring sizes varying from 10-14 as in (53). A reduced amide replaces the scissile bond at the active site, and the effect of the rings was to make the 14-membered ring compound as potent as its non-cyclic analogue for human renin, the 12-membered compound was 6-fold more potent against mouse renin, but the 10-membered ring analogue was inactive on both renins which has been explained by its conformation in solution being inconsistent with binding at the active site. The ring compounds showed resistance to cleavage by chymotrypsin. The peptide (54) with a γ-lactam conformational constraint and a hydroxyethylene isostere at the cleavage site inhibited<sup>118</sup> human renin with an IC<sub>50</sub> value of 6.5nM.

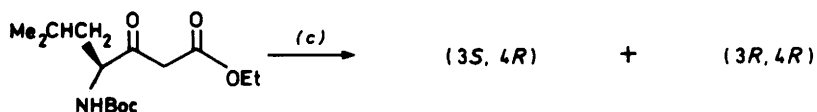
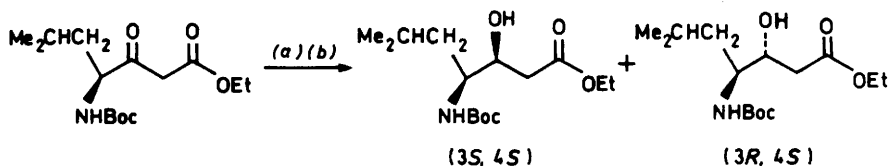
Amide isosteres have also become popular analogues in inhibitor studies as exemplified by the hydroxyethylene isostere<sup>119</sup> (55) for insertion in place of Leu-Val or the diol<sup>120</sup> containing inhibitor (56) which mimics the same bond. Molecular modelling has suggested that one of the hydroxyl groups should H-bond to Asp-32 and Asp-215 while the second bonds to Asp-215 in the renin. The diols show good selectivity for human renin over the related enzymes, cathepsin D, pepsin and gastricsin. When compared to their statine, Leuψ[CH<sub>2</sub>NH]Val and Phe-Phe analogues, the isosteres introduced<sup>121</sup> into H-Pro-His-Pro-Phe-His-Dhs-Ile-D-Lys-OH (Dhs = dehydrostatine), H-Pro-His-Pro-Phe-HisLeuψ[CH<sub>2</sub>S]Val-Ile-His-D-Lys-OH and the corresponding Leuψ[CH<sub>2</sub>SO]Val equivalent were not so successful. The last two examples were a 100-fold less potent than the ψ[CH<sub>2</sub>NH] counterpart,



(55)

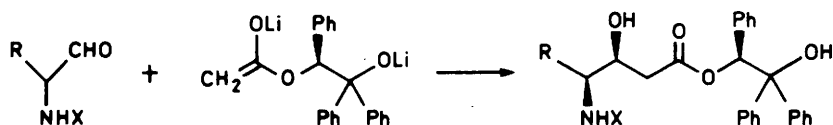


(56)

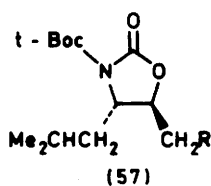


Method (a) H<sub>2</sub>(100 atm) RuBr<sub>2</sub>[*R*-BINAP] (3*S*, 4*S*)/(3*R*, 4*S*) > 99 : 1<sup>124</sup>  
 (b) NaBH<sub>4</sub>/K-selectride, -78 °C (3*S*, 4*S*)/(3*R*, 4*S*) = 90 : 10<sup>125</sup>  
 (c) i, LiBH<sub>4</sub> (3*S*, 4*R*)/(3*R*, 4*R*) = 63 : 37<sup>126</sup>  
 ii, NaBH<sub>4</sub> (3*S*, 4*R*)/(3*R*, 4*R*) = 75 : 25<sup>126</sup>  
 iii, KBH<sub>4</sub> (3*S*, 4*R*)/(3*R*, 4*R*) = 88 : 12<sup>126</sup>

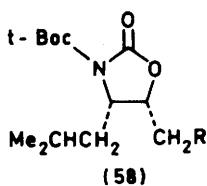
Scheme 4



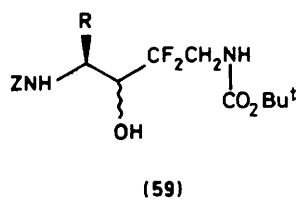
Scheme 5



(57)



(58)



(59)

but the dehydrostatine was equivalent to the Phe-Phe analogue but 100-fold less potent than its statine congener. When  $\psi[\text{CH}_2\text{O}]$  is inserted instead of the  $\psi[\text{CH}_2\text{S}]$  in the analogues above inhibition comparable to that of renin inhibitory peptide was obtained<sup>122</sup>, and when it is combined with a hydroxyethylene as in Boc-Phe-Pro $\psi[\text{CH}_2\text{O}]$ Phe-His-Leu $\psi[\text{CHOHCH}_2]$ Val-Ile-Amp (Amp = 2-aminomethylpyridine) the renin inhibition potency was  $\text{IC}_{50}$  of  $1.6 \times 10^{-9}\text{M}$ . Angiotensinogen based inhibitors of human renin modified by either Phe-Phe, Sta, Leu $\psi[\text{CH}_2\text{NH}]$ Val or Leu $\psi[\text{CHOHCH}_2]$ Val at the  $\text{P}_1\text{-P}_1'$  cleavage site gave the results<sup>123</sup> summarised in the Table.

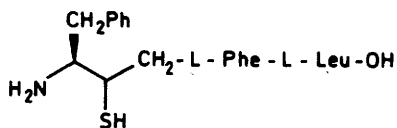
Table

| Analogue                                                       | Potency ( $\text{IC}_{50}$ )  |
|----------------------------------------------------------------|-------------------------------|
| Ac-Ftr-Pro-Phe-His-Phe-Val-FtrNH <sub>2</sub>                  | $5.0 \times 10^{-7}\text{M}$  |
| " " " " " Sta " "                                              | $3.8 \times 10^{-10}\text{M}$ |
| " " " " " Sta-NH <sub>2</sub>                                  | $3.8 \times 10^{-9}\text{M}$  |
| " " " " " Leu $\psi[\text{CHOHCH}_2]$ Val-NH <sub>2</sub>      | $3.1 \times 10^{-10}\text{M}$ |
| " " " " " Leu $\psi[\text{CH}_2\text{NH}]$ Val-NH <sub>2</sub> | $2.1 \times 10^{-8}\text{M}$  |

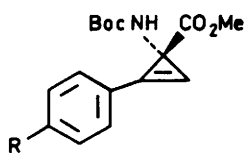
The great interest in statine as a  $\text{P}_1\text{-P}_1'$  cleavage site insert has spawned a number of methods for its synthesis. Scheme 4 summarises three syntheses<sup>124,126</sup> starting from  $\beta$ -keto esters; method (b) provides analogues of statine as well, while methods (c) have been used with *t*-butyl esters and isostatine precursors with improved selectivity. (3*S*,4*S*)-Statine has been prepared<sup>127</sup> via a nine-step synthesis from an isopropylidene glyceraldehyde starting material in 13% yield. A much shorter sequence of reactions summarised in Scheme 5 followed by transesterification, yields statine analogues in high optical purity<sup>128</sup>. Alternatively, a highly diastereoselective synthesis of (2*S*,3*R*)-BocNHCH(R)CH(OH)COCH<sub>2</sub>-CHMe<sub>2</sub> can be brought about<sup>129</sup> from a similar Boc-amino acid aldehyde by cyanohydrin formation followed by an iso-butyl-Grignard addition and hydrolysis. A highly stereocontrolled synthesis of the four stereoisomers of statine has been possible<sup>130</sup> via the formation of the cyclic intermediates (57) and (58). An  $\alpha,\beta$ -diamino- $\alpha,\alpha$ -difluoroketone isostere (59) related to statine has been described<sup>131</sup> as a potential serine protease inhibitor as a result of its synthesis from protected  $\alpha$ -amino acid aldehyde and BrCF<sub>2</sub>CO<sub>2</sub>Et over a 4-step synthetic route.

**5.3 Inhibitors of Other Enzymes.** The expected increase in effectiveness of thiol analogues in interacting with enzymic Zn has not been substantiated, as PhCH<sub>2</sub>CH(NH<sub>2</sub>)CH(R)X-Leu-OH (with X = O, R = SH) although found<sup>132</sup> to be an inhibitor of aminopeptidases B and M, and leucine aminopeptidase was only slightly better than the parent hydroxy analogues (X = O, R = OH). A similar result was found<sup>133</sup> in the 'thiobestatin' derivative (60). A naturally occurring

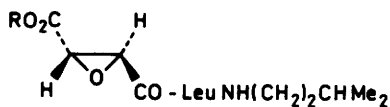
inhibitor of aminopeptidase B, OF 4949-III from the fungus *Penicillium rugulosum* has been synthesised<sup>134</sup>. The tripeptide D-Phe-Pro-Arg-NH<sub>2</sub> when treated<sup>135</sup> with phosphorus oxychloride or trifluoroacetic anhydride yields a C-terminal nitrile group which exhibits a strong inhibition of thrombin. The pseudotripeptide Ac-L-Lys(Ac)-D-NHCH(Me)-CH<sub>2</sub>-D-Ala-OH has been synthesised<sup>136</sup> as a potential D-Ala-D-Ala peptidase inhibitor, whereas inhibition of the *meso*-diaminopimelate adding enzyme involved in bacterial peptidoglycan biosynthesis has been recorded<sup>137</sup> for diastereoisomeric N $\alpha$ -propionyl dipeptides. Pr-L-Ala- $\xi$ -2-amino-4-phosphonobutyrate (probably in the L, D form) proved to be the best inhibitor. The bislactim method has been used in the synthesis of (61) which is of special interest<sup>138</sup> as a suicide inhibitor for pyridoxal phosphate-dependent enzymes. X-ray and n.m.r. data have been measured<sup>139</sup> for the epoxysuccinyl inhibitor loxistatin (62) and a <sup>13</sup>C n.m.r. study<sup>140</sup> was undertaken to determine the site of alkylation by the epoxide ring. The spectrum of the complex with papain displayed a new broad signal at 76.5 ppm establishing that the active site thiol group interacts with the C-3 carbon of (62). When X = Val in H-Phe-Gly-X-Sta-Ala-Phe-OMe the compound was found<sup>141</sup> to be a very potent inhibitor of pepsin ( $K_i < 1\text{nM}$ ), but when X = His the compound was two orders of magnitude less active ( $K_i = 150\text{nM}$ ). This has been explained in terms of the degree of protonation of the imidazole group of histidine under the conditions (pH 4). A positively charged side-chain at the P<sub>2</sub> is thought to be undesirable for maximum pepsin inhibition which differentiates it from renin inhibition. The octapeptide H-Thr-Pro-Pro-Thr-Pro-Ser-Pro-SerOH (the sequence 233-240 around the cleavage site of IgA1) has been shown<sup>142</sup> to be a competitive inhibitor of the two enzymes (proteinases Type 1 and Type 2) secreted by *Neisseria gonorrhoeae*. With Type 1 the peptide had IC<sub>50</sub> value of 0.25mM and 0.50mM with Type 2. Simultaneous blocking of the N- and C-terminal positions lowered activity to both enzyme types, while N-terminal acetylation only lowered affinity to Type 1 and C-terminal amidation improved binding to Type 2 but with no change to Type 1. Sequential replacement of the hydroxy-amino acid residues with cysteine units improved binding with Cys at position 6 (P<sub>3</sub>') being the most effective inhibitor. Compounds designed to be irreversible inhibitors of D-Ala-D-Ala ligase have been synthesised<sup>143</sup> and tested against Gram-positive and -negative bacteria. In the compounds represented by (63) the S, R configuration corresponded best to the D-Ala-D-Ala and compound (63) was shown to be the best antibacterial used in the study.  $\alpha$  and  $\gamma$ -Substituted phosphinothricins (64) can be synthesised<sup>144</sup> from conjugate addition of diethyl methylphosphinate to 2-cyclohexenone, and can be used as probes for studying the mechanism of glutamine synthetase inhibition by the naturally occurring inhibitor phosphinothricin. A report has appeared<sup>145</sup> of the first example of a fragment of an endogenous peptide exhibiting a higher potency than the parent compound. C-Terminal 15-residue fragments of neuropeptide Y



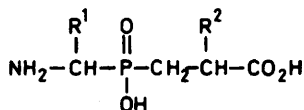
(60)



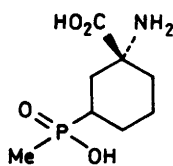
(61)



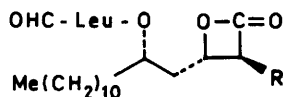
(62) R = H or Et



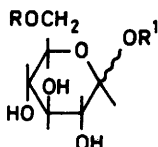
(63) R<sup>1</sup> = (S) - Me, R<sup>2</sup> = (R) - SMe



(64)

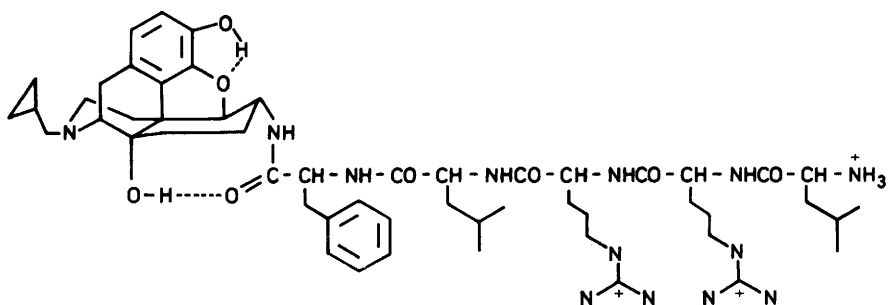


(65)



(66) R = H - Tyr - Gly - Gly - Phe - Leu -, R<sup>1</sup> = H

(67) R = H, R<sup>1</sup> = H - Tyr - Gly - Gly - Phe - Leu -



(68)

(69) H - Tyr - Gly - Gly - Phe - Leu - Arg - Arg - Ile - Arg - Pro - Lys - Leu - Lys - OH

(70) H - Tyr - Gly - Gly - Phe - Leu - Arg - Arg - Phe - Arg - Lys - Lys - Phe - Lys - OH

(71) H - Tyr - Gly - Gly - Phe - Leu - Arg - Arg - Phe - Arg - Pro - Lys - Phe - Lys - OH

were found to be up to ten times more potent than the full sequence in the inhibition of calmodulin-stimulated phosphodiesterase, a phenomenon related to the amphiphilic helical nature of the C-terminal half of the neuropeptide. The most active inhibitor against pancreatic and leukocyte elastase from a series of analogues synthesised<sup>146</sup> was  $\text{HO}_2\text{C}(\text{CH}_2)_3\text{COAla-Ala-Pro-NHPr}$ , and tetrahydrolipstatin (65) and an analogue, potent pancreatic lipase inhibitors, have been synthesised<sup>147</sup>.

## 6. Side-Chain Interactions studied by Residue Substitution or Deletion, and Similar Modifications

Topics under this category again tend to be rather random but sufficient order has been gleaned from some areas that it has been possible to categorise some reports under separate headings.

**6.1 Peptides with 'Opioid' Characteristics.** Selectivity in opioid receptor recognition is again a major theme in this area. Introduction of a t-butyl group on Thr<sup>6</sup> in H-Tyr-D-Thr-Gly-Phe-Leu-Thr-OH (DTLET) increased<sup>148</sup> selectivity for the  $\delta$ -opioid binding site while the same modification on Thr<sup>2</sup> gave complete loss of potency. A 400 MHz n.m.r. study indicated a loss of  $\beta$ -turn structure in the latter analogue probably induced by the steric hindrance of the bulky OBU<sup>t</sup> group in the 2-position which would also interfere with receptor binding. Yet in a similar study<sup>149</sup> in the hexapeptide series H-Tyr-X-Gly-Phe-Leu-Thr(OR)-OH it was the analogue with X = D-Ser(OBU<sup>t</sup>), R = H, or BU<sup>t</sup> which proved the most potent and selective  $\delta$ -probe to date. It can be deduced that the Thr(OBU<sup>t</sup>) has a greater steric influence than Ser(OBU<sup>t</sup>). Modifications at the 2 and 4-positions have been performed<sup>150</sup> on Tyr-D-Ala-Gly-NHCH(CH<sub>3</sub>)-CH<sub>2</sub>-CH(CH<sub>3</sub>)<sub>2</sub> a known  $\mu$ -selective peptide. Drastic loss of potency followed introduction of L-Ala and Aib at position 2, while substitution of D-Ser(OBU<sup>t</sup>) increased affinity for  $\delta$ -receptors. [D-Ala<sup>2</sup>, cysteamine<sup>5</sup>]-Enkephalin and its dimeric analogue (H-Tyr-D-Ala-Gly-Phe-NH(-CH<sub>2</sub>)<sub>2</sub>-S)<sub>2</sub> were synthesised to evaluate<sup>151</sup> interaction with opiate receptors. The latter was very potent in both  $\delta$  (11 times more potent than the monomer) and  $\mu$  assays (5 times more potent). It is suggested that the  $\mu$ -receptor might contain an essential thiol group, but no evidence of a metal ion site was found for the  $\delta$ -binding site. 3,5-Dimethoxy- $\alpha,\alpha$ -dimethylbenzyloxycarbonyl-[Trp<sup>4</sup>,Met<sup>5</sup>]-enkephalin has been synthesised<sup>152</sup> using solid phase techniques with polyethyleneglycol at C-terminal position.  $\beta$ -Turn conformational characteristics were elucidated by c.d. and i.r. for this analogue which had a high morphine-like activity in the rat. Both H-Tyr-D-His-Gly-Phe-Met-Arg-Arg-Val-NH<sub>2</sub> and H-Tyr-D-Arg-Gly-Phe-Leu-Arg-OH have been shown<sup>153</sup> to be more active than Met-enkephalin in g.p.i. bioassays. Substitution of DOPA instead of Tyr in position 1

of the des Met<sup>5</sup> derivatives H-X-D-Ala-Gly-R (with R = PhNH, PhNHNH, NO<sub>2</sub>C<sub>6</sub>H<sub>4</sub>NHNH, 2,4-diNO<sub>2</sub>C<sub>6</sub>H<sub>3</sub>NHNH) increased<sup>154</sup> biological activity *in vitro* and *in vivo*. Polar features incorporated<sup>155</sup> into the enkephalin analogues R-Tyr-X-Gly-Phe(4-NO<sub>2</sub>)-Pro-NH<sub>2</sub> with R = H, amidino; X = D-Met(Me<sup>+</sup>Cl<sup>-</sup>), D-Arg, D-Glu and into H-Tyr-D-NHCH(CH<sub>2</sub>R<sup>1</sup>)CO-Gly-Phe(4-NO<sub>2</sub>)-Pro-NH<sub>2</sub> in the form of R<sup>1</sup> = SO<sub>3</sub>Na, SSO<sub>3</sub>Na, SCH<sub>2</sub>CH<sub>2</sub>SO<sub>3</sub>Na gave analogues with predominantly peripherally mediated effects by antagonism of antinociception with the antagonist N-methyl nalorphine. Systematic insertion of one, two and three 6-amino-hexanoic acid residues (Ahx) into H-Tyr-Gly-Gly-Phe-Leu-(Ahx)<sub>n</sub>-Arg-OH gave some selectivity towards receptors<sup>156</sup>. When n = 3 the nonapeptide interacted strongly with δ- and moderately with μ-receptors, with n = 2 only moderate interaction with δ- and μ-receptors was seen, while shorter sequences showed low activity. Changes in the linking bonds between [Leu<sup>5</sup>]-enkephalin and C-terminal glucose have been investigated<sup>157</sup> by assay in the g.p.i. and m.v.d. tests. In general, derivatives of 6-0 conjugates (66) were less potent than 1-0 conjugates (67), but all were δ-receptor selective. [D-Met<sup>2</sup>, Pro<sup>5</sup>]-Enkephalin[N-1,5-β-D-glucopyranosyl]amide has given support<sup>158</sup> to the assumption that sugar molecules could improve affinity since it has an *in vitro* g.p.i. test potency of IC<sub>50</sub> = 64nM and its antinociceptive activity was 2000 and 200 times higher than morphine in rats and mice, respectively. A general method for insertion of isotopic labels into the S-methyl group of methionine has been reported<sup>159</sup> and involves treatment of a precursor homo-cysteine residue with Na/NH<sub>3</sub> and then treatment with the labelled CH<sub>3</sub>I, thus giving <sup>11</sup>C-, <sup>14</sup>C-, and <sup>3</sup>H-labelled S-methyl groups in Met-enkephalins and substance P analogues. Met-Enkephalin peptides have been detected in decalcified and depulped human teeth<sup>160</sup>.

Dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH<sub>2</sub>) labelled with tritium has been tested<sup>161</sup> for its relative affinity to μ- and δ-binding sites by determining its potency in displacing (<sup>3</sup>H)[D-Ala<sup>2</sup>, MePhe<sup>4</sup>, Gly<sup>5</sup>-ol]-enkephalin (DAGO - a selective μ-receptor ligand) and (<sup>3</sup>H)[D-Pen<sup>2</sup>, D-Pen<sup>5</sup>]-enkephalin (DPDPE a prototypical δ-receptor ligand). Dermorphin exhibited a selectivity ratio K<sub>i</sub>(DPDPE)/K<sub>i</sub>(DAGO) = 100 confirming its μ-selective qualities. Displacement of tritiated dermorphin by all alkaloid opiates or dermorphin-related peptides was monophasic consistent with competitive inhibition at a single binding site<sup>162</sup>. D-Ala<sup>2</sup> was found to be of crucial importance, while short analogues, e.g. N-terminal tri- and tetrapeptides were less potent. Pentapeptide dermorphin fragments H-Tyr-D-Ala-Phe-Gly-X-R with X = β-N<sup>7</sup>-uracilyl, β-N<sup>1</sup>-thyminyll or β-N<sup>9</sup>-adenyl-α-alanyl; R = NH<sub>4</sub>OH, produced by solution phase synthesis have shown<sup>163</sup> analgesic activity and bind to rat brain opiate receptors. However, desamino-Tyr<sup>1</sup> dermorphin analogues had negligible affinity<sup>164</sup>. Twenty one analogues based on H-Tyr-D-Arg-Phe-X (X = OH, alkyl ester, alkyl amide or another amino acid) have been tested<sup>165</sup> in mice. Alkyl amides with an OH group

on the alkyl moiety, or where  $X = \beta\text{-Ala}$  led to analogues more potent than morphine and confirmed the need for the presence of an 'O' atom in position 4.

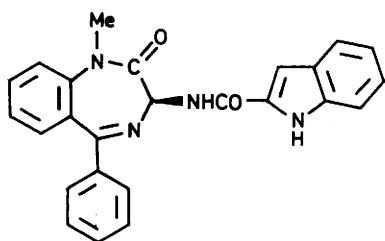
Opioid binding site affinities and selectivities available from the literature have been correlated with the estimated parameters of lipid membrane interaction<sup>166</sup>. The previously published report, that combination of  $\beta\text{-naltrexamine}$  with the address domain residues 4-8 of dynorphin A(1-8) in retro inverso form as in (68), produced a  $\kappa$ -selective analogue has been rationalised in terms of the 4-8 retro-inverso sequence endowing the opiate alkaloid 'message' with the necessary amphiphilic properties to meet the membrane requirements of  $\kappa$ -sites. Analogues of the dynorphin (1-13) peptide (69) have been synthesised by solution phase techniques<sup>167</sup>. With  $\text{Pro}^{10}$  replaced by  $\text{Lys}^{10}$  in (70), c.d. and i.r. studies confirmed a longer  $\alpha$ -helical structure for this compound when compared to (69) and (71) and the result of i.r. - a.t.r. studies with (70) on flat macroscopic 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphatidyl choline membranes confirmed the predicted structure. The strong affinity of (70) and (71) for opioid  $\kappa$ -receptors in guinea pig brain homogenates agreed with the postulated membrane structures. Analogue (70) also had a stronger affinity for  $\delta$ -receptors than (69) or (71).

**6.2 Cholecystokinin Analogues.** The demanding task of a total synthesis of human cholecystokinin (hCCK-33) has been achieved<sup>168</sup>. Critical to the protocol was the selective esterification of the  $\text{Tyr}^{27}$  phenolic group of unsulphated hCCK-33 with pyridine- $\text{SO}_3$  complex after the reversible masking of seryl side-chains by the  $\text{Bu}^t\text{Ph}_2\text{Si}$  group which can be selectively removed by fluoride ion. The binding properties of a series of CCK(27-33) analogues,  $\text{Boc-Tyr}(\text{SO}_3\text{Na})\text{-Nle-Gly-Trp-X-Asp}(\text{Na})\text{-PheNH}_2$  with  $X = \text{Phe, Ala, Orn(Z), Orn, or Glu(Na)}$  have been determined<sup>169</sup> for both mouse brain membranes and guinea pig pancreatic acini. Introduction of Phe, Ala or Orn led to compounds still displaying peripheral agonist properties, and the two former insertions displayed increased specificity for central receptors. When  $X = \text{Orn(Z)}$  the compound proved to be a new peripheral CCK antagonist. In  $\text{N}^\alpha$ -hydroxysulfonyl- $[\text{Nle}^{28,31}\text{-CCK}(26\text{-}33)]$  analogues systematically substituted at the C-terminal position with L-Leu, D-Phe or NMe-L-Phe, it is important for full agonist activity to have L-stereochemistry and an aromatic side-chain present at position-33<sup>170</sup>. Replacement of the C-terminal Phe in CCK(26-33) and CCK(27-33) with 2-phenylethyl alcohol, 2-phenylethylamine, Trp or D-Trp has given<sup>171</sup> derivatives with only a part of the CCK activity on amylase activity, with the D-Trp analogue behaving as a CCK-antagonist. Small C-terminal CCK fragments,  $\text{PhCO-D-Glu(PheNH}_2\text{)NPr}_2$ ,  $\text{PhCO-D-Glu(Asp-Phe-NH}_2\text{)NPr}_2$  have been shown to inhibit<sup>172</sup> gastric secretion, while the larger analogues  $\text{PhCO-D-Glu(Met-Asp-Phe-NH}_2\text{)NPr}_2$  and  $\text{PhCO-D-Glu(Trp-Met-Asp-Phe-NH}_2\text{)NPr}_2$  stimulated secretion. Leading on from previous

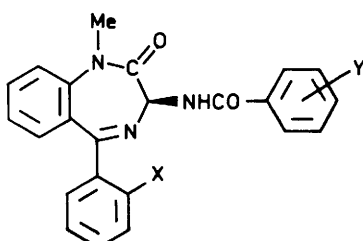
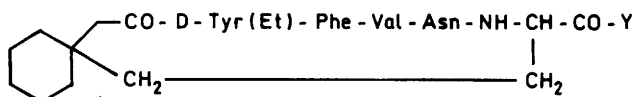
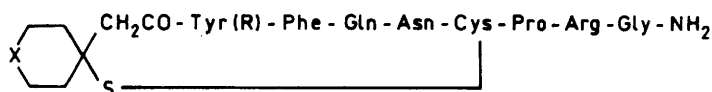


work on alkylbenzodiazepine antagonists of CCK, investigations<sup>173</sup> have provided more potent antagonists having structure such as (72). Compounds such as (73) have been finding use as pharmacological tools, with binding affinities approaching or equal to the natural CCK-8 ligand. N,O-Bis-Fmoc-D-Tyr-ONSu has been instrumental<sup>174</sup> in making D-Tyr-Gly-Asp-Tyr(SO<sub>3</sub>H)-Nle-Gly-Trp-Nle-Asp-Phe-NH<sub>2</sub> which could be radioiodinated to high specific radioactivity (2000 Ci/mmol) and bound with high affinity to the pancreatic CCK receptor.

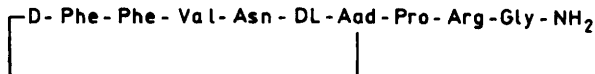
**6.3 Vasopressin Analogues.** Vasopression (V<sub>2</sub>) receptor antagonists introduced by Manning *et al* which usually require a  $\beta,\beta$ -alkylidene- $\beta$ -mercaptopropionic acid residue at position 1, have been modified<sup>175</sup> to include 2-methylene groups instead of the disulfide unit, in order to investigate metabolic stability and conformation. Each of the dicarba analogues in (74) were approximately equipotent with their sulfur analogues in antagonist activity, but had a reduced agonist activity. The 20-member carba ring can therefore represent an equivalent antagonist chromophore. Modifications at position 1 and 2 to create more lipophilic substitution on the  $\beta$ -carbon in position 1 have been built into<sup>176</sup> the analogues (75) with X = CHMe, CHCMe<sub>3</sub>, CHPh, NMe; R = H, Me. Some of the analogues, e.g., with X = CHMe, and with R = Me, are among the most selective and potent antagonist of the vasopressor response reported to date. Molecular modelling studies suggested that the conformational effects of the  $\beta$ -methyl- $\beta,\beta$ -pentamethylenepropionic acid<sup>1</sup> - Cys<sup>6</sup> cyclohexapeptide ring could be mimicked by a lactam bridge, so compound (76) has been synthesised<sup>177</sup> and the two diastereoisomers formed separated by h.p.l.c. Chiral gas chromatography and c.d. techniques were used to define chirality, but both peptides showed poor V<sub>2</sub>-receptor affinity *in vitro*, and an inability to inhibit or stimulate vasopressin induced adenylate cyclase formation. The question has been asked as to whether a cyclic structure is needed for V<sub>2</sub> (antidiuretic) and V<sub>1</sub> (vasopressor) vasopressin antagonists<sup>178</sup>. Taking Arg-vasopressin d(CH<sub>2</sub>)<sub>5</sub>[D-TyrEt<sup>2</sup>, Val<sup>4</sup>]AVP (77) as the standard, a series of 16 linear analogues related to (77) have been synthesised. By just opening up the S-S bond in (77) the corresponding linear analogue had 20% of the V<sub>2</sub>/V<sub>1</sub> antagonistic potencies of (77). With  $\alpha$ -L-aminobutyric acid (Abu) in position 6 and substitutions at position 1, including 1-adamantylacetic acid (Aaa), t-butylacetic acid, isovaleric acid, most of the linear peptides were as potent as (77) as V<sub>2</sub> antagonists. The linear analogue Aaa-D-Tyr(Et)-Phe-Val-Asn-Abu-Pro-Arg-Arg-NH<sub>2</sub> was as potent as any cyclic AVP V<sub>2</sub> antagonist to date. Extension of the Arg-vasopressin sequence by Ala, Ser-Ala, and Thr-Ser-Ala at the C-terminus has improved potency and duration of action<sup>179</sup>. Two analogues, [Gly<sup>7</sup>]- and [1- $\beta$ -mercaptopropionic acid, Gly<sup>7</sup>] Arg-vasopressin have raised the antidiuretic/vasopressor selectivity to 800-10,000 times higher than AVP<sup>180</sup>.



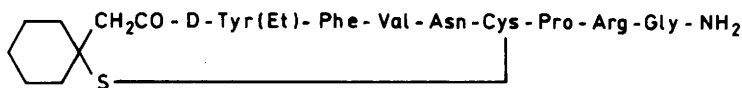
(72)

(73) X = H, Y = *p*-Br(74) Y = Pro-Arg-NH<sub>2</sub>, Arg-D-ArgNH<sub>2</sub>, or Arg-NH<sub>2</sub>

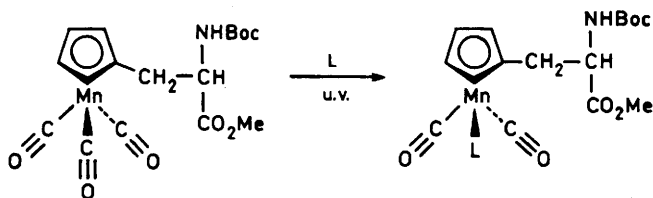
(75)



(76) Aad = amino-adipic acid



(77)



(78)

L = PMe<sub>3</sub>, PPh<sub>3</sub>, or P(C<sub>10</sub>H<sub>7</sub>)<sub>3</sub>

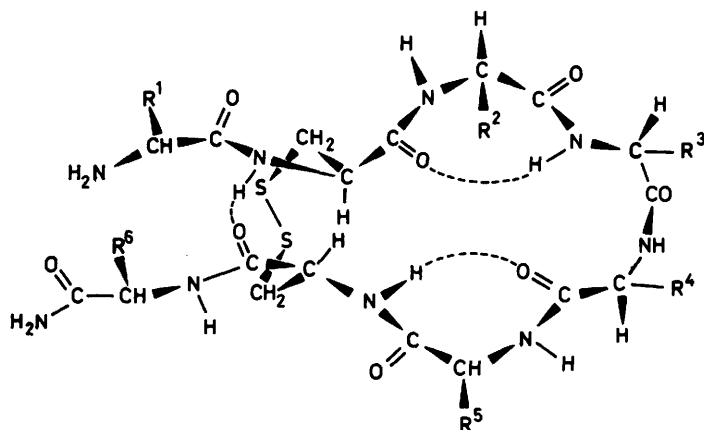
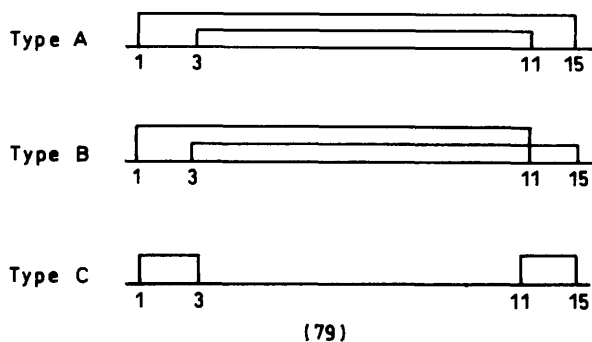
Scheme 6

**6.4 Miscellaneous Examples.** Substitution of D-amino acids into angiotensin II (AII) has been assessed<sup>181</sup> for antagonist activity. Thus D-substitutions into the interior 2-7 positions of [Sar<sup>1</sup>,Ile<sup>8</sup>]-AII gave rise to inactive compounds, while in position 8, [Sar<sup>1</sup>,D-Phg<sup>8</sup>]-, [Sar<sup>1</sup>,D-Peg<sup>8</sup>]- and [Sar<sup>1</sup>,D-Phe<sup>8</sup>]-AII analogues were considerably more potent *in vivo* than their *in vitro* affinities would indicate, suggesting that there is increased resistance to enzymic degradation. Sensitivity of potency to alteration in positions 2(Arg) and 6(His) have been monitored<sup>182</sup> using [Sar<sup>1</sup>,Ile<sup>8</sup>]-AII analogues. For both [des-Asp<sup>1</sup>]AII and [des-Asp<sup>1</sup>,Ile<sup>8</sup>]AII analogues, D-MeAla in position 2 gave analogues more potent than the native peptides. <sup>15</sup>N-Enriched angiotensin analogues have been synthesised<sup>183</sup> by classical solution methods and subjected to <sup>15</sup>N-n.m.r. analysis.

Modifications to a previously described series of LHRH antagonists have been made<sup>184</sup> in order to alter the hydrophobicity of the N-terminal acetyl-tripeptide position. Replacement of D-Trp<sup>3</sup> by the less hydrophobic D-Pal<sup>3</sup> [i.e., 3-(3-pyridyl)alanine] had only marginal effects on antagonistic and receptor binding potencies. Antagonists such as [Ac-D-Nal(2)<sup>1</sup>, D-Phe(4Cl)<sup>2</sup>, D-Pal(3)<sup>3</sup>, D-Cit<sup>6</sup>, D-Ala<sup>10</sup>]-LHRH and [Ac-D-Nal(2)<sup>1</sup>, D-Phe(4Cl)<sup>2</sup>, D-Pal(3)<sup>3</sup>, D-Hci<sup>6</sup>, D-Ala<sup>10</sup>]-LHRH (Nal(2) = 3-(2-naphthyl)alanine, Cit = citrulline, Hci = homocitrulline) were free from toxic effects in rats, and when the acetyl group was replaced by methoxycarbonyl(Moc) or Boc, the potency was increased by 170-260% and persisted for more than 2 hr. N,N-Dialkyl-D-homoarginines, prepared from D-Lys using carbodiimide couplings have provided<sup>185</sup> analogues of angiotensin II with high potency and prolonged duration of action, with one of the most potent being [N-Ac-D-Nal(2)<sup>1</sup>-D-pCl-Phe<sup>2</sup>, D-Trp<sup>3</sup>, D-hArg(Et<sub>2</sub>)<sup>6</sup>, D-Ala<sup>10</sup>]-LHRH (detirelix) which had an ED<sub>50</sub> of 0.7 μg in the rat antioviulatory assay. Cymantrenylalanine (Cym) (78) has already been incorporated before into peptides but as shown in Scheme 6 it has been possible<sup>186</sup> to increase the hydrophobicity by including phosphorus derivatives, which have been substituted for Gly<sup>6</sup> in GnRH (Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-GlyNH<sub>2</sub>). Analogues of melanin concentrating hormone (MCH) H-Asp-Thr-Met-Arg-Cys-Met-Val-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Glu-ValOH in which the disulfide ring was contracted from the 5-14 link to disulfides between 7-14, 8-14, and 10-14, were generally inactive at 10<sup>-6</sup>M or lower concentration<sup>187</sup>. *In vitro* stimulation of pituitary LH and FSH has previously been found to be a characteristic of human follicular gonadotropin releasing peptide (hF-GRP). Shorter sequences of hF-GRP have now been tested<sup>188</sup> for the same activity with the consequence that hF-GRP (5-14) retained moderate activity while the activity of hF-GRP (10-14) was markedly lower, and this situation prevailed also in radioimmunoassays. Binding studies<sup>189</sup> have shown that the α-amino groups of ovine prolactin and human

growth hormone is not relevant for binding to lactogenic liver receptors. The growth hormone from pituitary gland of the blue fox has been characterised<sup>190</sup> and found to have only 8% of the activity of bovine somatotropin in the receptor binding assay and only 10-14% of its immunoreactivity. In order to rationalise much of the non-systematic substitutions carried out on oxytocin-like peptides over a number of years, a series of computational 'fractionations' according to the method of Free and Wilson have been carried out on analogues that inhibit oxytocin on isolated rat uterus *in vitro*. Conclusions drawn from the study<sup>191</sup> were; (i) the intact ring structure is needed for peptide-receptor interaction, (ii) carba analogues display better affinities than peptides with an S-S ring, and (iii) better additivity is achieved when peptides are divided into sub-groups with vasopressin-like and oxytocin-like features. No evidence was obtained in favour of a Mg-potentiating effect on receptor binding. Application of the mild oxidant thallium(III) trifluoroacetate for cleaving S-protecting groups and the spontaneous formation of cystine has been proven<sup>192</sup> for oxytocin, urotensin II and human calcitonin gene-related peptide. All the disulfide analogues [types A, B and C in (79)] have been synthesised<sup>193</sup> to check the structure of porcine endothelin, H-Cys-Ser-Cys-Ser-Ser-Leu-Met-Asp-Lys-Glu-Cys-Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile-Trp-OH and subjected to h.p.l.c. comparisons. Type A was found to be identical with natural endothelin. Random oxidation of fully reduced endothelin formed a mixture of Type A and B in the ratio 3:1 with almost none of C.

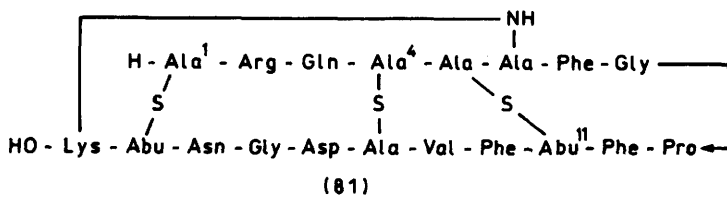
Analogues, [Glu<sup>21</sup>]-glucagon and [Lys<sup>17,18</sup>,Glu<sup>21</sup>]-glucagon were designed<sup>194</sup> to enhance the C-terminal amphipathic  $\alpha$ -helical conformation. A 3-fold and 5-fold increase, respectively, in receptor binding in these analogues relative to glucagon could be correlated with increased  $\alpha$ -helical content. Further substitutions in the 10-13 region of glucagon *via* the formation of {Phe<sup>13</sup>,Lys<sup>17,18</sup>,Glu<sup>21</sup>}- and [Phe<sup>10,13</sup>,Lys<sup>17,18</sup>,Glu<sup>21</sup>]-glucagon resulted in lower receptor binding. Initial studies<sup>195</sup> on the potentiation of glucose-induced insulin release by des His<sup>1</sup>-[Glu<sup>9</sup>]-glucagon amide in perfused isolated pancreatic islets revealed that the release of insulin had been increased by 30%. Ostrich insulin has been found<sup>196</sup> to have an identical sequence to the chicken hormone. Two monomers of ACTH (1-24) covalently linked *via* their C-termini using Lys-NH<sub>2</sub> as spacer, did not improve potency against ACTH (1-24) but strongly potentiated<sup>197</sup> the antagonistic effect of the fragments ACTH (11-24) and (7-24) in simulation of steroidogenesis in isolated adrenal cells. Four cyclotetradecapeptides related to gramicidin S *cyclo*-(Val-Orn-Leu-Leu-D-Phe-Pro-Leu)<sub>2</sub> have been synthesised<sup>198</sup> to investigate contribution of ring size and sequence around the Pro residue to antibiotic activity, but little correlation between their c.d. spectra and low activity could be found. Previously published analogues such as [D- $\alpha,\beta$ -diaminopropionic acid<sup>4,4'</sup>]gramicidin S *cyclo*-(Leu-Orn-Leu-Orn-Leu-D-Phe-Pro)<sub>2</sub> possessing



(80) R represents the side-chains of the residues:

$R^1$  : D-Phe or D-Nal;  $R^2$  : Phe or Tyr;  $R^3$  : D-Trp

$R^4$  : Lys;  $R^5$  : Val;  $R^6$  : Thr



increased basicity in the hydrophilic side of gramicidin S have been synthesised in greater quantities<sup>199</sup>.

Conformational conclusions, supported by data from force-field calculations, have been published<sup>200</sup> for a number of bioactive conformers of naturally occurring cyclic tetrapeptides such as HC-toxin, Cyl-1 and -2, chlamydocin, WF316 and [Gly<sup>4</sup>]HC-toxin. Solid phase methods have yielded<sup>201</sup> six retro-analogues of somatostatin with general formula *cyclo*(Xaa<sup>1</sup>-Phe<sup>2</sup>-Thr<sup>3</sup>-Lys<sup>4</sup>-Ybb<sup>5</sup>-Phe<sup>6</sup>) with Xaa = D- or L-Pro and Ybb = D- or L-Trp. The latest technology in 2D-n.m.r. analysis has been used to define the conformation of these compounds revealing an all-trans backbone conformation with a  $\beta$ -turn containing the Pro residue in the (i+1) position. High activity in the inhibition of cholate and phalloidin uptake by liver cells was exhibited but natural hormonal activities were suppressed. The most active analogue had Xaa = D-Pro and Ybb = Trp. This analogue has also been subjected<sup>202</sup> to modifications in the side-chains of Phe<sup>2</sup>, Lys<sup>4</sup> and Phe<sup>6</sup> in order to determine the influence of steric, lipophilic and charge effects on conformation and biological response. Little change was seen when these were altered, although the analogue with aromatic residues, e.g. BocTyr(Bzl) attached to Lys<sup>4</sup> had improved potency in the cytoprotective assay. It can be concluded<sup>203</sup> that the presence of the guanidyl moiety at position 1 in proctolin (H-Arg-Tyr-Leu-Pro-Thr-OH) is necessary for cardioexcitation of two insect species, since replacement of Arg by Lys, His, Phe(pNH<sub>2</sub>) or  $\gamma$ -Abu gave no activity. A convenient method to introduce N-alkyl amides at the C-terminal position has been developed<sup>204</sup> by attachment of peptide to the resin *via* an oxime link and then treatment of the peptide-oxime-resin with either ethylamine or cyclohexylamines to give the protected alkylamides. The sequence 34-43 of human epidermal growth factor was used as a test for the technique. Cyclohexylamides have been used<sup>205</sup> as C-terminal analogues of the molluscan neuropeptide H-Phe-Met-Arg-Phe-NH<sub>2</sub>. Replacement of Phe-NH<sub>2</sub> by D-Ala-cyclohexylamide showed only a relaxing effect on the anterior byssus retractor muscle while Met-Arg-Asp-dicyclohexylamide inhibited the induced contraction selectively. The N-terminal end has also been investigated<sup>206</sup> by the synthesis of analogues using water soluble active ester couplings. A hydrophobic or bulky group in position 1 contributed to the contractile effect, while the length of side-chain at position 2 contributed to a relaxing effect.

Biological evaluation of fragments of neuropeptide Y (NPY), a 36 amino-acid residue peptide amide known to be a potent vasoconstrictor has shown<sup>207</sup> that only the C-terminal 13-36 fragment retained some of the activities of NPY, but in another study<sup>208</sup> the unexpected finding of two fragments NPY (17-36) and (18-36) with substantial hypotensive action *in vivo* has been noted. The effect was slower in onset and of longer duration than for NPY itself, suggesting a different mechanism of action. The self-defence substance lepidopteran produced by the

silkworm *Bombyx mori* has been shown to be a 36-residue peptide containing a mixture of at least three congeners, and in structural activity studies<sup>209</sup> a minimal sequence required for biological activity has been defined as (1-18) together with the necessity to have an Arg<sup>1</sup>-Trp<sup>2</sup> sequence. In a series of analogue syntheses carried out by solid phase techniques<sup>210</sup> hexapeptides related to the C-terminal substance P sequence H-X-Phe-Phe-Gly-Leu-Met-NH<sub>2</sub> (with X = 2,4-diaminobutyric acid residue, Orn or Lys) showed contractile activity on guinea pig ileum. Physical methods applied to the flap region of human renin and recognition of antibodies raised against peptide fragments such as [77-93]-, [Ala<sup>80</sup>,Ala<sup>89</sup>][80-89]-, [Ala<sup>78</sup>Ala<sup>91</sup>][78-91]-, [Cys<sup>80</sup>,Cys<sup>89</sup>][80-89]- and [Cys<sup>78</sup>Cys<sup>91</sup>][78-91]-human renin have confirmed<sup>211</sup> that the flap seems to be a  $\beta$ -hairpin with a loop region of 4 residues leading to the CO group of Tyr<sup>83</sup> interacting with the NH's of Thr<sup>85</sup> and Gly<sup>86</sup>.

## 7. Conformational Information derived from Physical Methods

Advanced technology and techniques abound in a variety of conformational investigations on peptides. In this section the aim is to draw together a selection of papers which reflect the use of techniques which are contributing greatly to structure-activity studies but might also be of a fundamental nature which could in future benefit our understanding of peptide-receptor interactions. It is annually becoming more difficult to discuss the papers as if they solely represent one technique. The trend is for research groups to use a multi-technique approach deriving circumstantial evidence from a variety of methods. To give some substructure to the paragraphs, papers have been grouped under the main technique discussed in them.

**7.1 Nuclear Magnetic Resonance and Related Techniques.** Computational developments in n.m.r. have evolved at a brisk pace in recent years, so a background and overview of the 2D n.m.r. techniques, together with a discussion of the use of NOESY-TOCSY techniques for peptide sequences is timely<sup>212</sup>. The power of such techniques in molecular recognition studies have been well demonstrated<sup>213</sup> by the use of a truncated driven pseudo-NOESY experiment, to prove the folding-in of a hydrophobic side-chain to complete an anion binding pocket in the vancomycin-di-N-acetyl-L-Lys-D-Ala-D-Ala complex. <sup>15</sup>N-N.m.r. studies<sup>214</sup> has also showed the presence of H-bonding between Ac-D-Ala-D-Ala and vancomycin. 2D-N.m.r. investigations have detected<sup>215</sup> four distinct conformational species for gramicidin A in ethanol solution. Two of the species were left-handed parallel double-helices differing in relative arrangements of the polypeptide chains within the dimers. A third species was identified as a left-

handed antiparallel double-helix while a fourth was defined as being a right-handed parallel double-helix. A whole range of 1D,2D-techniques in  $^1\text{H}$  and  $^{13}\text{C}$ -n.m.r. have been used<sup>216</sup> to confirm the structure of the cyclic heptadepsipeptide didemnin A in DMSO solution.

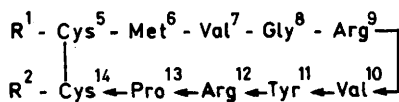
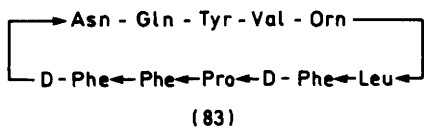
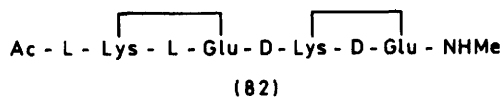
There have been several examples from the area of cyclic peptides which demonstrate the power of modern developments. The  $\delta$ -opioid enkephalin peptide H-Tyr-D-Pen-Gly-Phe-D-Pen-OH in aqueous solution has been shown<sup>217</sup> to exist primarily in two pairs of conformational forms, each pair differing by the sign of the disulfide chirality. One pair was of lower energy distinguished by an amphiphilic conformation with a type IV  $\beta$ -turn and transannular interactions between the aromatic side-chains of Tyr<sup>1</sup> and Phe<sup>4</sup> and the  $\beta,\beta$ -dimethyl groups of D-Pen<sup>2</sup>. The conformational influence of the latter residue on the disulfide ring system of opioid receptor selective somatostatin derivatives have been revealed<sup>218</sup> from proton n.m.r. Analogues of the superactive somatostatin mimetic D-Phe-Cys<sup>2</sup>-Phe<sup>3</sup>-D-Trp<sup>4</sup>-Lys<sup>5</sup>-Thr<sup>6</sup>-Cys<sup>7</sup>-Thr<sup>8</sup>-ol (SMS 201-995) have shown<sup>219</sup> different conformational characteristics in aqueous and DMSO solutions. In the series of compounds summarised under formula (80), their aqueous solution conformation contained  $\gamma$ -turns as depicted in (80). For all analogues in  $d_6\text{DMSO}$  there was a predominance of a type II'  $\beta$ -turn involving residues 3 to 6, and solvent effects predominated. In aqueous solution the molecules were more sensitive to conformational changes related to activity. In a conformationally-controlled structure-activity study<sup>220</sup> involving analogues of *cyclo*-(D-Pro-Phe-Thr-Lys(Z)-Trp-Phe) which is based on the retro-sequence 7-11 of somatostatin, each amino-acid has been substituted in turn by an L- or D-alanine residue and subjected to a 2D-n.m.r. determination. Backbone conformation in five analogues were identical to the parent *cyclo*-hexapeptide, and only in the case of replacement of Thr by Ala did a change occur in the backbone due to the removal of H-bonding characteristics of the threonine OH-group. To achieve high activity aromatic side-chains were proved to be necessary at the sites defined in the parent molecule. The structure of the polycyclic nonadecapeptide antibiotic (81) Ro 09-0198, has had to be solved<sup>221</sup> by n.m.r. due to the complication of having four side-chain cyclisations present in the molecule. An interesting separation of amino-acid residues into predominantly hydrophilic and hydrophobic types occurs within the molecule. Another 2D-n.m.r. study<sup>222</sup> on alamethicin has confirmed earlier reports (see last year's Report, Chapter 4) from solid state X-ray results and n.m.r., that the molecule is largely in a helical conformation. COSY and NOESY N.m.r. techniques used on 500MHz spectra<sup>223</sup> of [S-deoxo-Ile<sup>3</sup>-L-Ala]- and [S-deoxo-Ile<sup>3</sup>-D-Ala<sup>5</sup>]-amaninamide have shown that the rigidity of the bicyclic amatoxin framework can be preserved in the D- and L-Ala<sup>5</sup> analogues. The 10-fold decrease of biological activity has been explained by the methyl group of the L-Ala residue reducing accessibility to the ring for binding. A detailed



conformational analysis has now been carried out<sup>224</sup> on a series of lactam derivatives previously reported (see last year's Report, Chapter-3). C.d and n.m.r. measurements combined with molecular dynamics simulations and energy minimisation confirmed a  $\beta$ -turn conformation for compounds such as (82).

The enkephalins have served as model molecules for a number of conformational probes. In the so-called cryoprotective mixture (water/DMSO), Leu-enkephalin and its amide were investigated<sup>225</sup> over a temperature range of -30 to +54°C. Temperature coefficients of the amide protons were larger than those expected for H-bonded protons to detect several *intra*- and *inter*-residue n.O.e's, thus suggesting that there was no single folded conformation present although a short Gly<sup>2</sup>NH-Gly<sup>3</sup>NH distance implied support for a type I (or type I')  $\beta$ -turn. A 2D-reverse heteronuclear-correlated spectroscopic <sup>15</sup>N n.m.r. study<sup>226</sup> of Met-enkephalin at natural abundance has enabled deductions to be made on the basis of a nine hour accumulation of data. Chemical shifts, and both heteronuclear 1J(<sup>15</sup>N-<sup>1</sup>H) and homonuclear coupling constants between amide and  $\alpha$ -protons could be worked out. After synthesis of five <sup>17</sup>O-enriched Leu-enkephalins, each carrying the label at a different amide carbonyl oxygen and at the COOH group, the <sup>17</sup>O-n.m.r. spectra at 24.4 and 67.8MHz when measured<sup>227</sup> and analysed confirmed an intramolecular H-bond between the CO of Gly<sup>2</sup> and NH of Leu<sup>5</sup>. Solid state <sup>14</sup>N-n.m.r. of N-acetyl-L-Val-Leu-OH and H-L-Ala-Gly-Gly-OH without isotopic enrichment has given good agreement<sup>228</sup> with X-ray diffraction data for the same models. Sensitivity enhanced <sup>15</sup>N chemical exchange n.m.r. techniques applied to *cyclo*-(<sup>15</sup>N-Gly-D-Pro-Gly-<sup>15</sup>N-Ala-D-Pro) has shown<sup>229</sup> that the molecule undergoes slow conformational inversion at the Gly-Pro bond. <sup>1</sup>H and <sup>13</sup>C-N.m.r. spectra of glutathione in its free and La(III) complex forms were found<sup>230</sup> to be similar and in agreement with previous X-ray studies. Oligoglycyl peptides of known conformational forms in the solid state have been subjected<sup>231</sup> to <sup>13</sup>C-CP/MAS techniques to study the effect of H-bonds on the chemical shifts. <sup>13</sup>C-Chemical shifts of CO groups in a CO...NH type H-bond moved downfield with a decrease in the H-bond length, but those of CO...NH<sup>+</sup> type moved upfield. Deuterium n.m.r. spectroscopy has been used to study<sup>232</sup> the dynamics of the side-chain of methionine in polycrystalline L-[3-<sup>3</sup>-<sup>2</sup>H<sub>2</sub>]-methionine.

**7.2 X-Ray and Related Techniques.** Relating the pharmacophoric conformation of the enkephalins to that of the morphinoid molecules has been the subject of debate for a number of years. In an X-ray study<sup>233</sup> on a trihydrate crystal of Leu-enkephalin intramolecular orthogonal contact of the Tyr and Phe aromatic rings offers some resemblance to the morphine molecule. The conformation of sequences surrounding the asparagine residue involved in N-glycosylation of proteins has been the subject of model studies. Crystal structures<sup>234</sup> of two tripeptides, BocAsn(Me)-Ala-SerOMe and BocAsn(Me)-Pro-SerNHMe both showed



(84)  $\text{R}^1 = \text{H} - \text{Asp} - \text{Thr} - \text{Met} - \text{Arg} -$ ,  $\text{R}^2 = \text{HO} - \text{Val} \leftarrow \text{Gly} \leftarrow \text{Trp} -$

(85)  $\text{R}^1 = \text{H}$ ,  $\text{R}^2 = \text{OH}$

evidence of an Asx turn characterised by a H-bond between Ser-NH and AsnC $\alpha$ OY sites. The Pro-Ser sequence was  $\beta$ I-folded and the Ser hydroxyl group was H-bonded in both molecules. N-Formylmethionine peptides capable of stimulating cellular chemotaxis, have been subjected to comparable studies in the solid state and in solution. N-Formyl-L-Met-D-Phe-OH crystallised<sup>235</sup> in an extended  $\beta$ -sheet conformation with a *trans* planar amide bond with the methionine side-chain adopting the zig-zag *trans*-planar conformation. Model peptides of significance to studies on the substrate specificity of collagen polyhydroxylase, e.g., t-Boc-L-Pro-D-Ala-OH and t-Boc-L-Pro-D-Ala-L-Ala-OH, on X-ray analysis<sup>236</sup> showed the presence of an all *trans*  $\beta$ -turn conformation for the tripeptide while the urethane-proline bond in the dipeptide was in the *cis* form.

Cyclic peptides are a mainstream topic of Chapter 4 of this volume, so it is suffice in this context to report representative examples where X-ray crystallographic techniques have given useful information of interest to molecule designers. As part of a continuing programme in incorporating conformationally-restricted analogues of  $\beta$ -amino-acids into sequences the cyclotriptide *cyclo*-(N-methylanthaniloyl-L-Phe-L-Pro) has been studied<sup>237</sup> in an X-ray analysis. The 10-membered ring has a pseudosymmetric plane including three *cis*-amide bonds which is identical with the conformation in solution. A novel H-bonded conformation has been found<sup>238</sup> in the crystal structure of *cyclo*(Gly-L-Pro-D-Phe-L-Ala-L-Pro). It involves a  $\delta$ -turn formed between the amide proton of Gly and the CO group of Pro<sup>2</sup> as a consequence of the *cis* Gly-L-Pro bond. A type I  $\beta$ -turn with L-Ala<sup>4</sup> and L-Pro<sup>5</sup> in the corner position precedes the  $\delta$ -turn. A crystal structure of the disulfide bridge cyclic octapeptide *cyclo*-(Cys-Gly-Pro-Phe)<sub>2</sub> revealed<sup>239</sup> a Pro-Phe  $\beta$ -turn strongly resembling the backbone of  $\beta$ -amanitin and is roughly similar to previously reported *cyclo*-(Ala-Gly-Pro-Phe)<sub>2</sub> diastereoisomers. A crystal structure<sup>240</sup> of Boc-Cys-Val-Aib-Ala-Leu-Cys-NHMe has established for the first time the presence of an antiparallel  $\beta$ -sheet conformation for a peptide with a disulfide bridge. Uncomplexed valinomycin *cyclo*-(L-Val-D-Hyv-D-Val-L-Lac)<sub>3</sub> crystals grown from DMSO assume a quasi 3-fold symmetry<sup>241</sup> with three type II  $\beta$ -bends encompassing the three repeating L-Val-D-Hyv sequences. The conformation has a polar cavity which could attract and encapsulate a K<sup>+</sup> ion. Good correlation between the solution conformation from n.m.r. data and the crystal conformation from X-ray studies<sup>242</sup> has been found for the cyclohexadepsipeptide mycotoxin, destruxin B. Five *trans* and one *cis* peptide and ester units were found in the backbone.

**7.3 Circular Dichroism/Theoretical and Computational Methods.** Circular dichroism studies<sup>243</sup> have revealed that Leu-enkephalin analogues exist as rather random structures in aqueous solution in the pH ranges 2.5, 6.9 and 11.6, and in trifluoroethanol, whereas in  $\beta$ -casmorphin the Pro-Gly sequence results in a

Type-II  $\beta$ -turn conformation. Helical structures were shown to be preferred in the dynorphins.

The 3D-structure of tyrocidine A (83) has been 'modelled' *via* molecular mechanics, energy calculations and n.m.r.-derived distance and torsion angle constraints<sup>244</sup>. Two open-chain polyalanine models were used to represent separate halves of the backbone which were then scanned for suitable candidate structures. Side-chains were then added and the whole energy minimised to reveal a model with type II' and III  $\beta$ -turns at residues 4-5 and 9-10, respectively, and a backbone conformation closely resembling the crystal structure of gramicidin S. Molecular Dynamics analysis<sup>245</sup> and associated energy minimisations on the melanin concentrating hormone MCH (84) and its analogue MCH (5-14) (85) have revealed two major families of conformations supported by n.m.r. evidence. A strategy based on Scheraga's ECEPP programme has been evolved<sup>246</sup> for a study of cholecystokinin fragments CCK-5 (Gly-Trp-Met-Asp-Phe-NH<sub>2</sub>) which is biologically active and its analogues Gly-D-Trp-Met-Asp-Phe-NH<sub>2</sub> and Gly-Gly-Met-Asp-Phe-NH<sub>2</sub> which are not very active. One strongly preferred conformation has been revealed for the first two pentapeptides. Molecular mechanics calculations on *cis*, *trans*, *cis*, *trans* cyclotetraalanine and cyclotetraglycine has provided a means for predicting cyclic tetrapeptide conformations<sup>247</sup>. Correlation studies in biology and pharmacology and Q.S.A.R. will benefit greatly from information compiled<sup>248</sup> on 15 physicochemical descriptors of side-chains of 20 natural and 26 non-coded amino acids. It is shown that three principal components, one steric, one bulk and one electronic, account for 66% of the total variance in the available set of parameters. *Ab Initio* calculations<sup>249</sup> for N-acetyl-Ala-Gly-NH<sub>2</sub> have indicated the preference for three conformations, the most stable has two C<sub>7</sub> rings each including a 1 $\rightarrow$ 3 H-bond, another has a type I  $\beta$ -turn with a 1 $\rightarrow$ 4 H-bond (0.9 kcal/mol above the most stable) and a third having a type II  $\beta$ -turn and a 1 $\rightarrow$ 4 H-bond (1.8 kcal/mol above the most stable conformation). All three are expected to exist as a statistical ensemble. The 'folded' rotamer found in cyclic dipeptide containing benzylic side-chains has always been favoured, but in a new study<sup>250</sup> there is evidence that this conformer is only preferred as long as it is allowed by 'buckling' of the 'boat' form of the DKP ring. When the boat becomes highly buckled as in the case of a pyruvoyl derivative the folded rotamer is no longer favoured.

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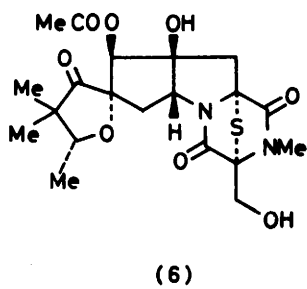
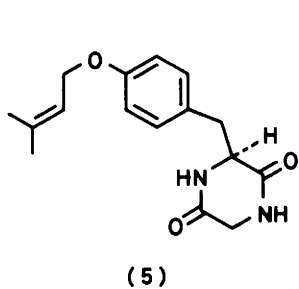
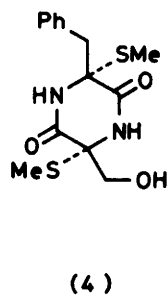
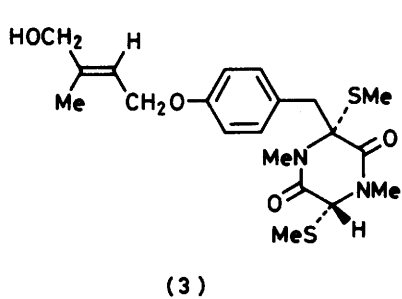
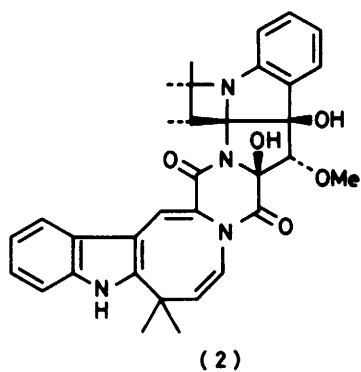
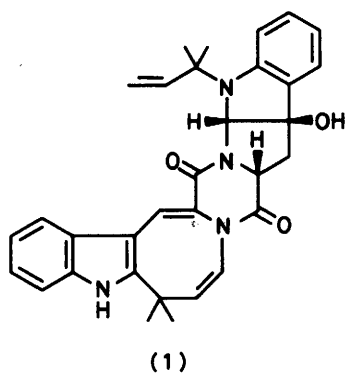
## 1 Introduction

Separate sections have been introduced this year for linear phosphonopeptides and for conformationally constrained peptides. The latter covers the growing use of structural approaches to inducing defined secondary structures or to rigidifying amide bond analogues by involving cyclic structures other than those found in conventional cyclic peptides. On the other hand, the sections on cyclic peptides containing 'thiazole and oxazoline rings' and 'other nonprotein ring compounds' have been conflated into 'cyclic peptides containing unusual constituents'. This reflects a current reduction in work on cyclic peptides containing heterocyclic rings in the backbone. Linear ' $\beta$ - and higher amino acids' also do not rate a separate section this year. The 300 reference milestone has also been reached in this report on the work of 1988.

## 2 Cyclic Peptides

### 2.1 Naturally Occurring Dioxopiperazines (Cyclic Dipeptides) -

Several cyclodipeptides have been isolated from liquid cultures of Alternaria alternata, the causal agent of the black leaf blight of spotted knapweed. One of these, maculosin, is active in the nicked-leaf bioassay at  $10^{-5}$ M. Maculosin, cyclo(Pro-Tyr), is phytotoxic only to spotted knapweed.<sup>1</sup> Two novel heptacyclic indole alkaloids, okaramines A and B (1) and (2) have been obtained from Penicillium simplicissimum. These compounds show insecticidal activity towards silkworms, A having a much lower activity than B.<sup>2</sup> No less than five co-metabolites of gliotoxin have been purified from Gliocladium virens. Three of these, (3), (4) and (5), are new natural products; (5) has been synthesised from cyclo(Gly-Tyr). This paper also gives details of the syntheses of the epi-tri- and epi-tetrasulphides gliotoxins E and G from gliotoxin.<sup>3</sup>



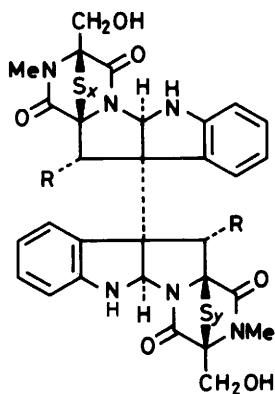
The first naturally-occurring epimonothiodioxopiperazine, sirodesmin H (6), has been isolated from Phoma lingam. It is only about one tenth as phytotoxic as sirodesmin PL, whose structure is identical except that it contains a disulphide bridge across the cyclodipeptide ring.<sup>4</sup> A new dimeric epipolythiodioxopiperazine, chetracin A (7a), with two tetrasulphide bridges has been obtained from Chaetomium nigricolor and C. retardatum. Two congeners of chaetocin (7b), chaetocins B and C (7c and 7d), have also been extracted from C. virescens. They are very active against Gram positive bacteria.<sup>5</sup>

The structure of neihumicin (8), a new antibiotic from Micromonospora nei huensis, has been proved by synthesis.<sup>6</sup> Some analogues of neihumicin have also been prepared. The most cytotoxic of these results from replacing the methoxy group by an ethoxy group; the potency is increased tenfold.<sup>7</sup>

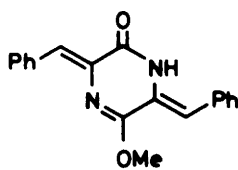
The stereochemistry of fumitremorgin C(9) has been confirmed by total synthesis from 6-methoxy-N-hydroxytryptophan via (10).<sup>8</sup> Details of the preparation of fumitremorgin B (11) have been reported, a key intermediate being (12). After numerous efforts, conditions for direct osmium tetroxide oxidation of (12) to the cis-diol, followed by N-prenylation gave fumitremorgin B in 66% yield.

The bicyclomycin-sodium methanethiolate addition product has been re-examined, and the structure originally assigned (13) now revised to (14), in which the dioxopiperazine ring has been opened.<sup>10</sup> The acid hydrolysis of bicyclomycin has been investigated in some detail. Two major 2(5H)-furanone fragments, (15) and (16), have been identified. In a comparable study with the 3'-ethylcarbamate derivative of bicyclomycin, (15) and (17) were obtained. It is thought that under acidic conditions a Michael addition of water to the olefinic group occurs after cleavage of the cyclodipeptide ring.<sup>11</sup> Bicyclomycin has now been found to bind to thiols at near neutral pH at 20 °C. It is suggested that drug activation may occur by initial ring cleavage of the hemiaminal bond to give (18). After thiol addition to the  $\alpha\beta$ -unsaturated carbonyl system, an intramolecular mixed-Claisen condensation can occur to produce (19) and ammonia.<sup>12</sup>

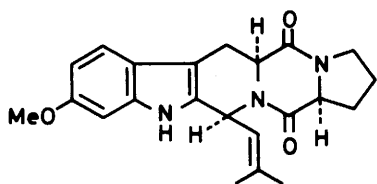
A versatile new approach to piperazine-2,5-diones has been developed (Scheme 1). This gives access to the deceptively simple yet difficult to prepare mono-ethers of cyclodipeptides.



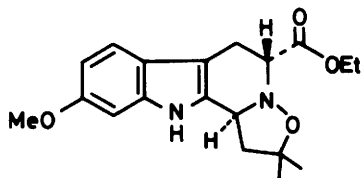
- (7) a;  $\text{R} = \text{OH}$ ,  $x = y = 4$   
 b;  $\text{R} = \text{H}$ ,  $x = y = 2$   
 c;  $\text{R} = \text{H}$ ,  $x = 3$ ,  $y = 2$   
 d;  $\text{R} = \text{H}$ ,  $x = 3$ ,  $y = 3$



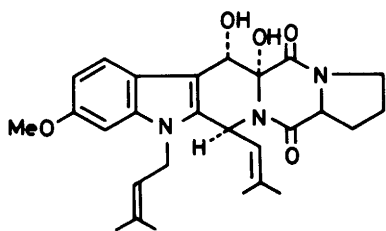
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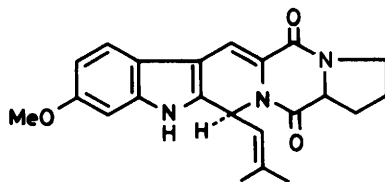
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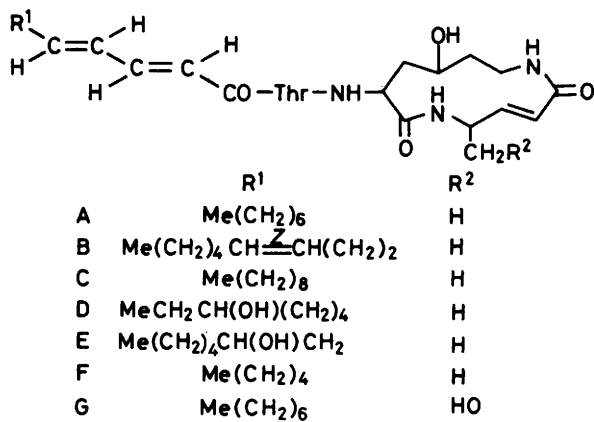
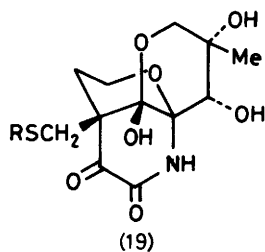
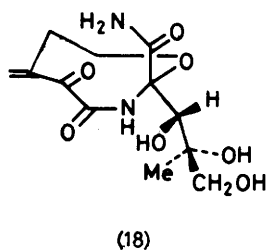
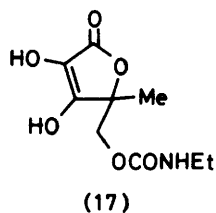
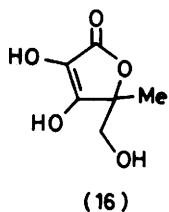
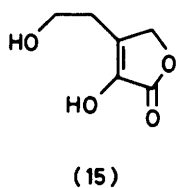
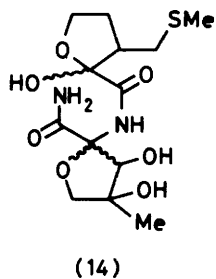
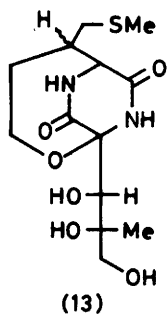
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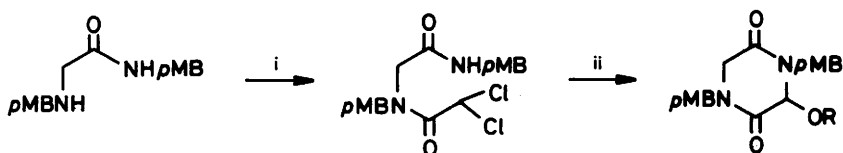
(11)



(12)



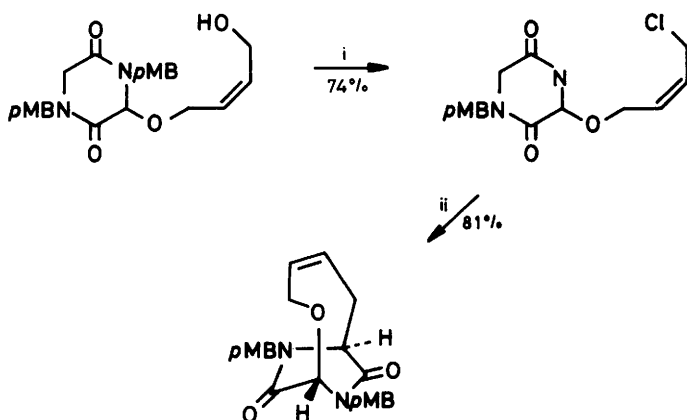




pMB = *p*-methoxybenzyl

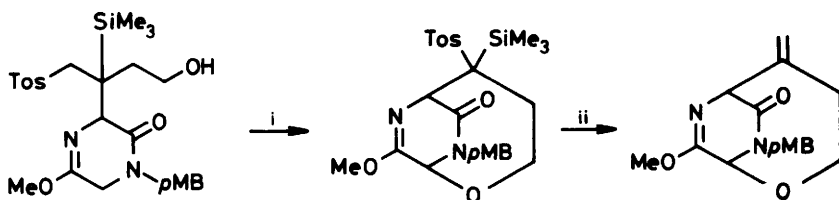
Reagents : i,  $\text{Cl}_2$ ,  $\text{CHCOCl}$ ,  $\text{K}_2\text{CO}_3$ ,  $\text{CH}_2\text{Cl}_2$  ; ii,  $\text{RO}^-$

Scheme 1



Reagents : i,  $\text{MsCl/LiCl}$ , collidine, DMF ; ii,  $(\text{TMS})_2\text{NLi}$  / THF,  $-78^\circ\text{C}$

Scheme 2



Reagents: i, DDQ ; ii,  $\text{F}^-$

Scheme 3

Suitable choices of substituent allows elaboration into bicyclic derivatives with a 5-atom bridge, as in Scheme 2.<sup>13</sup> Methods for elaborating the 4-atom bridge of bicyclomycin itself have further been explored. In particular, oxidation of a mono-imino ether using 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) has been found effective (Scheme 3).<sup>14</sup>

A group of antibiotic cyclodipeptides containing 12-membered rings, the glidobactins (20), have been isolated from Polyangium brachysporium from Greece.<sup>15,16</sup> Glidobactin A has been synthesised,<sup>15</sup> as have some analogues with modified fatty acids. The dodecanoyl and tetradecanoyl analogues exhibit better antitumour activity than the natural compounds.<sup>17</sup>

2.2 Other Cyclic Dipeptides - X-Ray analysis of cyclo(His-D-His) shows planar imidazole rings folded over the central ring and sandwiching it; n.m.r. indicates a similar conformation in solution.<sup>18</sup> The crystal structure of cyclo(Phe $\psi$ (CN<sub>4</sub>)Ala), in which the amide bond is replaced by a 1,5-disubstituted tetrazole, shows both rings to be essentially planar, with the phenyl ring adopting a flagpole orientation.<sup>19</sup> In the solid state cyclo-(Phe-NMe-4- $\alpha$ -aminobutyl) exists as two crystallographically independent molecules. The side chains are folded, but their orientations differ from that in the solution conformation.<sup>20</sup>

An n.m.r. study of cyclo(Trp-His) shows the indole-DKP ring interaction to be stronger than the imidazole-DKP ring interaction, but there also seems some sort of direct interaction between the aromatic rings.<sup>21</sup> Intramolecular van de Waals potential energy calculations on a number of dioxopiperazines containing Phe and Pro show a dependence between the DKP-ring and Phe side chain conformations. This is the first calculation to demonstrate that the folded rotamer is not always the most stable.<sup>22</sup>

A series of 33 cyclodipeptides have been made and their tastes compared with the corresponding linear peptides. In general dioxopiperazines have bitter tastes (except when they contain acidic amino acids or only Gly and Phe), and this bitterness is always more intense than the corresponding open chain compounds. The exception is Pro-Pro; here the linear compound is bitter and the cyclic one tasteless.<sup>23</sup> Cyclic analogues of the analgesic dipeptide derivative H-Arg-Trp(Nps)-OMe have been synthesised. The L-L diastereoisomer, cyclo-(Arg-Trp(Nps)), proved the most active, having ten times the

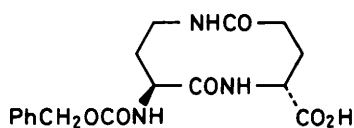
potency of kyotorphin.<sup>24</sup>

Several cyclodipeptides have been evaluated as catalysts for the asymmetric hydrocyanation of benzaldehyde and 3-hydroxy-benzaldehyde. Cyclo(R,R)- and (S,S)-(Phe-His) proved to be the most effective, giving enantiomeric excesses of 80-100% when reaction is carried out at -10 °C in toluene. Substitution of the Phe ring leads to a reduction in catalyst enantioselectivity.<sup>25</sup> A g.c.-m.s. analysis of the Curie-point pyrolysis products of cyclo(Val-Pro), cyclo(Ala-Pro), and cyclo(Gly-Ala) shows that from two to six primary products are formed with expulsion of simple molecules such as hydrogen, propene, and CO.<sup>26</sup> The products derived from the decomposition of aspartame are dominated by the cyclodipeptide at neutral and basic pH's. At neutral pH's, the amino acids in dioxopiperazines are more rapidly racemised than those in peptides. It is suggested that dioxopiperazines may constitute a temporary sink for degrading peptides in biogeochemical systems.<sup>27</sup>

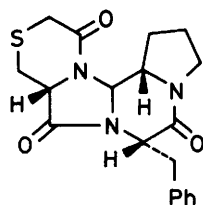
Theoretical calculations on Ac-Lys-Glu-D-Lys-D-Glu-NHMe are consistent with the presence of a  $\beta$ -turn, while a structure resembling a  $\beta$ -turn is favoured for Ac-Glu-Lys-D-Glu-D-Lys-NHMe.<sup>28</sup> The cyclic dilactam (21) has been prepared as a  $\beta$ -turn template, but its lack of crystallinity and poor solubility, together with a tendency to gel formation, seem likely to hinder its incorporation into peptides.<sup>29</sup> Eight further papers concerning the synthesis of a variety of amino acids from cyclo(Val-Gly) and cyclo(Val-Ala) by the bislactim ether method have been published.<sup>30-37</sup>

Removal of the S-protecting group from N-chloroacetyl-S-t-butylthio-Cys-Phe-Pro-ONp with tributylphosphine in aqueous solution at 20 °C gives the azacyclol (22). The same adduct is obtained starting from (23). The high stability of (22) is ascribed to the conformationally rigid polycyclic skeleton.<sup>38</sup> The crystal structure of the cyclic disulphide of phenacetyl-Cys-D-Pen-OMe (24a) shows a distorted trans lactam with a P-helical disulphide bridge. Individual molecules are H-bonded between the side-chain and lactam functions to form a  $\beta$ -pleated sheet array. The conformation in solution was similar, as is that of (24b), obtained by a 3-chloroperbenzoic acid oxidation.<sup>39</sup>

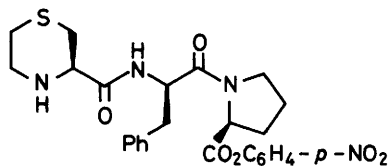
**2.3. Cyclic Tripeptides** - The crystal structure of cyclo(tri-D-azetidine-2-carboxylic-acid) shows a crown conformation for the central 9-membered ring, with the 4-membered rings in a



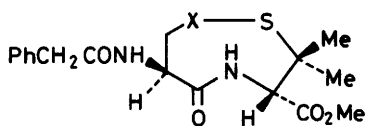
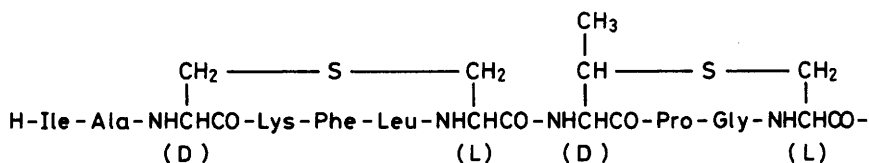
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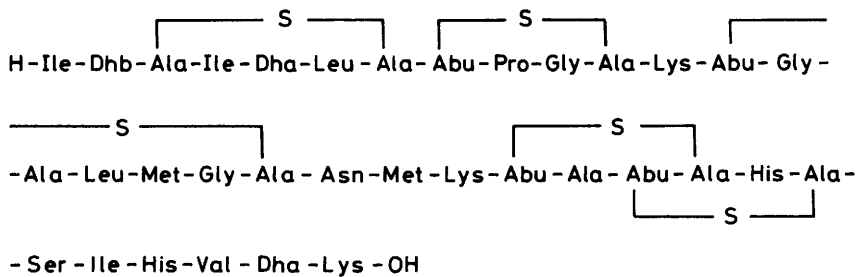
(22)



(23)

(24) a; X = S  
b; X = SO<sub>2</sub>

(25)



(26)

propeller-like arrangement.<sup>40</sup> X-Ray analysis of cyclo(MeAnt-Phe-Pro), where MeAnt = N-methylantranilic acid, shows three cis amide bonds and the aromatic ring at 63.3° to the best plane of the backbone and nearly perpendicular to the plane of the two adjacent amide bonds.<sup>41</sup>

2.4 Cyclic Tetrapeptides - A tentative identification of the antimitogenic bioactive conformer of the family of cyclic tetrapeptides which includes HC-toxin, Cyl I, II, and chlamydocin has been made. It contains a cis, trans, trans, trans, array of peptide bonds.<sup>42</sup> Three new cyclic enkephalin analogues, H-Tyr-D-Lys-Gly $\psi$ [CH<sub>2</sub>S]-Phe-L or D-Leu and H-Tyr-D-Pen-Gly $\psi$ [CH<sub>2</sub>S]-Phe-D-Pen-OH, have been reported. There is a large drop in potency in all cases compared to the parent peptides with no thiomethylene bonds, confirming suggestions that the Gly-Phe amide bond has a functional role.<sup>43</sup>

Three papers concern cyclic analogues with side-chain amide bonds. [D-Orn<sup>2</sup>, Asp<sup>5</sup>]-dynorphin A<sub>1-8</sub> is 45x more potent than linear dynorphin A<sub>1-8</sub>, but is now  $\mu$ -receptor selective;<sup>44</sup> [Orn<sup>5</sup>, Asp<sup>8</sup>]-dynorphin A<sub>1-13</sub>, however, has only a low biological activity.<sup>44</sup> Ac-[Nle<sup>4</sup>, D-Orn<sup>5</sup>, Glu<sup>8</sup>]- $\alpha$ -MSH<sub>4-11</sub>NH<sub>2</sub> is slightly more potent than Ac-[Nle<sup>4</sup>]-MSH<sub>4-11</sub>NH<sub>2</sub> in the frog skin bioassay, but Ac-[Nle<sup>4</sup>, D-Orn<sup>5</sup>, D-Phe<sup>7</sup>, Glu<sup>8</sup>]- $\alpha$ -MSH<sub>4-11</sub>NH<sub>2</sub> is 100 fold less potent.<sup>45</sup> Finally, the cyclic cholecystokinin analogues Boc-D-Asp-Tyr(SO<sub>3</sub>H)-Ahx-D-Lys-Trp-Ahx-Asp-Phe-NH<sub>2</sub> (Ahx = 2-amino-hexanoic acid) and its homologue where D-Glu replaces D-Asp both exhibit a high affinity and a high selectivity for central CCK binding sites. These compounds may help to clarify the respective roles of central and peripheral receptors for various CCK 8-induced pharmacological effects.<sup>46</sup>

The protected cyclotetrapeptides Boc-Cys-Pro-Tyr or Phe-Cys-NHMe have been synthesised as models for the redox-active disulphide loop of glutaredoxin. In CHCl<sub>3</sub> <sup>1</sup>H-n.m.r. indicates a type I  $\beta$ -turn for the Pro-Tyr or Phe segment, but in DMSO two conformational species in slow exchange are observed. These are assigned to type I and type II  $\beta$ -structures.<sup>47</sup> A new lanthionine-containing antibiotic (for which the general title of lantibiotic is proposed) has been isolated from Staphylococcus gallinarum. This [21]-peptide (25) is active against propionobacteria involved in acne disease, and differs from epidermin only in the replacement of Leu<sup>6</sup> of Ile.<sup>48</sup>

The total synthesis of the [34]-peptide lantibiotic

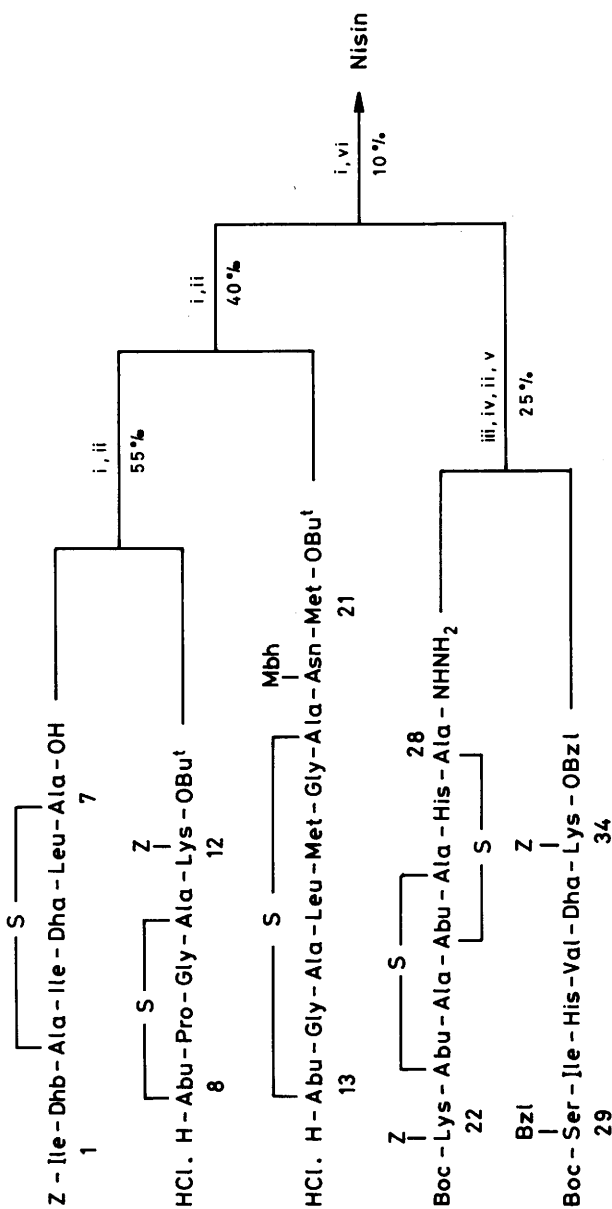
nisin (26) has been achieved. This antibiotic, which finds considerable use as a food preservative, was constructed from five fragments in a convergent synthesis (Scheme 4). The Dha residue, generally labile in acid media, proved stable to HF and TFA under anhydrous conditions. This important synthesis was made possible by development of a method for making lanthionine and methyl-lanthionine rings by desulphurisation of disulphide bridges with  $[P(NEt_2)_3]$ .<sup>49</sup>

A new method for elimination of water signals has made possible a complete  $^1H$ -n.m.r. assignment of all hydrogen atoms in the potent  $\delta$ -selective cyclopeptide  $[D-Pen^2, D-Pen^5]$ -enkephalin. Two pairs of very similar energy-minimised conformations differing primarily in the sign of disulphide helicity can be distinguished.<sup>50</sup>

**2.5 Cyclic Pentapeptides** - The crystal structure of cyclo(Gly-Pro-Phe-Ala-Pro) shows a cis Gly-Pro peptide bond in a conformation containing a  $\delta$ -turn and a type I  $\beta$ -turn.<sup>51</sup> Both diastereoisomers of the cyclic arginine vasopressin antagonist cyclo(D-Phe<sup>2</sup>, L- or D-Aad<sup>6</sup>) desCys<sup>1</sup>, Val<sup>4</sup> -AVP (where Aad =  $\alpha$ -aminoadipic acid) have only low biological activity,<sup>52</sup> but the human growth hormone releasing factor analogue cyclo(Asp<sup>8</sup>, Lys<sup>12</sup>)-[Asp<sup>8</sup>, Ala<sup>15</sup>]-GRF<sub>1-29</sub>-NH<sub>2</sub> retains significant biological activity. In the latter compound further substitution of Ala<sup>2</sup> by D-Ala<sup>2</sup> and/or amino-terminal replacement with desNH<sub>2</sub>-Tyr<sup>1</sup> or NMe-Tyr<sup>1</sup> gives highly potent compounds which are also active in vivo.<sup>53</sup>

Tests on the crystal structures of a cyclic pentapeptide and four cyclic hexapeptides have demonstrated that AMBER/OPLS force field calculations yield root-mean-square errors of only 0.1-0.2 Å for the position of non-hydrogen atoms.<sup>54</sup>

**2.6 Cyclic Hexapeptides** - A series of modifications of cyclo(D-Pro-Phe-Thr-Lys(Z)-Trp-Phe), a peptide which protects liver cells from phalloidin poisoning, have been prepared. Replacement of the  $\epsilon$ -Z group by  $-COPN_3C_6H_4$ ,  $-CO(CH_2)_2CO-AMC$  (where AMC = 7-amino-4-methyl coumarin), or Boc-Tyr(Bzl)- gives improved activity in each case.<sup>55</sup> Six analogues of the same cyclopeptide in which each amino acid is replaced in turn by Ala (of the same configuration) have also been prepared. A substantial decrease in biological effect was found where an aromatic amino acid (including Lys(Z)) was substituted. Conformationally, only the peptide in which Ala replaced Thr shows a change in the backbone.<sup>56</sup> The conformation



Reagents : i, water-soluble carbodiimide ; ii, TFA ; iii, azide coupling ; iv, Boc<sub>2</sub>O ; v, HCl ; vi, HF-anisole (9:1)

## Scheme 4

of the parent cyclo(D-Pro-Phe-Thr-Lys(Z)-Trp-Phe) comprises a  $\beta$ I,  $\beta$ II' structure involving respectively the Thr<sup>3</sup>CO-Phe<sup>6</sup>NH and Phe<sup>6</sup>CO-Thr<sup>3</sup>NH segments. This derived conformation has been refined by two molecular dynamics calculations.<sup>57</sup>

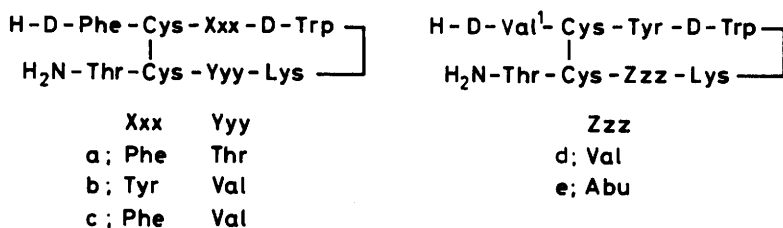
Six cyclic retro-analogues of somatostatin of the general types cyclo(Xxx-Phe-Thr-Lys-Yyy-Phe) and cyclo(Phe-Xxx-Thr-Lys-YyyPhe), where Xxx = D- or L-Pro and Yyy = D- or L-Trp, have been prepared. They lack any hormonal activity, but they do show a cytoprotective effect. The cytoprotective potency is greater when the Lys residue is  $\epsilon$ -Z-protected than when it is free. In dmsO-d<sub>6</sub> solution these all-trans compounds contain a  $\beta$ -turn with the Pro residue in position i+1.<sup>58</sup>

[Orn<sup>5</sup>, Asp<sup>10</sup>]-dynorphin A<sub>1-13</sub> is much less potent than linear dynorphin A<sub>1-8</sub>, and is now  $\mu$ -receptor selective.<sup>44</sup> A theoretical study of cyclo(Ala-Pro-D-Phe)<sub>2</sub> indicates that the methyl groups rotate more frequently during a simulation in the crystal environment than in the isolated peptide. In effect, the crystal environment is 'catalysing' the rotational isomerism of the methyl groups.<sup>59</sup> More details of the bicyclic nonapeptides reported last year (ref. 58) have appeared.<sup>60</sup>

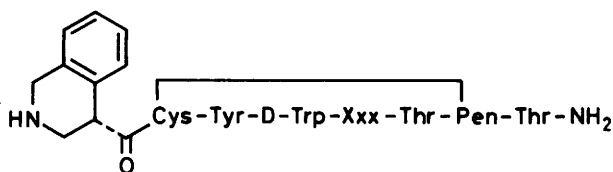
Five cyclic analogues of somatostatin active in inhibiting the secretion of growth hormone have been prepared (27a to e). In aqueous solutions of these compounds there seems to be conformational equilibria involving  $\gamma$ -turns, but in DMSO n.m.r. indicates a predominant conformation involving a type II'  $\beta$ -turn.<sup>61</sup> Three other cyclic somatostatin analogues (28) have proved to be the most potent and selective  $\mu$ -opioid antagonists known. Omission in these compounds of D-Trp<sup>4</sup>, Lys<sup>5</sup>, or Thr<sup>6</sup>, or modification of Thr<sup>8</sup> leads to compounds with greatly reduced selectivity.<sup>62,63</sup> A conformational study in DMSO-d<sub>6</sub> of a different three somatostatin analogues (29a to c) shows all three to adopt a  $\beta$ II'-turn for the Tyr<sup>3</sup>-Thr<sup>6</sup> segment. In (27a) and (27b) the disulphide has a left-handed chirality, but in (27c) it is right-handed.<sup>64</sup>

A study of some of the factors affecting the ease of cyclisation of disulphide-bridged cyclohexapeptides have involved preparing compounds (30a - d). The linear di-thiol precursors containing ionisable side-chains cyclised more easily than expected, but incorporation of pairs of Val residues made cyclisation more difficult. Ac-Cys-(Val)<sub>4</sub>-Cys-NHMe was also

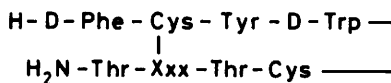




(27)



(28) Xxx = Lys, Orn, or Arg

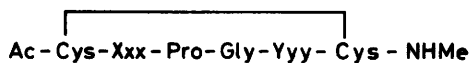


Xxx

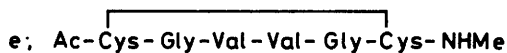
(29) a; Pen

b; Cys

c; D-Pen



|               |
|---------------|
| Xxx    Yyy    |
| a; Glu    Lys |
| b; Lys    Glu |
| c; Val    Val |
| d; Val    Gly |



(30)

prepared, but could not be converted to the monomeric cyclic disulphide. The relative propensities of the cyclic peptides for cyclisation were also studied by examining disulphide exchange reactions with (30e).<sup>65</sup> The crystal structure of Boc-Cys-Val-Aib-Ala-Leu-Cys-NHMe establishes for the first time in the solid state an antiparallel  $\beta$ -sheet conformation for a molecule with a disulphide bridge. A type II'  $\beta$ -bend exists at Aib<sup>3</sup>-Ala<sup>4</sup>.<sup>66</sup>

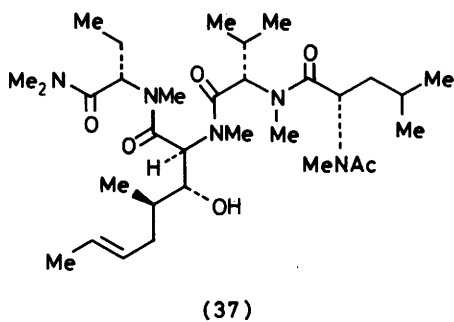
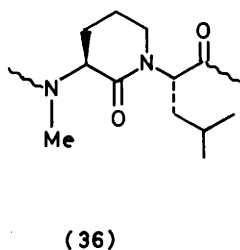
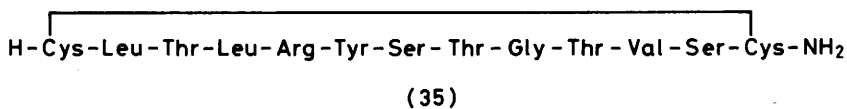
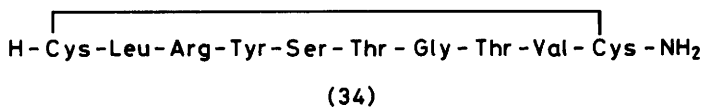
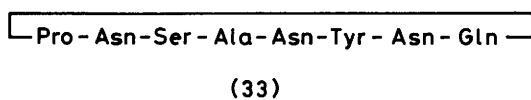
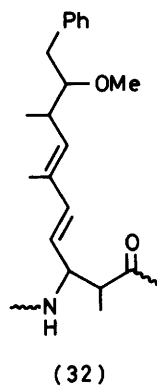
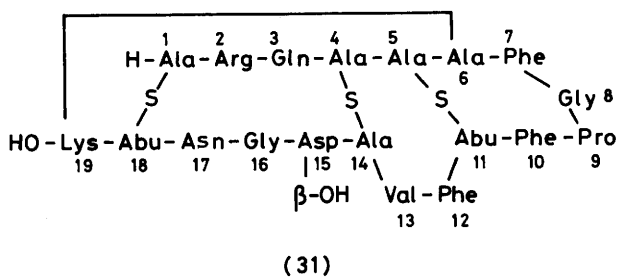
The structure of the lantibiotic Ro 09-0198 has been revised to (31), residues 6 and 10 being now reversed. N.m.r. shows that the hydrophilic and hydrophobic side chains are orientated on opposite sides of the molecule.<sup>67</sup> Lanthiopeptin has been shown to be identical to the revised structure for Ro 09-0198.<sup>68</sup>

**2.7 Higher Cyclic Peptides** - Two series of cycloheptapeptide analogues of gonadotropin releasing hormone antagonists have yielded as the most potent compounds cyclo(4-10) [Ac-D-Nal<sup>1</sup>, -D-Fpa<sup>2</sup>, D-Trp<sup>3</sup>, Asp<sup>4</sup>, D-Arg<sup>6</sup>, Dpr<sup>10</sup>]-GnRH, where Nal = 3-(2'-naphthyl)-alanine, Pal = 3-(3'-pyridyl)alanine, Cpa = (4-chlorophenyl)-alanine, Dpr = 2,3-diaminopropionic acid, and Fpa = (4-fluorophenyl)alanine. In the anti-ovulatory assay these were fully active in rats at 12.5  $\mu$ g and 2.5  $\mu$ g per rat respectively.<sup>69</sup>

The structure of cyanoginosin-RR from a Japanese strain of Microcystis aeruginosa has been reported as cyclo(D-Ala-Leu-erythro- $\beta$ -Me-D-isoAsp-Ala-Adda-D-Glu-NMe- $\Delta$ -Ala), where Adda is (32). It differs from cyanoginosin-LA, which was sequenced in 1984, in containing two Arg residue in place of Leu and Ala.<sup>70</sup> The highly selective  $\mu$ -opiate antagonist H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH<sub>2</sub> has been shown in solution to adopt a Tyr<sup>3</sup>-Thr<sup>6</sup> type II'  $\beta$ -turn.<sup>71</sup>

Amatoxins with position 5 substituted by L- or D-Ala have been studied by 500 MHz n.m.r. The results demonstrate that the rigidity of the bicyclic framework of amatoxin itself is preserved. The 10-fold decrease in biological activity of these analogues may be associated with the Ala methyl group pointing towards the indole ring of Trp, affecting the binding ability of the latter.<sup>72</sup>

A cyclo-octapeptide (33) from a Bacillus subtilis strain has been sequenced by a methodology called CAPS (computer aided peptide sequencing) analysis of its CID (collision-induced dissociation) spectrum of protonated molecular ions formed by FAB ionisation. The amino acid sequence and the origin



of the peptide strongly suggest it is iturine A-2.<sup>73</sup> A new series of cyclo-octapeptides based on the somatostatin 5-12 analogue cyclo(Asn-Phe-Phe-D-Trp-Lys-Lyr-Phe-Gaba) containing the substitutions [Aap<sup>9</sup>], [D-Lys<sup>9</sup>], [Trp<sup>8</sup>, D-Lys<sup>9</sup>], [Orn<sup>9</sup>], and [D-aThr<sup>10</sup>] have been synthesised (Aap = 2-aminoadipamic acid). All showed a considerable enhancement of stability towards rat intestinal juice in vitro, supporting Lys<sup>9</sup> to be the primary initial enzymic cleavage site in the parent compound.<sup>74</sup>

The crystal structure of cyclo(Cys-Gly-Pro-Phe)<sub>2</sub> shows a backbone with two  $\beta$ -turns which bears a strong resemblance to that of  $\beta$ -amanitin; it is roughly similar to backbones previously reported for cyclo(Ala-Gly-Pro-Phe)<sub>2</sub> diastereomers.<sup>75</sup> Analogues of melanin concentrating hormone H-Asp-Thr-Met-Arg-Cys-Met-Val-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Glu-Val-OH containing smaller cyclic structures in which Cys<sup>14</sup> is retained have been prepared. However, all of these compounds, including [Ala<sup>5</sup>, Cys<sup>10</sup>]-MCH<sub>5-17</sub>, [Ala<sup>5</sup>, Cys<sup>8</sup>]-MCH<sub>5-17</sub>, [Ala<sup>5</sup>, Cys<sup>7</sup>]-MCH<sub>5-17</sub>, [Cys<sup>10</sup>]-MCH<sub>10-17</sub>, were inactive in the fish-skin assay and only [Ala<sup>5</sup>, Cys<sup>10</sup>]-MCH<sub>5-17</sub> was active in the frog-skin bioassay.<sup>76</sup> [Orn<sup>5</sup>, Asp<sup>13</sup>]-dynorphin A<sub>1-13</sub> has been prepared, but it is much less potent than linear dynorphin A<sub>1-13</sub> and less  $\mu$ -selective.<sup>44</sup> Only one gramicidin S analogue has been reported this year. [ $\Delta$ Phe<sup>4,4'</sup>]-gramicidin S shows very strong activity against gram positive bacteria, but is not very effective towards gram negative ones.<sup>77</sup>

Two cyclopeptides related to the human renin flap region, (34) and (35), have been made. Structural analysis by i.r. and n.m.r. and recognition by polyclonal human renin antibodies provides support for a  $\beta$ -hairpin secondary structure earlier proposed as a speculative model.<sup>78</sup> Methods for the unequivocal synthesis of both parallel and antiparallel dimers of the test peptide H-Gly-Cys-Phe-Val-Pro-Cys-Gly-OH have been explored. Various combinations of the S-protecting groups fluorenylmethyl, acetamidomethyl, thiopyridyl, and 3-nitro-2-pyridinesulphenyl were used.<sup>79</sup>

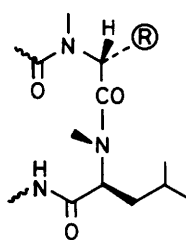
Full details of the synthesis of several cyclosporin analogues reported last year have appeared. These are [MeThr<sup>1</sup>, Abu<sup>2</sup>]-, [MeSer<sup>1</sup>, Abu<sup>2</sup>]-, [Hyp<sup>1</sup>, Abu<sup>2</sup>]-, [Dab<sup>1</sup>, Abu<sup>2</sup>]-, [MeThr<sup>1</sup>, Nva<sup>2</sup>]-, [MeSer<sup>1</sup>, Nva<sup>2</sup>]-, [MeThr<sup>1</sup>, Nle<sup>2</sup>]-, [MeSer<sup>1</sup>, Nle<sup>2</sup>]-, [Hyp<sup>1</sup>, Nle<sup>2</sup>]-, and [MeSer<sup>1</sup>Thr<sup>2</sup>]-CS. Stepwise assembly of the linear [11]-peptides was found to give a higher yield than a fragment condensation approach. The diphenyl-phosphinic mixed

anhydride method was used for linear peptide preparation, and on cyclisation the Castro BOP reagent gave the best yields; the highest was 65%, achieved in the case of [Hyp<sup>1</sup>, Abu<sup>2</sup>]-CS. No biological activities are given.<sup>80,81</sup> Another CS-analogue, [lactam<sup>3,4</sup>]-CS, has been prepared. In this compound the Sar<sup>2</sup>MeLeu<sup>3</sup> has been replaced by the unit (36) with the idea of stabilising a  $\beta$ -turn. However, it has only a weak immunosuppressive activity, as has [MeAla<sup>3</sup>]-CS. [D-MeAla<sup>3</sup>]-CS, however, is nearly as active as CS itself. [Lactam<sup>3,4</sup>]-CS has a conformation in solution indistinguishable from CS, so its low activity suggests steric hindrance with the CS receptor.<sup>82</sup>

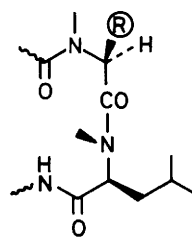
A peptide (37) that approximates the critical binding/immunosuppressive domain within cyclosporin has been prepared, but lacks the ability of CS to bind to the cytoplasmic protein cyclophilin.<sup>83</sup> Because of its poor solubility, it is difficult to study the conformation of cyclosporin in aqueous solution. As an alternative, the ability of a series of monoclonal antibodies raised against CS to recognise different parts of the N-terminal amino acid were determined from the crossreactivity of CS analogues modified in position one. The results indicate that the side chain of the first residue is probably folded back under the molecule, as occurs in the crystal structure.<sup>84</sup>

It has been observed that multiply lithiated compounds are solubilised in THF by lithium halides. This effect has been exploited for the alkylation of sarcosine CH<sub>2</sub> groups in both open-chain and cyclic oligopeptides. The largest peptide examined, cyclosporin, can be converted to a THF-soluble hexalithioderivative without any epimerisation and alkylated by different electrophiles in the presence of either excess lithium diisopropylamide or of up to 30 equivalents of lithium chloride. Depending on the conditions of deprotonation, an enolate is generated that is preferentially attacked either from the Re side (38) or with Si (39) selectivity. Substitutions in the Re position retain immunosuppressive properties, those in the Si orientation lose activity. This loss is ascribed to steric hindrance of the group introduced with the adjacent NMe group, causing a change in backbone conformation.<sup>85</sup>

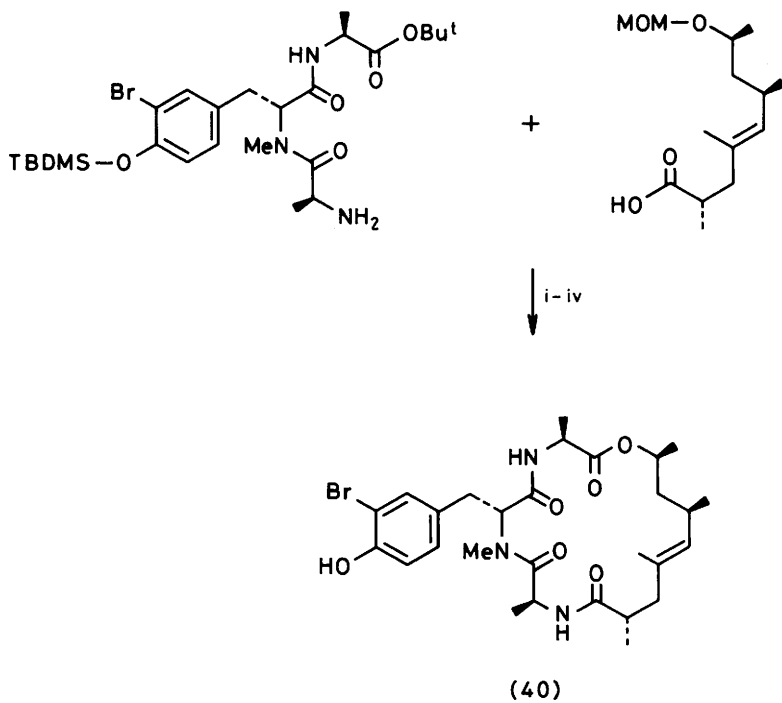
The [12]-peptide cyclo(Leu-Phe-Pro)<sub>4</sub> has been found to complex selectively with alkaline earth metal ions. In the free state, the skeletal conformation is non-symmetrical, but when complexed with Ba(II), a C<sub>4</sub> symmetrical conformation



(38)



(39)



Reagents : i, DCC, HOBT ; ii, HSCH<sub>2</sub>CH<sub>2</sub>SH, TFA ; iii, DCC, DMAP ; iv, Bu<sub>4</sub>N<sup>+</sup>F<sup>-</sup>

**Scheme 5**

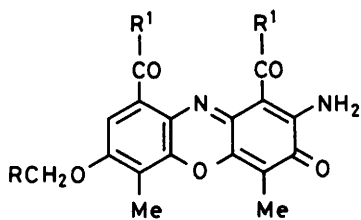
having all peptide bonds trans and four  $\beta$ -turns containing transannular bonds is formed.<sup>86</sup> Four [14]-cyclopeptides related to gramicidin S have been synthesised. Cyclo(Val-Orn-Leu<sub>2</sub>-D-Phe-Pro-Leu)<sub>2</sub>, cyclo(Val-Orn-Leu-D-Leu-D-Phe-Pro-Leu)<sub>2</sub>, and cyclo(Val-Orn-Leu<sub>2</sub>-D-Phe-Pro-D-Leu)<sub>2</sub> show one quarter to one eighth of the antibiotic activity of GS, but cyclo(Val-Orn-Leu-D-Leu-D-Phe-Pro-D-Leu)<sub>2</sub> is inactive. No clear relationship of CD spectra to activity was found.<sup>87</sup>

**2.8 Cyclodepsipeptides** - The first synthesis of geodiamolide B (40), isolated in 1987, has been achieved. In the final steps the polypropionate fragment was coupled with the tripeptide unit containing the unique amino acid (R)-3-bromo-N-methyltyrosine (Scheme 5).<sup>88</sup> Novel actinomycin D analogues (41) substituted with aziridine and cyclopropyl functions have been prepared, but have only 1/4 to 1/40 the cytotoxicity of the parent, the most active being the cyclopropyl compound. They also intercalate into DNA with a weaker affinity.<sup>89</sup> Another series of actinomycin D analogues (42) exploring the effect of fusing an oxazole ring onto the phenoxazine chromophore has been reported. These compounds are very active in priming superoxides in the presence of microsomal enzymes as well as in inhibiting the synthesis of DNA. They are also more effective than actinomycin D towards several experimental tumours and show a reduced toxicity in tumour-free mice.<sup>90</sup>

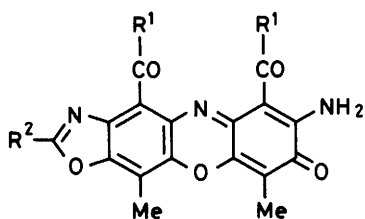
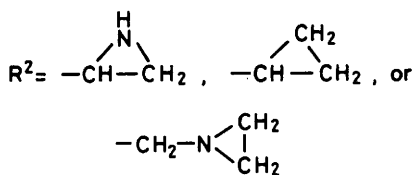
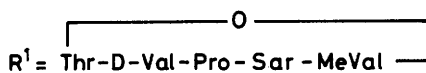
A new cyclohexadepsipeptide antibiotic A 83586C from Streptomyces karnatakensis harvested in Guam (43) has potent activity against Gram-positive bacteria. X-ray analysis shows that it differs from azinotricin only in the presence of N-hydroxyAla in place of N-hydroxy-O-methylSer.<sup>91</sup>

A protected  $\alpha,\gamma$ -diaminobutyryl congener of the cyclohexadepsipeptide virginiamycin S<sub>1</sub> (44) has been made using BOP-Cl for imino acid peptide bond formation and a water-soluble carbodiimide-HOBt cyclisation (to the amino group of Phg). Rupture of the MePhe-MeA<sub>2</sub>bu peptide bond with TFA was a problem during synthesis.<sup>92</sup> The selective benzylation of the picolinic acid residue of virginiamycin S<sub>1</sub> has been explored. With benzylbromide/KF/DMF the 1'-N-benzyl derivative was obtained quantitatively, but in the presence of 1,8-bis(dimethylamino)-naphthalene as the base benzyl bromide gives a 27% conversion to the 1'-O-benzyl ether.<sup>93</sup>

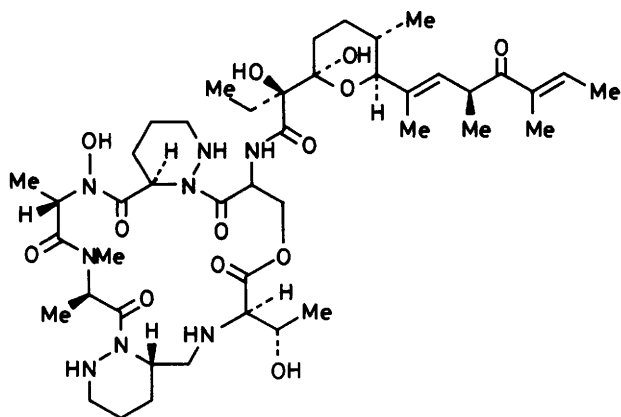
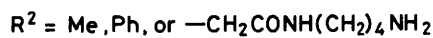
A second synthesis of the didemnin ring system has been



(41)

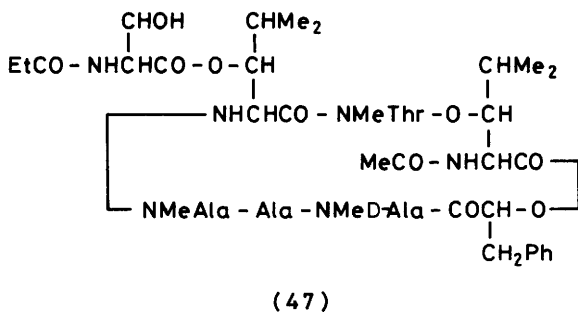
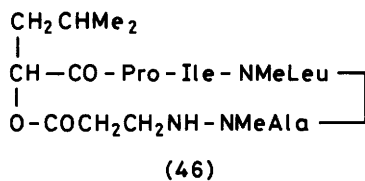
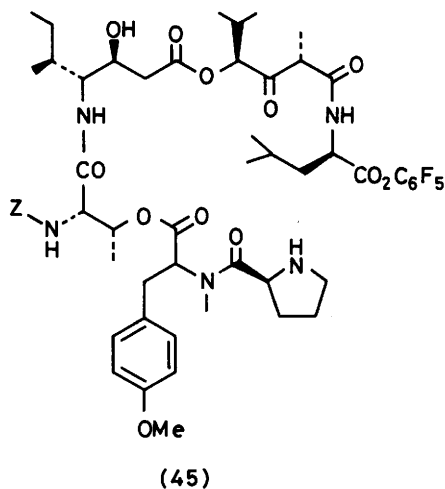
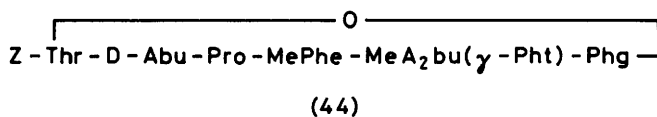


(42)



(43)



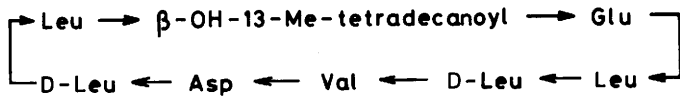


achieved by ring closure of a pentafluorophenyl ester (45) in 70% yield without high dilution.<sup>94</sup> The product was elaborated into didemnins A, B, C, and N-prolyldidemnin A by replacing the Z-group with H-(R)-MeLeu, H-Lac-Pro-(R)-MeLeu, H-Lac-(R)-MeLeu, and H-Pro-(R)-MeLeu respectively.<sup>95</sup> The crystal structure of didemnin B, the most potent of these antiviral and cytotoxic cyclodepsipeptides, shows the backbone to be folded in the shape of a bent figure-eight. The side chain forms a  $\beta$ -bend and is folded back towards the cyclic backbone, giving the molecule an overall globular character. There is one transannular hydrogen bond.<sup>96</sup>

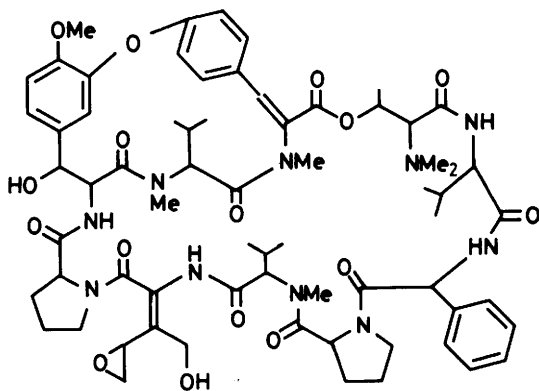
X-Ray analysis of destruxin B (46), isolated in 1964, shows an asymmetric backbone with one cis peptide unit and five trans amide and ester groups.<sup>97</sup> A novel cycloheptadepsipeptide (47) from a Far Eastern evergreen plant Ardisia crenata sims shows inhibition of platelet aggregation and lowers blood pressure. Of its constituents N,O-Dimethylthreonine is a novel amino acid, and N-methyldehydroalanine has only been reported once before. The amino acid configurations remain to be elucidated.<sup>98</sup> The biosynthesis of the lipopeptide antibiotic surfactin (48) has been investigated using <sup>14</sup>C-labelled precursors. The results are consistent with a peptide synthesising multienzyme which activates its substrate amino acids simultaneously as reactive aminoacyl phosphates.<sup>99</sup>

A new cyclodepsipeptide dityromycin (49), isolated from a Streptomyces strain, contains an unusual epoxydehydroleucine residue. The configuration of the two alkene bonds has not yet been determined. The facile acid-induced cleavage of the peptide bond adjacent to the epoxy-residue is suggested to occur via (50).<sup>100</sup> A tripeptide fragment of jaspamide, isolated in 1986, with the sequence (S)-Ala-(R)-2-bromoabryl-(R)- $\beta$ -Tyr has been made,<sup>101</sup> and two different routes for the preparation of Ala-D-NMe-3-iodoTyr-Ala, a segment of geodiamolide A, have been developed.<sup>102</sup>

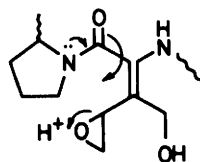
Two cyclic nonadepsipeptides have been obtained from a strain PBJ-5356 related to the genus Cytophaga. These compounds, katanosins A and B (51) show curative effects in mice infected with pathogenic bacteria.<sup>103</sup> Dilute alkali inactivates them, giving the biologically inactive linear [11]-peptide which was sequenced by Edman degradation.<sup>104</sup> Lysobactin from a species of Lysobacter differs from katanosin B only in that L-alloThr



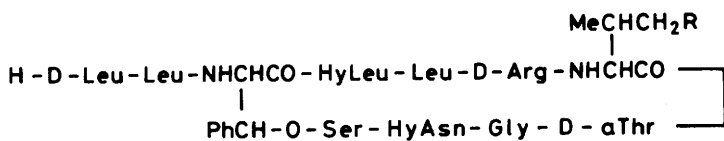
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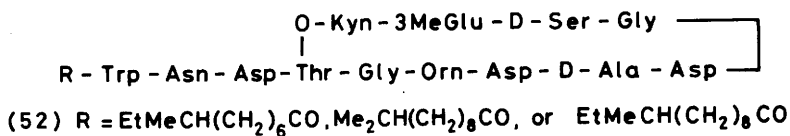
(49)



(50)



(51) A, R = H; B, R = Me



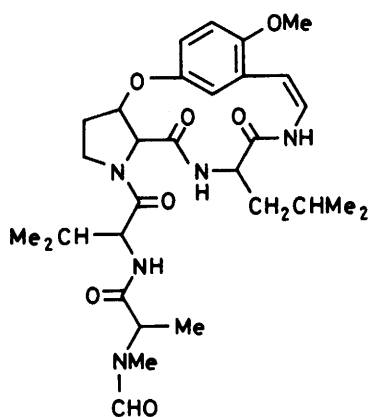
replaces D-alloThr.<sup>105</sup> It has been found that treatment of members of the A 21978C lipopeptide complex (52) (reported in 1987) with Actinoplanes utahensis removes the fatty acid. Of a series of acyl analogues prepared from the deacylated antibiotic, the n-decanoyl derivative gave the best survival in the mouse acute toxicity test. This compound, named daptomycin, is undergoing further study.<sup>106</sup>

2.9 Cyclic Peptides Containing Unusual Constituents - Two new cyclopeptide alkaloids have been reported. Ruganosanine A (53) from the Indian shrub Zizyphus rugosa is the third N-formyl peptide alkaloid to be found,<sup>107</sup> while tscheschamine (54) from Zizyphus sativa is an addition to the growing list of the nummularine C type of 13-membered cyclopeptide.<sup>108</sup> Two independent syntheses of the cyclopeptide OF 4949-II (55) have been accomplished. This compound is an inhibitor of aminopeptidase B from Ehrlich ascites carcinoma cells first isolated 1986.<sup>106,110</sup>

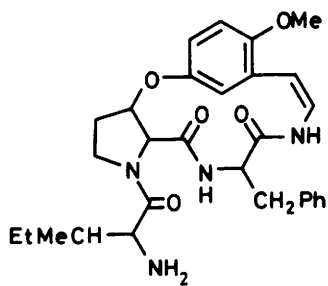
$\alpha$ -Chymotrypsin catalyses the hydrolysis of the cyclopeptide (56) very efficiently, but the ortho derivatives (57a and b) are cleaved much more slowly. The relative cleavage rates of (56) and (57a) are 330:1.<sup>111</sup> The homologous o-amino-methyl-phenylacetic acid residue has also been incorporasted into the somatostatin analogue (58a), but the compound is biologically inactive.<sup>112</sup> However, one isomer of a disubstituted derivative (58b) is equipotent to somatostatin in an in vivo test, the other being inactive. This gives positive evidence for a proposed cis-peptide bond in the bioactive conformation of cyclic somatostatin analogues.<sup>113</sup>

It has now been proved that the first intermediate peptide in bacitracin biosynthesis, enzyme-bound Ile-Cys, contains a thiazoline ring by liberation of the free dipeptide from the enzyme and oxidation to a compound (Scheme 6) previously isolated from a bacitracin hydrolysate.<sup>114</sup> Three dicarbavaso-pressin antagonist analogues (59a-c) are approximately equipotent with their disulphide congeners, but their increased metabolic stability makes them potentially of therapeutic use.<sup>115</sup>

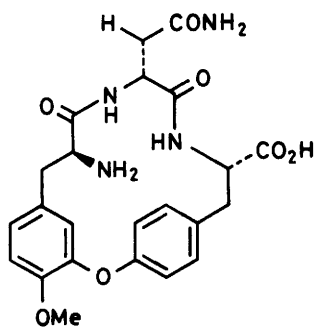
The synthesis of the parent monocyclic peptide skeletons of the bicyclic antitumour antibiotics bouvardin, deoxybouvardin, and RA's I, IV, VI, and VII (60a-c) have been prepared. One of these compounds, (60c), showed observable, albeit marginal, cytotoxic activity, as did the smaller cyclic peptide cyclo-



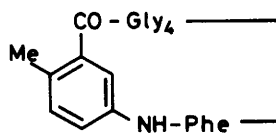
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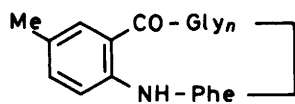
(54)



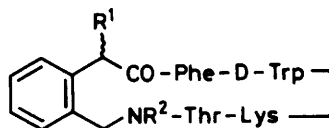
(55)



(56)



(57) a;  $n = 4$   
b;  $n = 6$

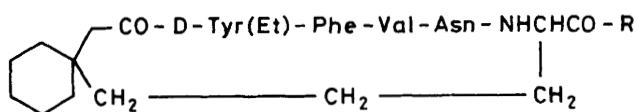


(58) a;  $R^1 = R^2 = H$   
b;  $R^1 = CH_2Ph, R^2 = Me$



Reagents : i,  $\text{MnO}_2$

**Scheme 6**



(59) R

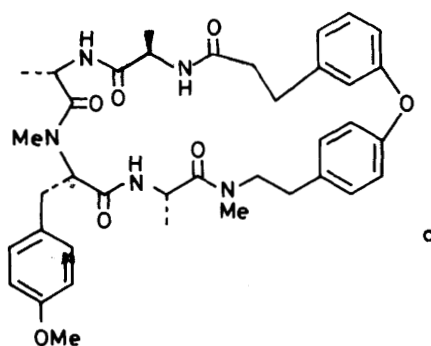
a ; -Pro-Arg-NH<sub>2</sub>

b ; -Arg-D-Arg-NH<sub>2</sub>

c ; -Arg-NH<sub>2</sub>

*cyclo* (D-Ala-Ala-NMeTyr(OMe)-Ala-NMeTyr-NMeTyr) a

*cyclo* (D-Ala-Ala-NMeTyr(OMe)-Ala-NMeGly-NMeGly) b



(60)

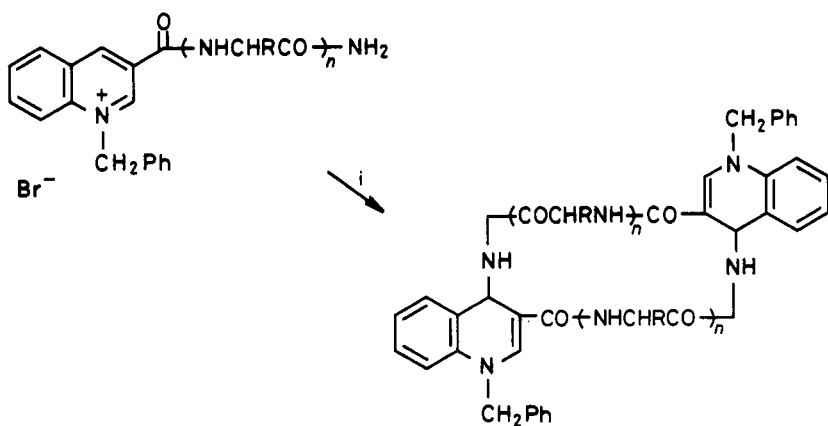
(D-Ala-Ala-NMeTyr(OMe)-Ala).<sup>116</sup> Amides of di- and tripeptides substituted with a 3-quinoline-carbonyl group at the N-terminus have been prepared and quaternised. Under basic conditions these salts dimerise to give cyclic peptides (Scheme 7). Only neutral amino acids were used (Gly, Leu, Val, Ile, and Phe). This extends work done with amino acid derivatives (see SPR 20 ref. 101).<sup>117</sup>

A study of the biosynthesis of nosiheptide (61), isolated in 1977 and used as a growth promotant for poultry, shows that in addition to expected origins the pyridine ring is formed by a unique 'tail to tail' condensation of two serine residues and the indolic moiety by a novel intramolecular reaction of tryptophan. It is therefore derived entirely from amino acids.<sup>118</sup> A new cyclopeptide scytonemin A (62) which possesses potent calcium antagonistic properties has been extracted from a blue-green alga of the Scytonema sp. It contains three modified proline residues and an unusual  $\beta$ -amino acid. Mild acid hydrolysis results in selective cleavage of the homoseryl amide bond to give a linear decapeptide.<sup>119</sup> Full details of the crystal structure of ascidiacyclamide have been published.<sup>120</sup>

### 3 Modified Linear Peptides

3.1 Enzyme inhibitors - This year's crop of novel renin inhibitors are listed in Table 1. In general only the most potent or potentially medically useful compound in a paper is reported. The structure-activity relationship of acyl-His-trifluorinated leucinol derivatives as human renal renin inhibitors has been discussed on the basis of the deduced tertiary structure of human renin.<sup>133</sup>

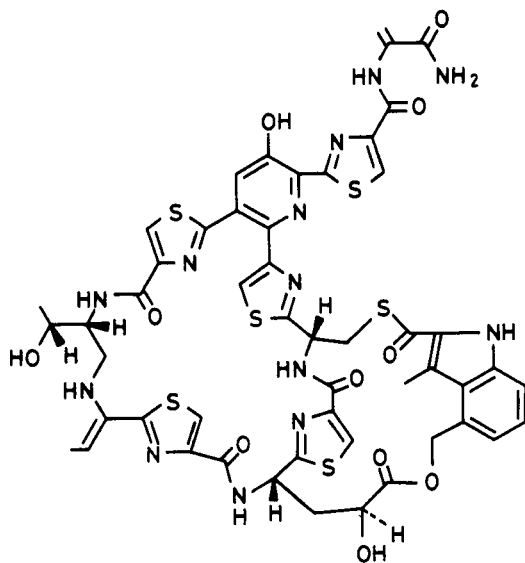
The Michael-type addition of dipeptides to 4-oxo-4-phenyl-2-butenic acid derivatives, used in the preparation of ACE inhibitors, has been found to be a reversible reaction (Scheme 8). The forward reaction is favoured at 20 °C, and the reverse reaction at 80 °C.<sup>134</sup> Of a series of potent phosphinyloxyacyl proline ACE inhibitors, the most active compounds (63) has intravenous and oral activities superior to those of captopril in the normotensive rat.<sup>135</sup> Analogues of ACE inhibitors such as captopril, enalaprilat, and phosphinic acid compounds with hydrophobic substituents on the 4-position of the proline residue are more potent in vitro than their parents. Two compounds of this type, zofenapril (64) and sofinapril (65)



$n = 2 \text{ or } 3$

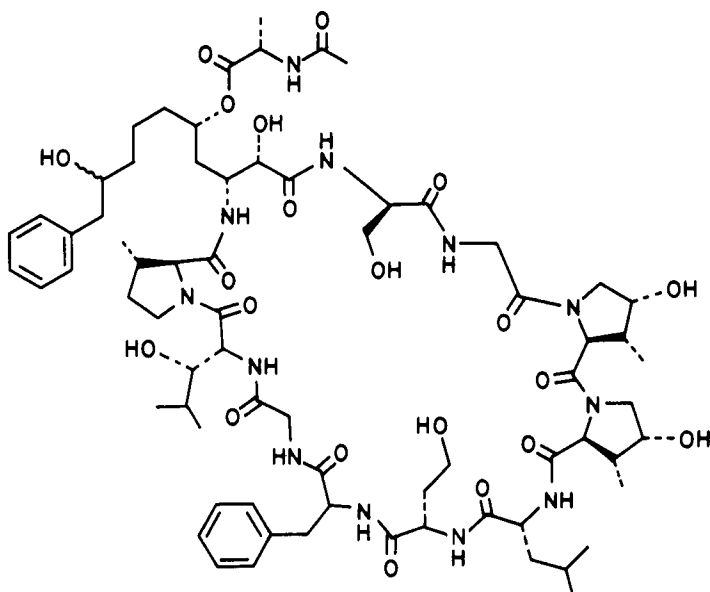
Reagent :  $i$ , OH

**Scheme 7**



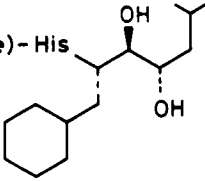
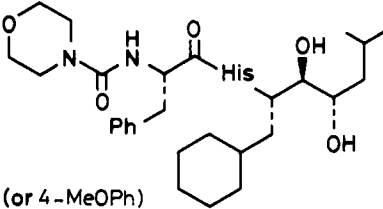
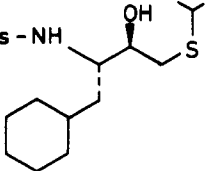
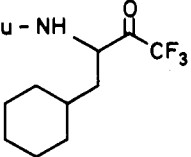
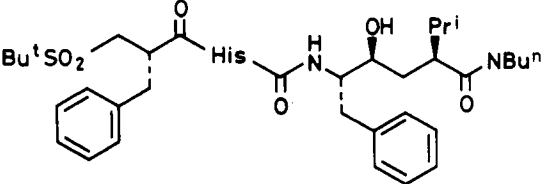
(61)

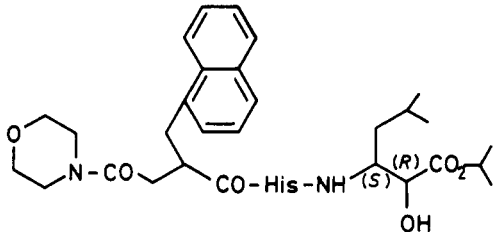
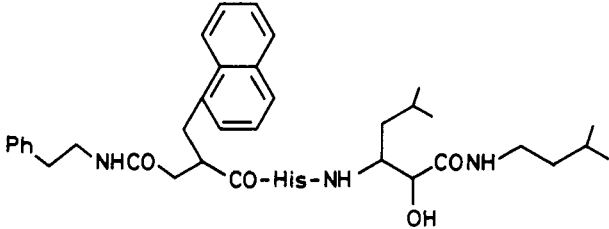
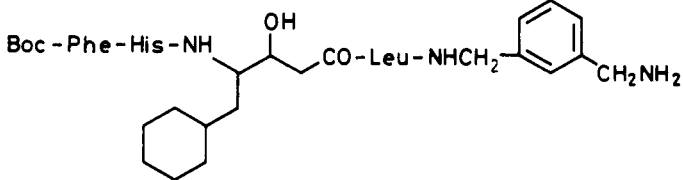
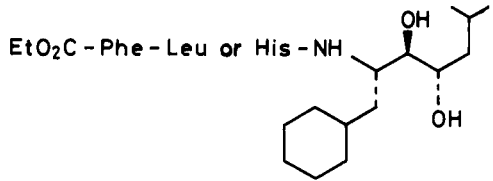


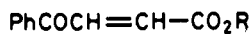


(62)

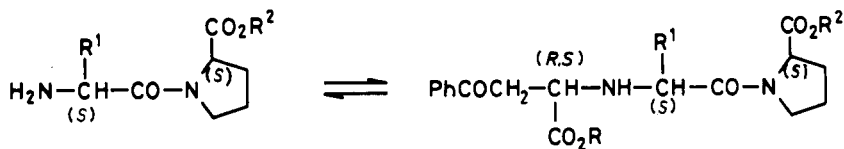
**Table 1**      Novel renin inhibitors

| <u>Compound</u>                                                                                                   | <u>IC<sub>50</sub>(nM)</u> | <u>Ref.</u> |
|-------------------------------------------------------------------------------------------------------------------|----------------------------|-------------|
| $\beta$ -Val - Phe(4-OMe) - His  | 0.6                        | 121         |
| <br>(or 4-MeOPh)                 | 0.35 (0.76)                | 122         |
| Boc - Phe - His - NH             | 4                          | 123         |
| Boc - Phe - Leu - NH           | 250                        | 124         |
|                                | 2                          | 125         |

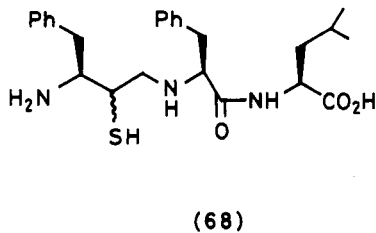
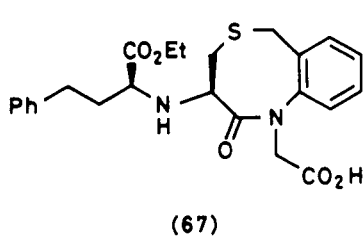
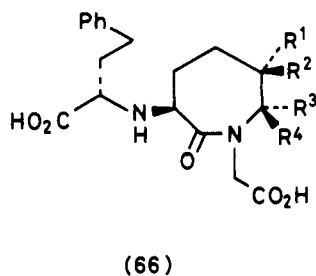
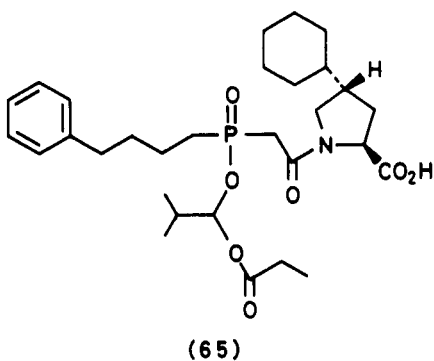
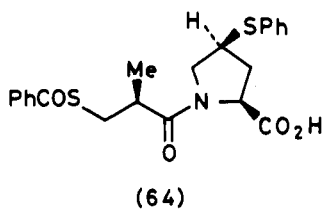
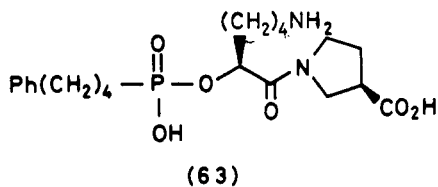
|                                                                                                                                                    |                                      |     |
|----------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|-----|
| $\begin{array}{c} \text{CHO} \\   \\ \text{Ac-Trp-Pro-Phe-His-Sta-Ile-NH}_2 \end{array}$                                                           | 0.1                                  | 126 |
| $\text{Ac-His-Pro-Phe-Val-Sta-Leu-Phe-NH}_2$                                                                                                       | 30                                   | 127 |
|                                                                   | 25                                   | 128 |
|                                                                   | 250                                  | 129 |
|                                                                  | 1                                    | 130 |
|                                                                 | 0.3 and<br>0.5                       | 131 |
| $\begin{array}{l} \text{H-His}\Psi[\text{CH}_2\text{CH}_2]\text{His-OH and} \\ \text{H-His}\Psi[\text{CH}_2\text{CH}_2]\text{-Asp-OH} \end{array}$ | 0.7 and 0.5<br>$\mu\text{g ml}^{-1}$ | 132 |



+



Scheme 8



are undergoing clinical trials as oral antihypertensive agents.<sup>136</sup>

A series of perhydroazepin-2-ones (66) have been examined as ACE inhibitors. With a phenyl substituent at the 6R, 6S, or 7S position, potent *in vitro* inhibition was seen. The most active compound, IC<sub>50</sub> 3.1 nM, was (66) (R<sup>1</sup> = Ph; R<sup>2</sup>=R<sup>3</sup>=R<sup>4</sup>=H).<sup>137</sup> The ACE inhibitory effect of (67) is similar to the 7-membered ring lower homologue earlier reported.<sup>138</sup> Three and five step convergent syntheses useful for the large scale preparation of enalapril and lisinopril respectively without the need for classical protecting group chemistry have been developed. The requisite dipeptides were prepared via *N*-carboxyanhydrides.<sup>139</sup>

Of a series of active centre directed peptide inhibitors of plasmin with a lysine residue at the P1 position, H-D-Ile-Phe-Lys-4-benzoylanilide inhibits plasmin activities towards H-D-Val-Leu-Lys-p-nitroanilide and fibrin with IC<sub>50</sub>'s of 0.069 mM and 0.18 mM respectively, but is cleaved by plasmin to release benzoylaniline.<sup>140</sup> The inhibition of cathepsin B by a number of Z-Phe-Ala ketones and the analogous aldehyde has been investigated. Although the aldehyde is almost as potent a competitive reversible inhibitor as leupeptin, the trifluoromethyl ketone showed a comparatively weak and slow-binding reversible inhibition.<sup>141</sup> Peptidyl *O*-acyl hydroxamate inactivators of cathepsin B are most rapid when they contain lipophilic *O*-acyl groups, as exemplified by *O*-mesitoyl-*N*-Z-Phe-Ala-hydroxamate.<sup>142</sup>

Tripeptide anilides have proved more potent than piperidine amides as inhibitors of human leukocyte elastase (HLE), the most active being Suc-Tyr-D-Leu-D-Val-p-benzoylanilide (K<sub>i</sub> 0.06 mM). All compounds tested inhibited HLE more strongly than human leukocyte cathepsin G except for Suc-Tyr-Leu-Val-p-benzoylanilide.<sup>143</sup> Peptide boronic acids such as methoxy-succinyl-Ala<sub>2</sub>-Pro-(L)-Boro-Phe-OH inhibit the activity of chymase (a serine protease from connective tissue mast cells) forty to eighty-fold more than 'atypical chymase' from mucosal mast cells, and do not inhibit trypsin. They have therapeutic potential for treatment of allergic inflammations catalysed by chymase.<sup>144</sup>

Of a series of *N*-(1-carboxy-3-phenylpropyl) peptide derivatives synthesised, *N*-[1(R,S)-carboxy-3-phenylpropyl]-Ala<sub>2</sub>-Tyr-p-aminobenzoate proved the best inhibitor of rat brain metalloendopeptidase, having a K<sub>i</sub> of 16 nM. Data indicate that the carboxy group co-ordinates with the active site zinc atom

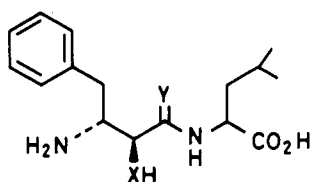
and the remaining part of the inhibitor with the substrate recognition site of the enzyme.<sup>145</sup> Some retro-inverso analogues of hydroxamate inhibitors of brain metalloendopeptidase of the general formula  $\text{HONR}'(\text{CH}_2)_n\text{-CH}(\text{CH}_2\text{Ph})\text{NHCOCHR}^2\text{CO}_2\text{H}$  ( $\text{R}' = \text{H}$  or  $\text{Me}$ ,  $\text{R}^2 = \text{H}, \text{Me}$ , or  $\text{CH}_2\text{Ph}$ ) have been made. With  $n=0$  a large decrease in inhibitory potency occurred, but when  $n=1$  the potencies were similar to compounds with a natural amide bond.<sup>146</sup>

The peptidyl diaminothiol (68),  $\text{IC}_{50}$  1nM, has proved the most active inhibitor of rat brain aminopeptidase of a quite extensive group of compounds of this type tested.<sup>147</sup> Some sulphur-containing analogues of bestatin have been examined for their inhibitory effects on aminopeptidases M, B, and leucine aminopeptidase. The 2-thiol compound (69a) is a potent inhibitor of all three (although only slightly more potent than the 2-hydroxy parent), whereas the thioamide (69b) is only a modest inhibitor of the three enzymes. These results suggest that the S atoms in these compounds do not interact strongly with the active-site zinc atom of these aminopeptidases when the inhibitors are bound to the enzyme.<sup>148</sup>

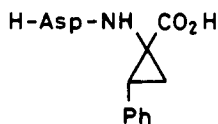
Some derivatives of the pentapeptide  $\text{Gln-Val}_2\text{-Ala-Gly}$  have been tested as thiol proteinase inhibitors. The  $\text{N-succinyl p-nitroanilide}$  showed better activity than the  $\text{N-Z p-nitroanilide}$ , but lacked any protective effect against T-kininogen-induced inhibition.<sup>149</sup> Variation of three of the amino acid residues of a good pepsin substrate has led to the development of  $\text{H-Phe-Gly-Val-(S,S)-Sta-Ala-Phe-OMe}$  as a good pepsin inhibitor ( $\text{K}_i < 1 \text{ nM}$ ). The activity is consistent with the proposal that statine functions as a bioisosteric replacement for a substrate dipeptidyl unit.<sup>150</sup>

Thirty analogues of leupeptin ( $\text{Ac-Leu-Leu-Argal}$ ) of the form  $\text{R-Leu-Argal}$  have been synthesised and examined for their inhibitory properties. The benzoyl and 1-naphthalenesulphonyl compounds are 8x more inhibitory towards papain, the Z-pyroglutamyl 10x more inhibitory to trypsin and plasmin, and the DL-Z-pipecolyl 25x more inhibitory to kallikrein than leupeptin itself. Against urokinase, only pyroglutamyl-Leu-Argal showed potent inhibitory activity.<sup>151</sup>

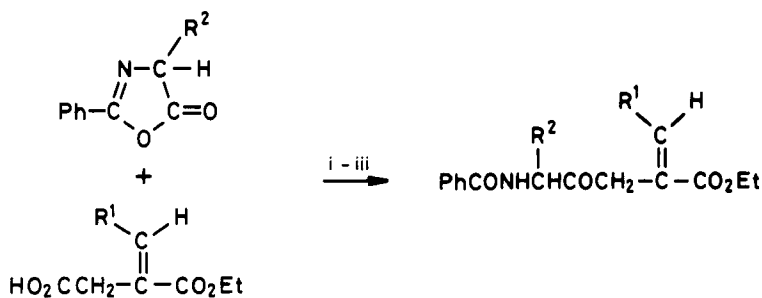
**3.2 Dehydropeptides** - Synthesis, reactions, and spectroscopic investigations in the field of dehydro-amino acids and peptides between 1982 and October 1987 has been reviewed. A survey of natural compounds of this type is included.<sup>152</sup> The crystal



(69) a;  $\text{X} = \text{S}, \text{Y} = \text{O}$   
 b;  $\text{X} = \text{O}, \text{Y} = \text{S}$

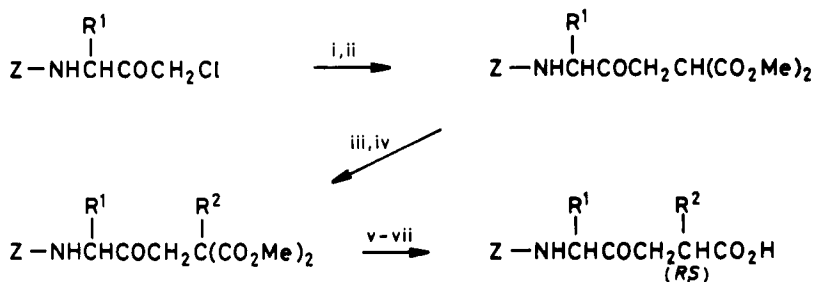


(70)



Reagents: i, base; ii, pyridine - DMAP; iii,  $\text{MeCO}_2\text{H}$

**Scheme 9**



Reagents: i,  $\text{NaI}$ ; ii,  $\text{NaCH(CO}_2\text{Me)}_2$ ; iii,  $\text{NaOMe}$ ; iv,  $\text{RBr}$ ; v,  $6\text{ M NaOH}$ ; vi,  $\text{HCl}$ ; vii, dioxan,  $100^\circ\text{C}$

**Scheme 10**

structures of Boc-Gly- $\Delta$ Phe-Gly-OMe<sup>153</sup> and Boc-Pro- $\Delta$ Leu-OMe<sup>154</sup> have been reported; both adopt type II  $\beta$ -bend conformations. 270 MHz <sup>1</sup>H-n.m.r. n.O.e. data for Boc-Ala-Leu- $\Delta^Z$ Phe-Leu-OMe are consistent with a dynamic equilibrium between an Ala- $\Delta^Z$ Phe type II  $\beta$ -turn and a second species with  $\Delta^Z$ -Phe adopting a partially extended conformation. For the isomeric Boc-Leu- $\Delta^Z$ Phe-Ala-Leu-OMe the results are compatible with a highly folded consecutive  $\beta$ -turn structure and an almost completely extended conformation.<sup>155</sup>

The decapeptide [Phe<sup>5</sup>, $\Delta$ Ala<sup>6</sup>]-LH-RH and des-Gly<sup>10</sup> [Phe<sup>5</sup>, $\Delta$ Ala<sup>6</sup>]-LH-RH have been synthesised. The unsaturated residue was introduced with Boc-Phe- $\Delta$ Ala-OH using a mixed anhydride coupling. Both analogues are inactive, suggesting that flexibility at position 6 is crucial for retention of the biological activity of LH-RH.<sup>156</sup> Formyl-Met-Leu- $\Delta^Z$ Phe-OMe, a dehydro-analogue of the chemotactic tripeptide, shows high potency in stimulating superoxide production by rabbit neutrophils. N.m.r. results in dmsd-d<sub>6</sub> support the presence of a largely extended conformation in agreement with earlier results for the parent peptide.<sup>157</sup> Z- $\Delta^1$ -dehydroglutamine has been prepared starting from  $\alpha$ -oxoglutaric acid and coupled with esters of Gly, Ala, Phe, and Leu to give the corresponding protected dipeptides.<sup>158</sup>

3.3 Peptides Containing  $\alpha,\alpha$ -Dialkylamino Acids - The synthesis of peptides containing  $\alpha$ -methylserine has been explored. Boc-Ala<sub>3</sub>- $\alpha$ MeSer-OMe was prepared in solution without side chain protection, but in solid phase synthesis side chain hydroxyl protection was found to be necessary.<sup>159</sup> [NMeAib<sup>1</sup>]- and [Aib<sup>1</sup>,Aib<sup>2</sup>]-angiotensin II have been prepared; the former has a low activity, but the latter is totally inactive.<sup>160</sup> [Lys<sup>8</sup>,Acc<sup>9</sup>]- and [Arg<sup>8</sup>,Acc<sup>9</sup>]-vasopressin (where Acc = 1-amino-cyclopropane-1-carboxylic acid) have also been synthesised, but they only have low biological activity.<sup>161</sup> All four isomers of (70), cyclopropane analogues of aspartame, have been made and found to lack taste. It is thought that the rigid positioning of the phenylalanine prevents a flat arrangement of the molecule and the orthogonal projection of the phenyl ring.<sup>162</sup> A high resolution solid-state <sup>13</sup>C-n.m.r. study of Z-Aib<sub>n</sub>-OMe (n = 3 to 8) and Z-Aib<sub>n</sub>-Leu-Aib<sub>2</sub>-OMe (n = 0 to 5) shows all the compounds to adopt an incipient or fully developed <sub>3</sub><sub>10</sub>-helical structure, as judged from the characteristic



splitting of the C $\beta$ -signals as well as the conformation-dependent displacement of the C $\alpha$  and C=O peaks.<sup>163</sup> Other conformational studies on Aib-containing peptides are listed in Table 2.

Table 2 Application of physical methods to peptides containing  $\alpha,\alpha$ -dialkylamino acids

| Peptide                                                                                                                                | Technique used                                          | Ref. |
|----------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|------|
| Alamethicin                                                                                                                            | $^1\text{H}$ -n.m.r.                                    | 164  |
| Z-Ile-NH-X-CO-NHC $_6\text{H}_4\text{p}$ -CO $_2\text{Et}$<br>(X=CMe $_2$ , CMePr $^i$ , CPr $_2^n$ , CMeBzl,<br>-(CH $_2$ ) $_3$ to6) | $^1\text{H}$ -n.m.r., $\underline{\text{X}}$ -ray, c.d. | 165  |
| Emericin fragments                                                                                                                     | $^1\text{H}$ -n.m.r.                                    | 166  |
| Boc-(R)-Eta-Hyp(Bzl)-Ala and<br>Aib-Phol                                                                                               | $\underline{\text{X}}$ -ray                             | 167  |
| (Eta= $\alpha$ -ethylalanine)                                                                                                          |                                                         |      |
| Boc-Ala-Aib-Ala-OMe $\psi$ [CSNH]<br>analogues                                                                                         | $\underline{\text{X}}$ -ray                             | 168  |
| Tfa-Deg $_2$ - $_5$ -OBu $^t$                                                                                                          | $\underline{\text{X}}$ -ray                             | 169  |
| Tfa-Deg $_2$ - $_5$ -OBu $^t$<br>(Deg=diethylglycine)                                                                                  | $^1\text{H}$ -n.m.r.                                    | 170  |
| Boc-(Aib-Val-Ala-Leu) $_2$ -Aib-OMe                                                                                                    | $\underline{\text{X}}$ -ray                             | 171  |
| Boc-(Aib-Ala-Leu) $_3$ -Aib-OMe                                                                                                        | $\underline{\text{X}}$ -ray                             | 172  |
| Boc-Trp-Ile-Ala-Aib-Ile-Val-Aib-<br>-Leu-Aib-Pro-OMe                                                                                   | $\underline{\text{X}}$ -ray                             | 173  |
| Boc-Met-Acc-OMe<br>(Acc=1-aminocyclohexane-1-carboxylic<br>acid)                                                                       | $\underline{\text{X}}$ -ray                             | 174  |
| Z-(+)- $\underline{\text{E}}$ -cyclopropane-Phe-Leu-OMe                                                                                | $\underline{\text{X}}$ -ray                             | 175  |

**3.4. Amide-Bond Analogues** - Two new methods for forming keto-methylene dipeptide isosteres have been developed. One involves condensation of an oxazolone with an  $\alpha$ -substituted succinoyl chloride half ester; dehydropeptides can be formed directly (e.g. Scheme 9).<sup>176</sup> A second process utilises  $\underline{\text{N}}$ -Z-amino acid halomethyl ketones and dimethyl malonate as starting materials to give products which are further alkylated (Scheme 10). Under the conditions used, the configuration of the  $\underline{\text{N}}$ -terminal amino

acid remains unchanged.<sup>177</sup> This method has been extended to accommodate the synthesis of  $\psi[\text{COCH}_2]$  dipeptides containing basic amino acids in the C-terminal position.<sup>178</sup> Thioacylation of amino acids by amino acid thioesters to form thiopeptides has been found to work well, but the reaction is less useful when amino acid esters or peptides are used except when the reacting nucleophile is the amino group of Gly or the imino group of Pro.<sup>179</sup>

A number of highly active peptides containing amide bond surrogates have been reported. The hexapeptide Ac-Trp(N-CHO)-Pro-Phe-MeHis-Phe $\psi[\text{CH}_2\text{NH}]$ -Phe-NH<sub>2</sub> is a 'super potent' and very selective human renin inhibitor,<sup>180</sup> and Boc-Phe-Pro $\psi[\text{CH}_2\text{O} - \text{Phe-His-Leu}\psi[\text{CHOH-CH}_2]\text{Val-Ile-(2-aminomethyl)-pyridine}$  is another highly active renin inhibitor ( $\text{IC}_{50}$  1.6 nM).<sup>181</sup> Ketomethylene analogues also feature in novel ACE inhibitors, cyclobutylcarbonyl-Tyr-Pro $\psi[\text{COCH}_2]$ -Gly-Pro-Lys or Nle-Phe $\psi[\text{COCH}_2]$ -Gly-Pro-OH having  $\text{IC}_{50}$ 's of 3.4 and 8 nM respectively,<sup>182</sup> and in an inhibitor of substance P degradation in rat hypothalamus preparations, [pGlu<sup>6</sup>, (RS)Phe<sup>8</sup> $\psi[\text{COCH}_2]$ Gly<sup>9</sup>]- substance P<sub>6-9</sub> OMe having an  $\text{IC}_{50}$  value of 1.8  $\mu\text{M}$ .<sup>183</sup>

Each peptide bond in the C-terminal octapeptide region of [Leu<sup>14</sup>]-bombesin has been replaced in turn by  $\text{CH}_2\text{NH}$  group using recently developed solid-phase methods.<sup>185</sup> One of these analogues [Leu<sup>13</sup> $\psi[\text{CH}_2\text{NH}]$  Leu<sup>14</sup>]-bombesin, is a specific bombesin receptor antagonist which blocks long-term growth promoting effects of bombesin-like peptides.<sup>184,186</sup>

Other amide bond analogues which have been reported this year are listed in Table 3.

---

Table 3 Analogues containing pseudo-peptide bonds

---

| Peptide                                                                                                                                | Context                    | Ref. |
|----------------------------------------------------------------------------------------------------------------------------------------|----------------------------|------|
| Boc-Pro $\psi[\text{CSNH}]$ -Leu-Gly-NH <sub>2</sub>                                                                                   | $\bar{\text{X}}$ -ray      | 187  |
| Boc-Ala $\psi[\text{CH}_2\text{S}]$ -Phe-OH                                                                                            | $\bar{\text{X}}$ -ray      | 188  |
| H-Leu $\psi[\text{CHOHCH}_2]$ Val-OH                                                                                                   | Synthetic method           | 189  |
| Boc-Trp-Leu $\psi[\text{E CH=CH}]$<br>L & D-Asp-Phe-NH <sub>2</sub>                                                                    | CCK <sub>4</sub> analogue  | 190  |
| Boc-Tyr(SO <sub>3</sub> H)-Nle $\psi[\text{COCH}_2, \text{NHCO},$<br>and $\text{CH}_2\text{CH}_2]$ Gly-Trp-Nle-Asp-Phe-NH <sub>2</sub> | CCK <sub>4</sub> analogues | 191  |

|                                                                                                                                                                                                      |                          |     |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|-----|
| Ac-D and L-Trp(CHO)-Pro-Phe-His                                                                                                                                                                      | Fluorescence             |     |
| $\psi$ [CH <sub>2</sub> NH]Phe-Val-Tyr-NH <sub>2</sub>                                                                                                                                               | spectroscopy             | 192 |
| tBuCO-Pro-Gly $\psi$ [CH <sub>2</sub> NH]NHMe or Et                                                                                                                                                  | Conformation             | 193 |
| $\psi$ [NHCONH] analogues of Leu-enkephalin                                                                                                                                                          | Enzyme resistance        | 194 |
| $\psi$ [CH <sub>2</sub> S] analogues of allyl <sub>1</sub> or <sub>2</sub> -<br>or H-Tyr-D-Ala-Phe-Gly-Phe or<br>Phg-NH <sub>2</sub>                                                                 |                          | 195 |
| MeOSuC-Ala <sub>2</sub> Pro $\psi$ [B(OH)N]Val<br>and Phe-OH                                                                                                                                         | $\alpha$ -Lytic protease | 196 |
| H-Tyr-Gly or D-Ala-Gly $\psi$ [NHCO]Phe-<br>-Leu-OH                                                                                                                                                  | Enkephalin analogues     | 197 |
| $\psi$ [CH <sub>2</sub> NH] and $\psi$ [CH <sub>2</sub> NAC] isosteres<br>of [NAC-D-Nal <sup>1</sup> , D-Phe <sup>2,3</sup> , D-Arg <sup>6</sup> ,<br>Phe <sup>7</sup> , D-Ala <sup>10</sup> ]-LH-RH | LH-RH antagonists        | 198 |

**3.5  $\gamma$ -Glutamyl Peptides** - Two new  $\gamma$ -glutamyl peptides have been isolated from Rhynchosia albiflora.  $\gamma$ -Glu- $\alpha$ -methylene- $\beta$ -amino-propionic acid has previously been found in two other species, but ethyl  $\gamma$ -Glu- $\beta$ -aminoisobutyrate is the first report of a natural  $\gamma$ -Glu amino acid ester.<sup>199</sup> H- $\gamma$ -Glu-Arg-OH has been found in Sphagnum palustre from Japan; it has hitherto only been isolated from the onion.<sup>200</sup>  $\gamma$ -Glu-DOPA has been obtained from L-glutamine and L-DOPA in 79% yield using the  $\gamma$ -glutamyl transpeptidase of E. coli K-12. This strain has 5-6 fold higher enzyme activity than the wild-type strain.<sup>201</sup>

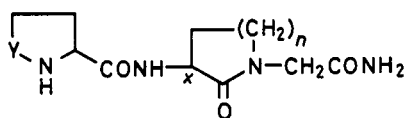
A series of N-acyl- $\gamma$ -D-glutamyl peptide derivatives containing a C-terminal fragment of cholecystokinin have been prepared and their effects on gastric acid secretion investigated. PhCO-D-Glu(Phe-NH<sub>2</sub>)-NPr<sub>2</sub> and PhCO-D-Glu(Asp-Phe-NH<sub>2</sub>)-NPr<sub>2</sub> are inhibitors, but are less effective than proglumide (PhCO-DL-Glu-NPr<sub>2</sub>). PhCO-D-Glu(Met-Asp-Phe-NH<sub>2</sub>)-NPr<sub>2</sub> and PhCO-D-Glu(Trp-Met-Asp-Phe-NH<sub>2</sub>)-NPr<sub>2</sub>, on the other hand, stimulate gastric secretion.<sup>202</sup> PhCO-Glu(Phe-NH<sub>2</sub>)-NPr<sub>2</sub>, however, is a more potent inhibitor than proglumide, while PhCO-Glu(Asp-Phe-NH<sub>2</sub>)-NPr<sub>2</sub> stimulates acid secretion.<sup>203</sup> Z, 4-chlorobenzoyl, and isonicotinoyl-D-Glu(Phe-NH<sub>2</sub>) are all more potent inhibitors than proglumide.<sup>202</sup>

**3.6 Conformationally Constrained Peptides** - The synthesis of peptides containing relatively rigid ring systems to control to some degree at least the conformation adopted has become

increasingly popular. The tetrazole ring has been shown by X-ray to closely resemble a cis-peptide bond in bond lengths and angles. H-Pro-Leu-[CN<sub>4</sub>]-Gly-NH<sub>2</sub>, however, is unable to enhance the binding of dopamine receptor antagonists to dopamine receptor, in contrast to H-Pro-Leu-Gly-NH<sub>2</sub> itself.<sup>204</sup> Of a series of other analogues of this tripeptide (71a-c), only (71b) has significant activity, being 10,000 times more active than the parent. With the Gly residue incorporated into a ring (72a-c), however, none of the materials was active.<sup>205</sup> The 2-imidazoline ring has been incorporated as an amide bond replacement in enkephalin to replace the Tyr-Gly, Gly-Gly, and Gly-Phe segments (73) and in an ACE inhibitor (74).<sup>206</sup>

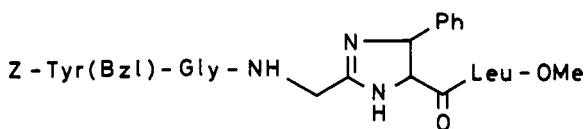
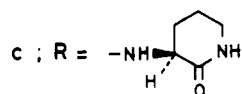
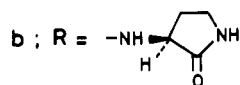
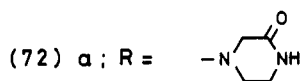
Dipeptide isosteres containing  $\gamma$ - or  $\delta$ -lactams (75) have been prepared. Diastereoisomeric mixtures at the lactam quaternary centre were separable by chromatography. The Phe-Phe surrogate could be extended to a tripeptide at both its N- and C-terminus without problems.<sup>207</sup> The bicyclic system (76) has been prepared as a type I  $\beta$ -turn mimetic. A key step in the synthesis was a previously unreported intramolecular Diels-Alder reaction of an azo-dicarbonyl system (Scheme 11).<sup>208</sup> Another structure designed to induce a  $\beta$ -turn is a novel spiro-cyclic structure which has been incorporated into the nonapeptide H-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-OH to give (77). <sup>1</sup>H-n.m.r. evidence is in agreement with a single strong hydrogen bond as depicted.<sup>209</sup>

A linear tetracyclic system has been incorporated into the centre of a peptide to stimulate the formation of anti-parallel pleated sheets. The <sup>1</sup>H-n.m.r. spectrum in dmsO-d<sub>6</sub> is in agreement with the  $\beta$ -turn,  $\beta$ -sheet conformation (78).<sup>210</sup> The synthesis has also been described of a relatively rigid functionality (79) ( $\alpha$ -Temp) containing three amide carbonyls oriented with the pitch and spacing of an  $\alpha$ -helix. It was designed to enhance the probability of helix formation in an attached peptide.  $\alpha$ -Temp has been coupled to peptides to give  $\alpha$ -Temp-Ala<sub>4</sub>-OMe and  $\alpha$ -Temp-Ala<sub>2</sub>-Phe-Lys(Boc)-Lys(Boc)-NHMe, and in MeCN these compounds have been shown to adopt distorted  $\alpha$ -helical conformations with a bifurcated hydrogen bond.<sup>211</sup> A new series of conformationally restricted analogues of angiotensinogen (80) have been prepared. When n=3, the compound was inactive, and when n=7, it was equipotent with the parent. However, when n=5 it was 6-fold more potent than the parent.<sup>212</sup>

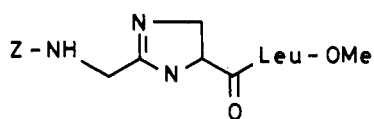


H-Pro-Leu-R

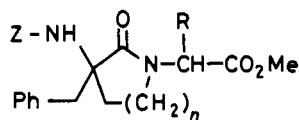
|      |                 |   |   |
|------|-----------------|---|---|
| (71) | Y               | x | n |
| a ;  | CH <sub>2</sub> | S | 1 |
| b ;  | CH <sub>2</sub> | R | 1 |
| c ;  | CH <sub>2</sub> | S | 2 |
| d ;  | CO              | S | 1 |
| e ;  | CO              | R | 1 |



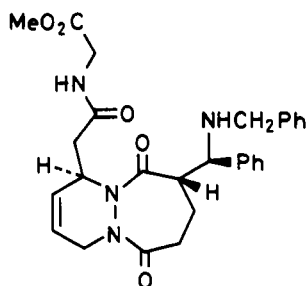
(73)



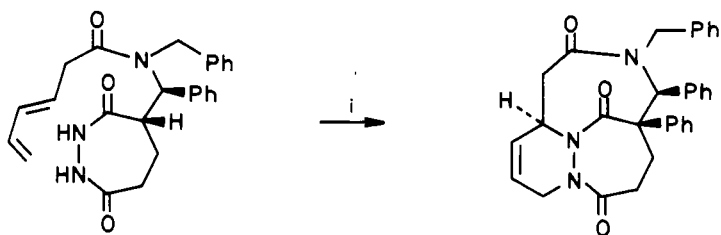
(74)



(75) n = 1 or 2

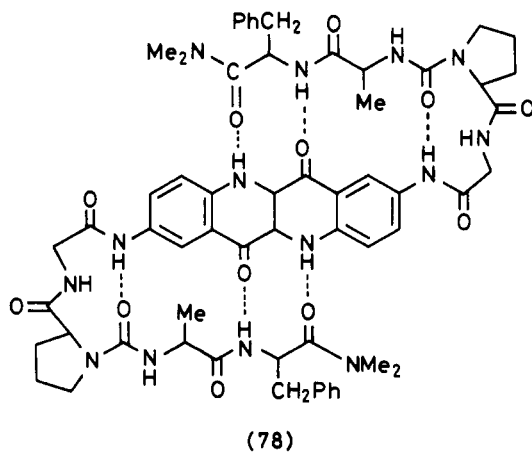
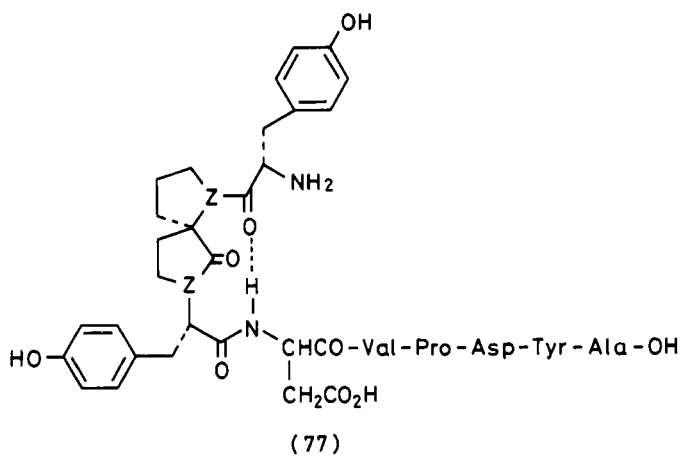


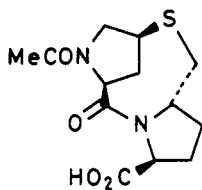
(76)



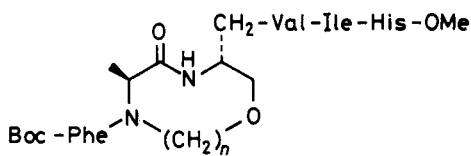
Reagent : i,  $\text{PhI}(\text{OAc})_2$

**Scheme 11**

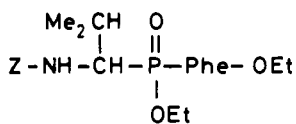




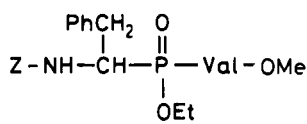
(79)



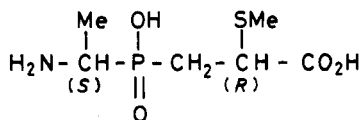
(80)



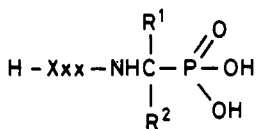
(81)



(82)

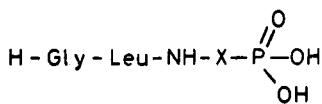


(83)



(84)

| Xxx    | R <sup>1</sup>                     | R <sup>2</sup>                  |
|--------|------------------------------------|---------------------------------|
| a; Leu | Me                                 | Et                              |
| b; Phe | Me                                 | CHMe <sub>2</sub>               |
| c; Pro | c-C <sub>3</sub> H <sub>5</sub>    | c-C <sub>3</sub> H <sub>5</sub> |
| d; Ala | -(CH <sub>2</sub> ) <sub>4</sub> - |                                 |



(85)

|    |                                                            |
|----|------------------------------------------------------------|
| a; | X = -CH <sub>2</sub> -C(=CH <sub>2</sub> )=CH <sub>2</sub> |
| b; | X = -C(=CH <sub>2</sub> )=CH <sub>2</sub> -                |

3.7 Phosphono-peptides - Two novel hydrophilic antifungal peptide antibiotics have been isolated from Bacillus subtilis, H-Arg-L-2-amino-5-phosphono-3-cis-pentenoic acid (Arg-APPA) and H-Val-Arg-APPA, named rhizocticins A and B. Also found were small amounts of tripeptides containing Ile or Leu in place of Val (rhizocticins C and D). D-APPA has previously been found in the plumbemycins, threonine antagonists.<sup>213</sup>

A convenient method for the synthesis of amide esters and mixed esters of phosphonic acids involves oxidising trimethylsilyl phosphonites with  $\text{CCl}_4$  to generate phosphonochloridates in the presence of the amine or alcohol nucleophile to minimise side reactions. In this way, for example, (81) and (82) were prepared in 74% and 67% yields respectively.<sup>214</sup> Phosphonodepsipeptides have been prepared too by reacting [1-(benzyloxy-carbonylamino)alkyl]phosphonic acids with  $\alpha$ -hydroxy acid p-nitrobenzyl esters in the presence of thionyl chloride in DMF.<sup>215</sup>

A series of phosphonodipeptide analogues related to H-D-Ala-D-Ala-OH have been synthesised. The best antibacterial activity was shown by (83), whose MIC's range from 4-128  $\mu\text{g/mL}$  on a series of nine bacterial organisms.<sup>216</sup> The  $^{31}\text{P}$ -n.m.r. resonances in 5 N-phospho and N-phosphono derivatives of Ala-Pro indicate the presence of s-cis and s-trans resonances for the Pro carbons in the same intensity ratio as observed by  $^{13}\text{C}$ -n.m.r.<sup>217</sup> Aminoalkyl phosphonic acids can be converted into their diethyl esters using triethyl orthoformate. The amino group is formylated in the process, but the formyl group can be selectively removed with methanolic HCl. Using the products, a number of dipeptide phosphonates have been prepared by the mixed anhydride method, using HBr/HOAc for cleavage of the protecting groups. Several of these dipeptides involved  $\alpha$ -dialkyl amino phosphonic acids (84).<sup>218</sup>

The LD diastereoisomer of Pr-Ala-Apb-OH (where Apb=2-amino-4-phosphonobutyric acid) has proved to be a moderate inhibitor of the meso-diaminopimelate-adding enzyme from E. coli,<sup>219</sup> the LL compound being much less effective. Ac-Ser(P)-Ser(P)-Ser(P)-OH ( $\text{P}=\text{PO}_3\text{H}_2$ ) which reproduces a recurrent segment of most caseins, has been synthesised. After partial dephosphorylation by acid phosphatase it serves as an efficient substrate for casein kinase-2. Dephosphorylation beyond 30% hinders subsequent phosphorylation.<sup>220</sup> The related octapeptide Ac-Glu-Ser(P)-Leu-



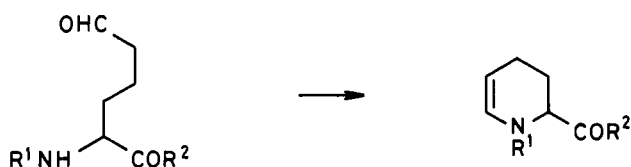
-Ser(P)-Ser(P)-Ser(P)-Glu-Glu-NHMe, whose sequence corresponds to positions 14-21 and 5-12 of bovine and human  $\beta$ -casein respectively, has also been prepared in a stepwise solution phase synthesis using Boc-Ser( $\text{PO}_3\text{Ph}_2$ )-OH to incorporate phosphoserine residues. The phenyl protecting groups were cleaved hydrogenolytically.<sup>221</sup> The structure of antibiotic A53868A from Streptomyces luridus has been revised from (85a) to (85b) on the basis of the pattern of C-P coupling constants in the  $^{13}\text{C}$ -n.m.r. spectrum. Phosphorus is directly bonded to the non-protonated carbon.<sup>222</sup>

The tripeptide H-Pro-Tyr(P)-Val-OH has been constructed as a model substrate for tyrosine specific kinases. Deprotection of the methyl groups used to protect the  $\text{O}$ -phosphotyrosine with HF proved unsatisfactory, but use of 1M TMSBr/TFA/m-cresol was effective.<sup>223</sup>

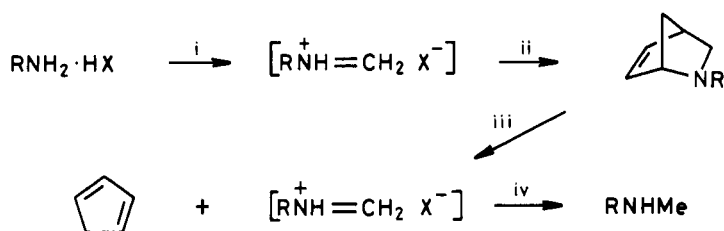
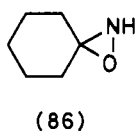
3.8 Peptides Containing Modified Protein Constituents - Allysine, which arises naturally from lysine in some proteins, is an important crosslink precursor. Two independent routes to prepare allysine derivatives have now been developed. In peptides, an unexpected nucleophilic attack of the allysine amide nitrogen upon the aldehyde was found, giving derivatives of 5,6-dehydropipecolic acid (Scheme 12). To prevent this reaction, Boc-Pro-allysine-Pro or Gly-ONb has been prepared with the allysine protected as its dimethyl or diethyl acetal.<sup>224</sup>

A smooth and mild procedure is reported for the  $\text{N}$ -amination of amino acid and peptide derivatives with cyclohexanespiro-3'-oxaziridine (86). The process is racemisation free.<sup>225</sup> Primary amides can be efficiently converted to nitriles using the Burgess reagent  $\text{MeO}_2\text{CNSO}_2\text{NEt}_3$ . Its chemoselectivity makes it useful for peptides, secondary amide bonds being unaffected. Boc-Gly-Phe-NH $_2$ , for example, can be converted by this reagent to Boc-Gly-NHCH( $\text{CH}_2\text{Ph}$ )CN in 91% yield.<sup>226</sup>

A novel method for the  $\text{N}$ -methylation of peptides features a room temperature retro-Diels Alder reaction of  $\text{N}$ -substituted 2-azanorbornenes (Scheme 13). Both steps are conducted in an acid medium; there is no racemisation.<sup>227</sup> An investigation of 30 peptides containing a number of  $\text{N}$ -alkylated amino acids has revealed a facile acidolysis between the second and third residue in a sequence of three L-imino acid residues. This has implications for synthetic strategy as cleavage can occur under conditions used for deprotection of Boc-peptides or

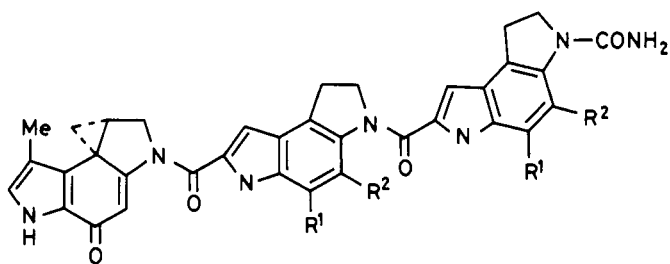


Scheme 12



Reagents: i,  $CH_2O, H_2O, 20^\circ C$ ; ii, cyclopentadiene,  $H_2O$ ; iii,  $CHCl_3 : TFA, 1:1, 20^\circ C$ ; iv,  $HSiEt_3$

Scheme 13



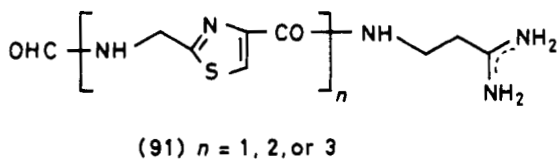
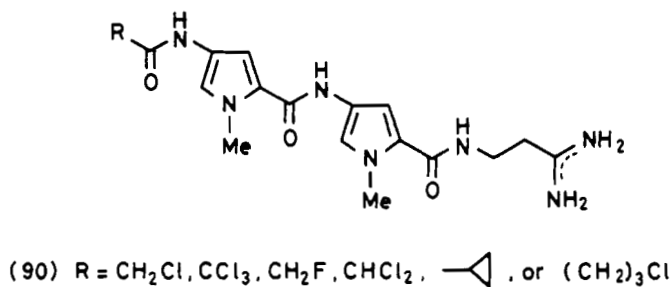
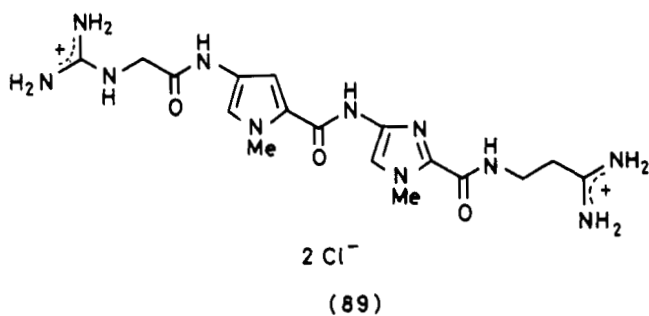
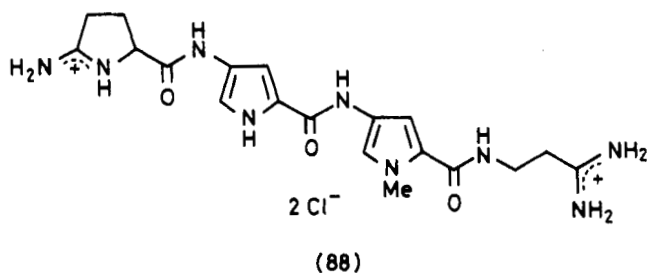
(87) a;  $R^1 = OMe, R^2 = OH$   
b;  $R^1 = R^2 = H$

t-butyl esters.<sup>228</sup> C-Terminal N-alkylated amino acid residues in peptide acids are also prone to acid hydrolysis. A mechanism has been proposed in which nucleophilic attack of the conjugate base of the terminal acid on the protonated preceding cis-peptide bond occurs.<sup>229</sup>

The dipeptide Pr-Ala-D-Met sulfoximine-OH is a moderately effective inhibitor of the meso-diaminopimelate-adding enzyme from E. coli, but the potential affinity labeller Pr-Ala-D-Gly-(CH<sub>2</sub>Cl)-OH is a better inhibitor.<sup>219</sup> Of a series of polar enkephalin-related peptides synthesised, H-Tyr-D-Arg-Gly-Phe-(4-NO<sub>2</sub>)-Pro-NH<sub>2</sub> has been selected as worthy of further investigation in view of its pharmacological profile.<sup>230</sup> The analogue of the leech anticoagulant peptide hirudin [Phe(4-Cl)<sup>56</sup>]-hirudin<sub>54-65</sub> only has low potency, while [Phenylglycyl<sup>56</sup>]-hirudin<sub>54-65</sub> is nearly inactive.<sup>231</sup> [DOPA<sup>1</sup>]-Angiotensin II also has only a low biological activity.<sup>146</sup> The affinity inactivators Ac-Leu-Orn(BrAc)-Arg-Ala-Ser-Leu-Gly-OH and Ac-Leu-Arg-Orn(BrAc)-Ala-Ser-Leu-Gly-OH have been used as probes to study the catalytic subunit of cyclic AMP-dependent protein kinase. The former alkylates Thr-197 and Glu-346 and the latter Cys-199 and Glu-346. Overall it was concluded that Thr-197 and Cys-199 are located at, or near, the active site.<sup>232</sup>

3.9 Peptides Containing Other Unusual Amino Acids - Full details of the formal total synthesis of the potent anti-tumour antibiotic CC-1065 (87a) have been published. All six ring nitrogen atoms are derived ultimately from sodium azide.<sup>233</sup> A bis-des-hydroxy, bis-des-methoxy analogue of CC-1065 (87b) has also been made. It shows a lower induced c.d. in the presence of DNA than CC-1065, indicating that the o-catechol substituents are important in stabilising its non-covalent binding to DNA. However, it has a similar biological activity to CC-1065, including the usual phenomenon of delayed death in mice.<sup>234</sup>

The total synthesis of anthelvencin C (88), first isolated in 1965 from Streptomyces venezualae, has been achieved; this establishes unambiguously the natural configuration as (4S)-(+)-. Attempts to prepare the same compound with the second pyrrole ring N-methylated gave only an unstable hygroscopic material.<sup>235</sup> A high resolution <sup>1</sup>H-n.m.r. study of the binding of a prototype lexitropsin (89) to the deoxynucleotide d(GGCAATGCG)<sub>2</sub> shows the drug binding centrally to the minor groove. Unlike the parent antibiotic netropsin, the lexitropsin is subject to



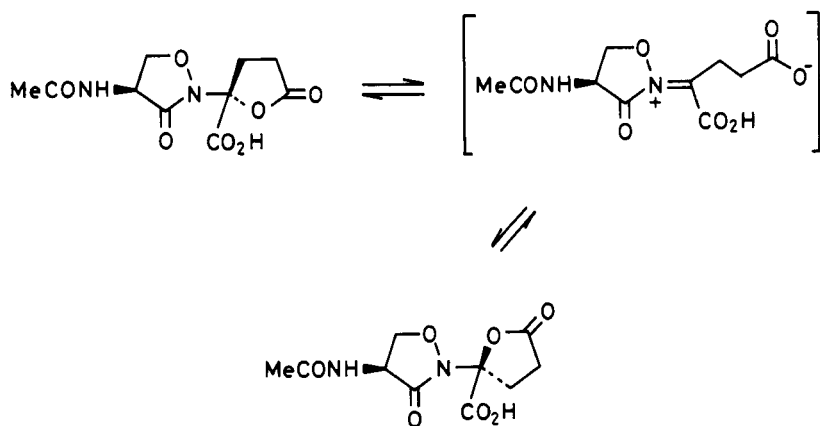
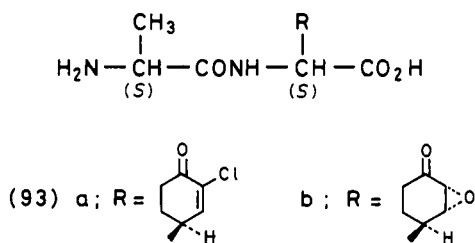
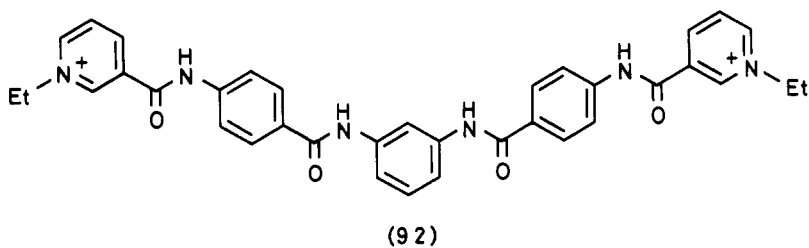
significant propeller twisting in both free and bound forms.<sup>236</sup>

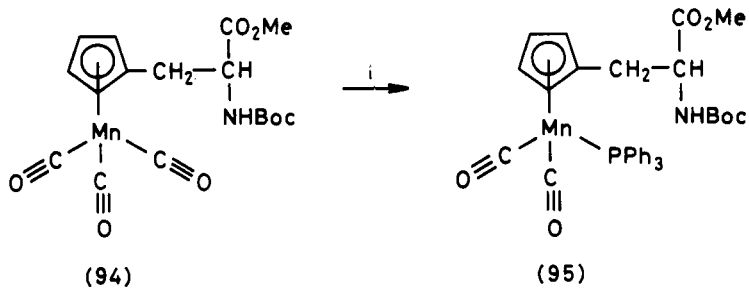
A group of oligopeptides have been synthesised that are related to distamycin and netropsin but which bear alkylating functions (90). The chloroacetyl compound proved the most potent; it is 15-45 times more active than netropsin and 3-18 times more active than distamycin against a variety of murine and human tumour cell lines.<sup>237</sup> Three sulphur-containing analogues (91) of netropsin and distamycin have been made in which N-Me pyrrole is replaced by thiazole, a unit occurring in other highly cytotoxic natural cyclopeptides. No biological activities are given.<sup>238</sup> Another novel synthetic analogue of distamycin (92) recognises only B-DNA, to which it binds via the minor groove. Unlike distamycin, it binds with comparable affinities to A-T and G-C base pairs in a natural DNA. The backbone curvature of this ligand is less than in distamycin.<sup>239</sup>

A new antifungal dipeptide antibiotic chlorotetain (93a) has been isolated from a Bacillus subtilis strain. Bacilysin (93b) was earlier isolated from the same source, but it has been established that chlorotetain is not an artefact produced on isolation.<sup>240</sup> The novel antibiotic lactivicin from Empedobacter lactamgenus and Lysobacter albus has  $\beta$ -lactam-like properties. In aqueous solution it exists as an equilibrium mixture of two epimers in roughly equal quantities (Scheme 14).<sup>241</sup>

Analogues of angiotensin have been prepared with residue 8 replaced by cymantrenylalanine, prepared using (94), or its triphenylphosphine derivative (95), (Scheme 15). On rabbit aorta strips these analogues are pure antagonists with higher affinities than most other analogues substituted in position 8 with bulky amino acids.<sup>242</sup> Two groups have incorporated cobalt carbonyls into amino acids. They facilitate i.r. detection as they have intense absorption at 2091, 2048, and 2021  $\text{cm}^{-1}$ . [ $\text{Aha}^{11}$ ]-substance P and [ $\text{Aha}^{10}$ ]-neurokinin A have been prepared (Aha = 2-amino-4-hexynoic acid) and subsequently derivatised (Scheme 16). A similar complex was prepared from neurokinin containing (96) in place of Lys residue.<sup>243</sup> The dipeptides Boc-Phe-Aha-OMe and Ac-Aha-Phe-OMe have also been derivatised with  $\text{Co}_2(\text{CO})_8$  to explore FT-i.r. as a non-radioactive method to study peptide-receptor interaction.<sup>244</sup>

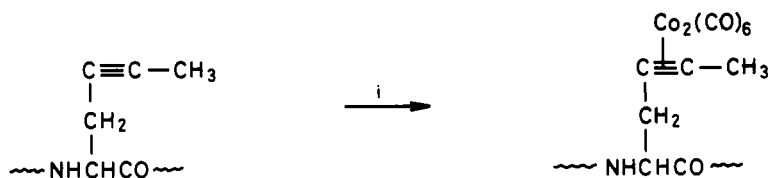
A new tripeptide immunomodulator (97) from a Discosia species contains an unusual central  $\beta$ -amino acid. The peptide has been synthesised, the  $\beta$ -amino acid being introduced via





Reagent: i,  $\text{PPh}_3$ ,  $h\nu$

**Scheme 15**



Reagent: i,  $\text{Co}_2(\text{CO})_8$

**Scheme 16**



methyl (2S,3S)-1-tosyl-3-methyl-2-aziridinecarboxylate.<sup>245</sup> A number of taurine-containing dipeptides have been prepared. All showed high cross-reactivities with antisera against haptenic taurine  $\gamma$ -Glu-Tau (glutaurine), which occurs in mammalian parathyroids.<sup>246</sup> Dipeptide derivatives of the general structure X-Phg-Yyy-OMe (where Phg = L- or D-phenylglycine) show separate methyl ester peaks in the  $^1\text{H}$ -n.m.r. for L and D C-terminal amino acid enantiomers. X can be a protecting group, hydrogen, or another amino acid residue.<sup>247</sup>

Other peptides containing unusual amino acids are listed in Table 4.

---

Table 4      Peptides containing non-protein amino acids

---

|                                                                                                                                                                                                    | Ref. |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| N-Ac[D-Nal(2) <sup>1</sup> ,D-pCl-Phe <sup>2</sup> ,D-Trp <sup>3</sup> ,D-homoArg(Et <sub>2</sub> ) <sup>6</sup> ,D-Ala <sup>10</sup> ]-LH-RH                                                      | 248  |
| Antide analogue containing 3-(4-pyrazinyl-carbonylamino)cyclohexyl)alanine                                                                                                                         | 249  |
| LH-RH antagonists containing D-citrulline and D-homocitrulline                                                                                                                                     | 250  |
| Adamantaneacetyl-D-Tyr(Et)-Phe-Val-Asn-Abu-Pro-Arg <sub>2</sub> -NH <sub>2</sub>                                                                                                                   | 251  |
| HO <sub>3</sub> S-Asp-Tyr(OSO <sub>3</sub> <sup>-</sup> )-Nle-Gly-Trp-Nle-Asp-Phe-NH <sub>2</sub>                                                                                                  | 252  |
| Boc-[Nle <sup>28</sup> ,Orn(Z) <sup>31</sup> ]-CCK <sub>27-33</sub>                                                                                                                                | 253  |
| Ac-[Nle <sup>4</sup> ,Phg <sup>7</sup> or Tic <sup>7</sup> ]- $\alpha$ -MSH <sub>4-11</sub> NH <sub>2</sub> (where Phg=phenylglycine, Tic=tetrahydro-isoquinoline carboxylic acid)                 | 254  |
| Dermorphin tetrapeptides containing Phg, PheAbu,homoPhe,Nal(1) and Nal(2)                                                                                                                          | 255  |
| L- $\alpha$ -Aminoadipoyl-Cys-D-[4-H <sup>2</sup> ]isodehydrovaline                                                                                                                                | 256  |
| Nps-Val-D-Hiv-D-Val-Lac-OBzl and dichloroacetyl-(Lac-Ala) <sub>3</sub> -OBzl                                                                                                                       | 257  |
| Boc-Ala-(R)Iva-OMe                                                                                                                                                                                 | 258  |
| ACE inhibitory di- and tripeptides containing DL- <u>erythro</u> - $\alpha$ -amino- $\beta$ -phenylbutyric acid, DL- <u>erythro</u> - $\beta$ -phenylserine, and $\alpha$ -aminocyclohexanoic acid | 259  |
| Z-L-Aminosuccinyl-Phe-NH <sub>2</sub>                                                                                                                                                              | 260  |

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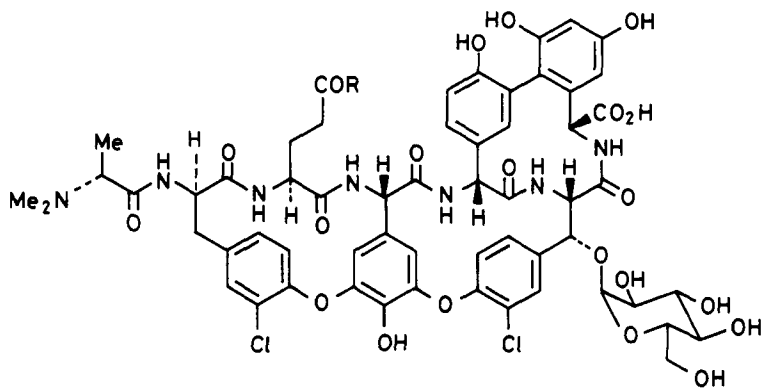
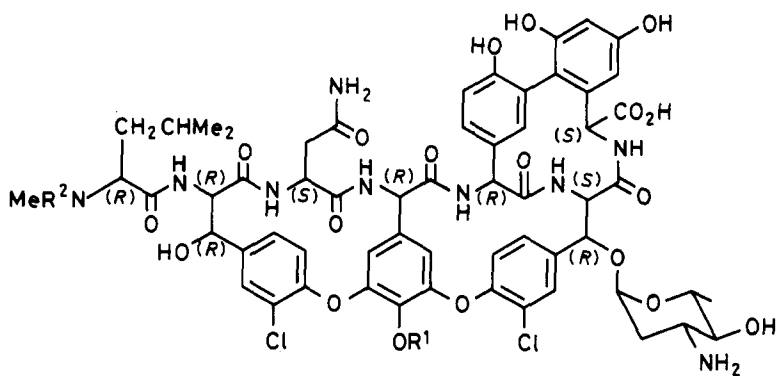
#### 4 Conjugate Peptides

4.1 Glycopeptide Antibiotics - Two groups have determined the structure of the vancomycin-like antibiotic OA-7653 (98) from Streptomyces hygroscopicus; glucose is the sole carbohydrate component. Neither Ac-D-Ala-D-Ala-OH nor Ac<sub>2</sub>-Lys-D-Ala-D-Ala-OH binds as strongly to OA-7653 as to vancomycin, possibly because of the lack of one of the ring sugar residues.<sup>261,262</sup> The chloro-orienticins (99), new vancomycin-type antibiotics, have been obtained from Amycolatopsis orientalis. Some are more active than vancomycin itself.<sup>263</sup>

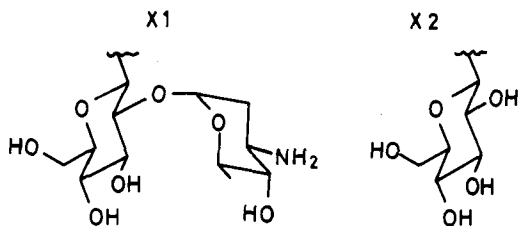
Four different N-acetylated derivatives of vancomycin and two partial hydrolysis products have been obtained, and found to still complex with Ac<sub>2</sub>-Lys-D-Ala-D-Ala-OH, but none as effectively as vancomycin itself.<sup>264</sup> Mono-N-aracyl derivatives of the vancosamine moiety of vancomycin are in general more active than aliphatic N-acyl derivatives. The most potent are p-octylbenzoyl and p-octyloxybenzoyl.<sup>265</sup> In solution, the N-terminal region of vancomycin has been found to be much more mobile than the C-terminal region. In particular, the amide bond connecting residues 2 and 3 rotates 180° between two conformers of comparable energy.<sup>266</sup>

Vancomycin and related antibiotics can be selectively cleaved by TFA at -15 °C for 40 h. This removes only the amino sugar vancosamine. The second sugar, glucose, can be removed with TFA at 50 °C for 3 h.<sup>267</sup> A synthetic analogue of the carboxylate-binding pocket of vancomycin has been made (100). Changes in the <sup>1</sup>H-n.m.r. spectrum on addition of one equivalent of cyanoacetic acid are consistent with an association which involves proton transfer from acid to amine and complexation of the carboxylate anion by multiple hydrogen bonding to amide groups on the receptor.<sup>268</sup>

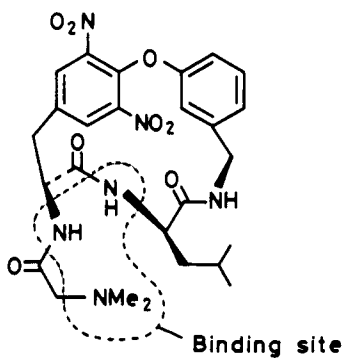
A synthetic model for the metal binding site of bleomycin with a 4-methoxypyridine nucleus and a t-butyl groups has been found to be comparable to bleomycin in terms of dioxygen activation. On coupling this to a tetrapeptide to give (101), the product obtained shows a potent DNA-cleaving activity in vitro (~ 10% of that of bleomycin). It has a somewhat random cleavage pattern.<sup>269</sup> The intercalating agent 4'-(9-acridinylamino)-methanesulphon-m-anisilide is active against a wide range of tumours and is in clinical use. It has now been linked to the complexing part of bleomycin to give (102), which has been

(98) R = OH or NH<sub>2</sub>

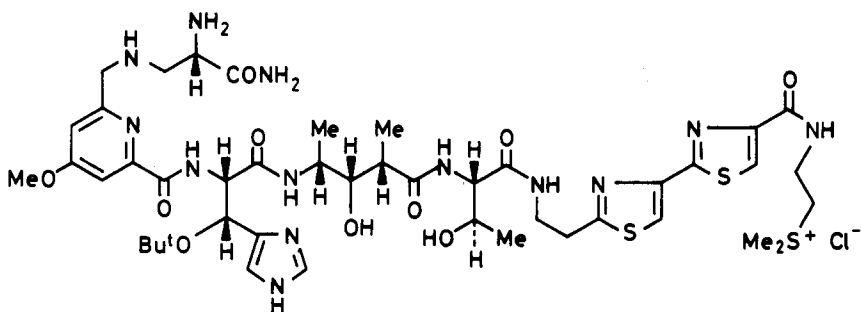
|   | R <sup>1</sup> | R <sup>2</sup> |
|---|----------------|----------------|
| A | X1             | H              |
| B | X2             | H              |
| C | H              | H              |
| D | X1             | Me             |
| E | X2             | Me             |



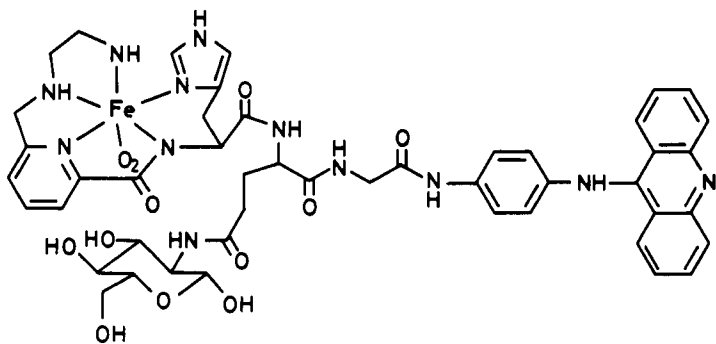
(99)



(100)



(101)



(102)

found to induce a high level of single-strand breakage of duplex DNA.<sup>270</sup>

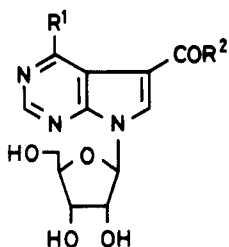
Dipeptide conjugates of sangivamycin (103a) and desamino-sangivamycin acid (103b) have been prepared. Sangivamycin is a natural nucleoside antibiotic active against L1210 leukaemia and Lewis lung carcinoma in mice. No biological activities of the dipeptide conjugates have been reported.<sup>271</sup>

4.2 Other Glycopeptides - The synthesis of glycopeptides has been reviewed.<sup>272</sup> This is timely in view of the increasing attention being paid to this topic. The presence of glycolipids and glycoproteins in opioid receptors is well established. In an attempt to improve the delivery effectiveness, a glycosyl-enkephalin has been prepared. [D-Met<sup>2</sup>,Pro<sup>5</sup>]-enkephalin[N<sup>1,5</sup>- $\alpha$ -D-glucopyranosyl]-amide had 2000x and 200x the antinociceptive activity of morphine in rats and mice respectively.<sup>273</sup> D-Glucose has also been linked to [Leu<sup>5</sup>]-enkephalin as an ester through the C-terminal carboxyl group. The 1-O-glucoside was somewhat more active than [Leu<sup>5</sup>]-enkephalin, but the 6-O-derivative is considerably less potent.<sup>274</sup>

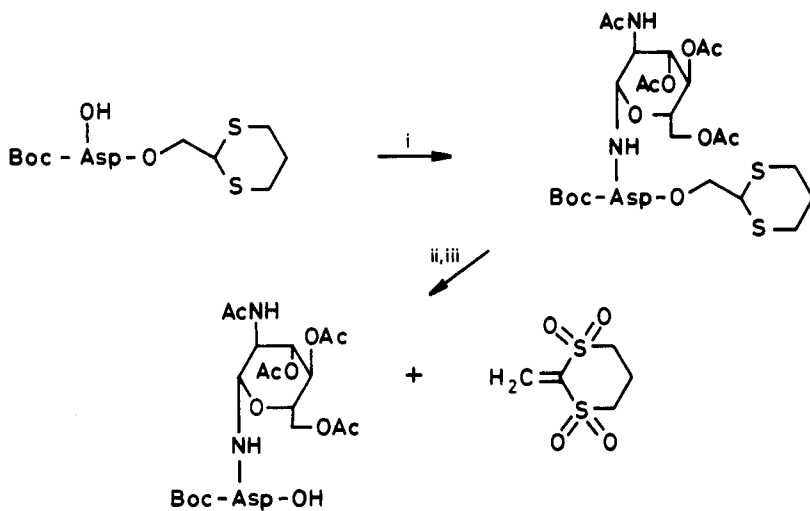
Vespulakinin is a naturally occurring derivative of bradykinin from the yellowjacket isolated in 1976. A glycopeptide containing the N-terminal segment of vespulakin, Z-Thr(Bu<sup>t</sup>)-Ala-Thr-[ $\alpha$ -Gal(Bzl)<sub>4</sub>]-Thr(Bu<sup>t</sup>)-NHNBoc, has now been prepared.<sup>275</sup> 1,3-Dithian-2-ylmethyl esters have been used for C-protection during the synthesis of per-acetylglucosaminyl-asparagine dipeptides (Scheme 17). These esters are made by Al isopropoxide catalysed transesterification of amino acid methyl esters, and can be cleaved at pH 8 after oxidation of the sulphur atoms to sulphones.<sup>276</sup>

The solid phase synthesis of a glycotriptide has been carried out using an allylic anchor group. The final product (104) can be cleaved to free the glycopeptide under almost neutral conditions using [(Ph<sub>3</sub>P)<sub>4</sub>Pd] in morpholine-THF (1:10 v/v). Boc, Fmoc, OAc groups, and glycosidic linkages remain intact during this cleavage.<sup>277</sup> Partial sequences of ovine submaxillary mucin, H-Ala-Gly-Ala-Gly-Thr( $\alpha$ -D-GalNAc)-Ala-OH, and porcine glycophorin, H-Ala-Thr( $\alpha$ -D-GalNAc)-Val-Thr( $\alpha$ -D-GalNAc)-Ala-Gly-OH, have also been prepared using solid phase synthesis. In this case Boc-amino acids, Fmoc-Thr( $\alpha$ -D-GalNAc(OAc)<sub>3</sub>)-OH, and a 4-alkoxy-benzyl resin were used.<sup>278</sup>

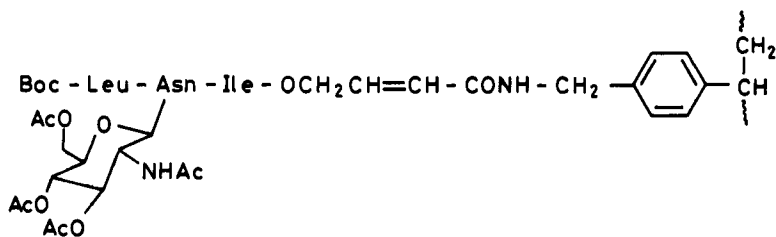
Sequence 3-6 (105) of the protein core of a proteodermatan



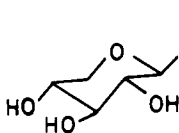
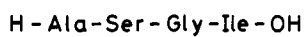
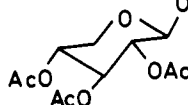
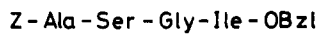
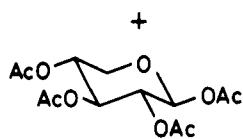
b;  $R^1 = OH$ ,  $R^2 = -Lys - Gln - NH_2$



### Scheme 17



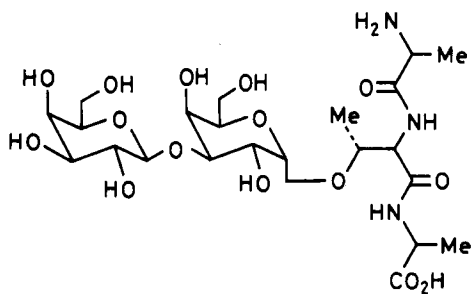
(104)



(105)

Reagents : i, trimethylsilyl triflate ; ii, Pd/H<sub>2</sub>; iii, NaOMe-MeOH, pH 8.5

**Scheme 18**



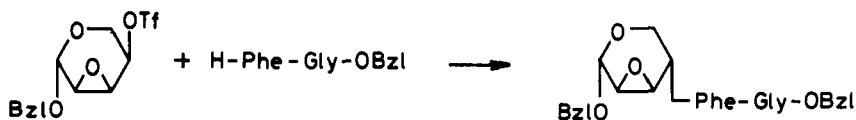
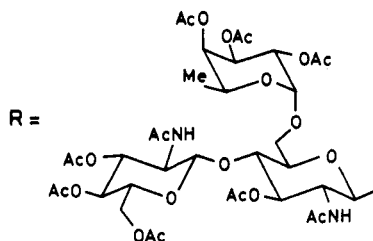
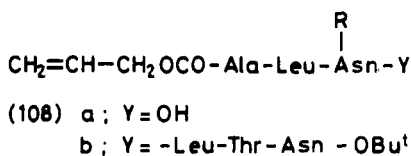
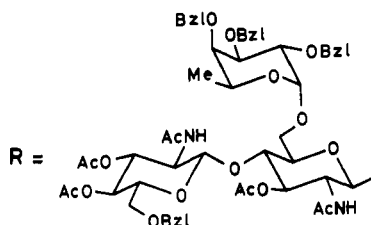
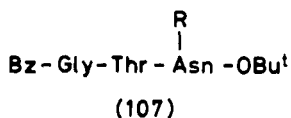
(106)

sulphate has been prepared by direct glycosidation of the protected tetrapeptide (Scheme 18),<sup>279</sup> and a close analogue of the repeating unit of the antifreeze glycoprotein of the polar fish (106) has been prepared (in protected form) by coupling a protected disaccharide chloride with Z-Ala-Thr-Ala-2,2,2-trichloroethyl ester (yield 63%).<sup>280</sup> Attempted selective cleavage of the t-butyl ester group of (107) with TFA leads to complete cleavage of the fucoside bond of the fucosyl-chitobiose unit. However, (108a) was converted to the peptide acid in 95% yield with TFA, enabling the preparation of (108b), which corresponds to a segment of the linking region of the envelope protein of a leukaemia virus.<sup>281</sup>

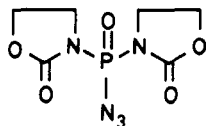
An  $\alpha$ -N glycodipeptide has been prepared by reacting a 4-O-triflyl- $\beta$ -L-ribofuranoside derivative with a free  $\alpha$ -amino group, inversion at C-4 occurring during the process (Scheme 19).<sup>282</sup> New  $\epsilon$ -N-fluorescently labelled MDP derivatives for use as biologic probes have been prepared by reacting  $N^{\alpha}$ -(N<sub>2</sub>AcMur)-Lys-D-iGln-OH and  $N^{\alpha}$ -(N<sub>2</sub>AcMur)-Ala-D-iGln-Lys-OH with 2-(fluoresceinylamino)-4,6-dichloro-s-triazine. The tripeptide product produces an exaggerated antibody response, but the dipeptide gives a response similar to the MDP control.<sup>283</sup>

A simple synthesis of MDP, nor-MDP, and murabutamide using a new coupling agent BOPA (109) has been developed. In the coupling the azide group of BOPA is replaced by hydroxyl, giving a water-soluble byproduct.<sup>284</sup> Although N<sub>2</sub>Ac-norMur-Abu-D-Gln-OBu is inactive in the delayed hypersensitivity test, its  $\alpha$ -benzylglycoside is comparable in activity to MDP. Conversely, N<sub>2</sub>AcMur-Abu-Gln-OBu is more active than MDP, but its  $\alpha$ -benzylglycoside less potent.<sup>285</sup> A <sup>1</sup>H-n.m.r. study of MDP in dmsd-d<sub>6</sub> confirms the S-shaped conformation with two adjacent  $\beta$ -turns. The second of these turns can be correlated with the undesirable pyrogenic activity of MDP, but as this turn requires the  $\alpha$ -carboxamide of D-iGln for its stabilisation, it does not occur in murabutamide. [D-Ala]-MDP, however, forms a cyclic structure with the iGln  $\alpha$ -carboxamide protons and the acetamido group in close proximity, and this is thought to account for its loss of anti-infective activity.<sup>286</sup>

4.3 Non-Carbohydrate Conjugate Peptides - 9-Acridinyl (Acr) groups have been linked to the  $\alpha$ -amino groups of some peptides through  $\epsilon$ -aminohexanoic acid (Aha), and the DNA-photocleavable group 4-nitrophenylsulphonyl (Npso) to the  $\epsilon$ -amino group of some



Scheme 19



(109)

- (110) a ; Acr - Aha - Lys - Glu - Leu - Phe - Glu - Asp - Leu - Gln - Lys -  
-Leu - Ala - Lys - Gln - Phe - Tyr - Gly - OH  
b ; Acr - Aha - His - Pro - Gly - Asp - Ser - Gly - OH  
c ; Acr - Aha - Ser - Gly - Lys (Npso) - Gly - OH  
d ; Acr - Aha - Lys (Npso) - Gly - Lys - Gly - OH

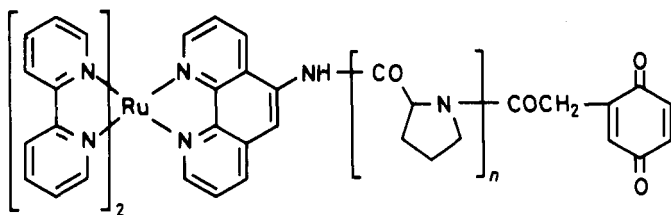
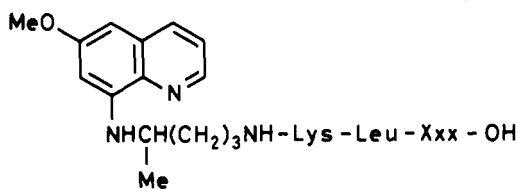


lysine-containing peptides (110a to d). Calf thymus DNA binds 1 mol of (99b) for every four base pairs, whereas (110a) seems to aggregate with the DNA already at a ratio of ligand concentration to base pairs of less than 0.2 to form a precipitate. In a plasmid relaxation assay, (110c) has no ability to photocleave DNA, but (110d) has photonuclease activity with some evidence of sequence preference.<sup>287</sup>

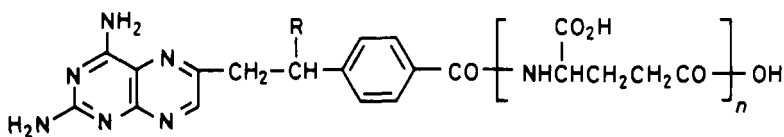
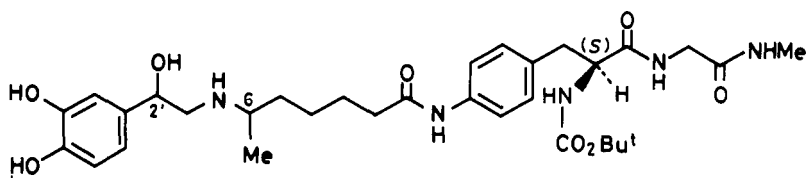
Oligoproline bridges have been used to link polypyridyl Ru(II) complexes with *p*-benzoquinone (and *p*-dimethoxybenzene as a control) (111). Luminescence experiments have established that the efficiency of electron transfer quenching falls as the number of prolyl residues increases.<sup>288</sup> Three peptide derivatives of primaquine have been synthesised (112) and tested for radical curative antimalarial activity in rhesus monkeys. All three showed greater activity than that expected for the primaquine content of each prodrug, the D-Val compound seeming the least toxic and the most active.<sup>289</sup>

The experimental anti-cancer drugs 10-deazaminopterin and its 10-ethyl derivative have been linked to the  $\alpha$ -amino group of oligo- $\gamma$ -glutamates (113). In general, the antifolate activity decreases with increasing chain length.<sup>290</sup> The complexones  $n\text{-C}_{10}\text{H}_{21}\text{CH}(\text{CH}_2\text{SCH}_2\text{CO-Gly-Gly-OMe})_2$  and  $n\text{-C}_{10}\text{H}_{21}\text{C}(\text{CH}_2\text{SCH}_2\text{CO-Gly-Gly-OMe})_3$  and the corresponding di- and triacids have been prepared as simple analogues of electron-transfer proteins which can bind various metal-ion oxidants.<sup>291</sup> A new analogue of the anti-arrhythmic peptide H-Gly-Pro-Hyp-Gly-Ala-Gly-OH from bovine atria, Hpp-Pro<sub>2</sub>-Gly-Ala-Gly-OH (where Hpp = N-3-(4-hydroxyphenyl)propionyl), has proved even more effective in inhibiting calcium-induced arrhythmias than Hpp-Pro-Hyp-Gly-Ala-Gly-OH.<sup>292</sup>

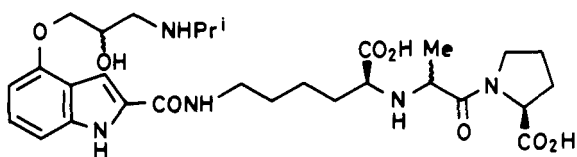
The opioid binding site affinities and selectivities of  $\text{N}^{\beta}$ -(D-Leu-D-Arg<sub>2</sub>-D-Leu-D-Phe)-naltraxamine, an alkaloid-peptide hybrid, have been correlated with estimated parameters of lipid membrane interaction. The results agree with an earlier proposed mechanism of opioid receptor subtype selection.<sup>293</sup> Seventy four tripeptide amides of 7-amino-4-methylcoumarin (Mec) have been prepared and tested to find specific substrates for blood-clotting proteases. The most sensitive substrates for individual enzymes are listed in Table 5.<sup>294</sup>

(111)  $n = 0 - 4$ 

(112) Xxx = Val, D-Val, or D-Ala

(113) R = H or Et,  $n = 1 - 6$ 

(114)



(115)

Table 5 Specific substrates for enzymes

| Substrate                 | Enzyme                     |
|---------------------------|----------------------------|
| Boc-Asp(OBzl)-Pro-Arg-Mec | human $\alpha$ -thrombin   |
| Z-pGlu-Gly-Arg-Mec        | bovine factor Xa           |
| Boc-Gln-Gly-Arg-Mec       | bovine factor XIIa         |
| Boc-Asp(OBzl)-Ala-Arg-Mec | bovine activated protein C |
| Boc-Gly-Phe-Arg-Mec       | plasma kallikrein          |
| Boc-Glu(OBzl)-Ala-Arg-Mec | human factor XIa           |

In Ac-Ala-(Xxx-Ala<sub>2</sub>)<sub>2</sub>-NH-polyethylene glycol monomethyl ether, (S)-Iva was found to have a comparable helix-inducing effect to Aib. No significant helix-promoting effect could be seen for  $\alpha$ -MeSer in polar solvents, but there is evidence for helices in CHCl<sub>3</sub> and in the solid state.<sup>295</sup> Two nucleopeptides, H-Phe-Tyr(pATAT)-NH<sub>2</sub> and H-Ala-Ser(pATAT)-Ala-OAllyl have been prepared. During synthesis, the exocyclic amino-function of adenosine was protected with the 2-nitrophenylsulphenyl group. Basic hydrolysis of the N-acyl groups normally used for nucleoside base protection is not compatible with phosphodi- or tri-ester linkages to Ser or Thr hydroxyls.<sup>296</sup>

The (6R,2'R)-isoproterenol analogue (114) has been prepared. It shows long-lasting positive inotropic activity after intravenous as well as oral administration and is at least three times as potent as rac-iso-proterenol.<sup>297</sup> A combined ACE inhibitor- $\alpha$ -adreno-receptor antagonist (115) has been synthesised and shows both activities in vitro and in vivo. It thus constitutes potentially a novel type of antihypertensive agent.<sup>298</sup> S-(2-Chloroacetyl)glutathione has been synthesised as a putative metabolite of 1,1-dichloroethen. In aqueous buffer it does not hydrolyse to release glutathione, but undergoes a two step rearrangement to give a cyclic product with the Glu-NH<sub>2</sub> and the -SH linked. In its reaction with thiols it differs strikingly from ClCH<sub>2</sub>COC<sub>2</sub>Cl.<sup>299</sup>

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# 5

## $\beta$ -Lactam Antibiotic Chemistry

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BY A. V. STACHULSKI

### 1. Introduction

The unique properties of the  $\beta$ -lactam ring continue to attract growing interest from the world's chemists. Highly stereoselective syntheses of monocyclic intermediates for the preparation of carbapenems and other bicyclic systems continue to be extensively explored. The elucidation of the biosynthetic pathways of natural  $\beta$ -lactams is also accelerating, and last year featured a noticeable renaissance of interest in the chemistry of 'classical' penicillins and cephalosporins.

I have retained the appendix used in the two previous volumes and somewhat expanded it to include miscellaneous, essentially routine  $\beta$ -lactam syntheses as well as syntheses for structure-activity relationships. The sub-section illustrating broader uses of  $\beta$ -lactams in synthetic and structural investigations is retained from vol.20. The main sectional divisions are unchanged.

A major review on the biosynthesis of penicillins and cephalosporins<sup>1</sup> by Baldwin and Abraham summarises our current knowledge (references to 1987) of the field to which they themselves have contributed so much, including characterisation of the enzymes involved. A brief resumé of the biosynthesis of  $\beta$ -lactams is presented in the general context of biosynthesis of alkaloids and microbial metabolites,<sup>2</sup> while another report examines both natural and new semisynthetic cephalosporins.<sup>3</sup>

Synthetic reviews have covered the synthesis of  $1\beta$ -substituted carbapenems, a still growing area,<sup>4</sup> and the use of sulphur-containing heterocycles in the chiral synthesis of  $\beta$ -lactams.<sup>5</sup> A review of the application of organometallic reagents in  $\beta$ -lactam chemistry<sup>6</sup> covers both the construction and rearrangement of the ring. Another growing tendency, the use of  $\beta$ -lactams to produce synthons for other natural products has also been reviewed<sup>7</sup> while a review on the Wittig reaction of carbonyl compounds other than aldehydes and ketones<sup>8</sup> is relevant to both

$\beta$ -lactam carbonyl reactivity and bicyclic  $\beta$ -lactam synthesis. The published proceedings of two international conferences,<sup>9</sup> omitted from last year's review, also contain interesting  $\beta$ -lactam chemistry.

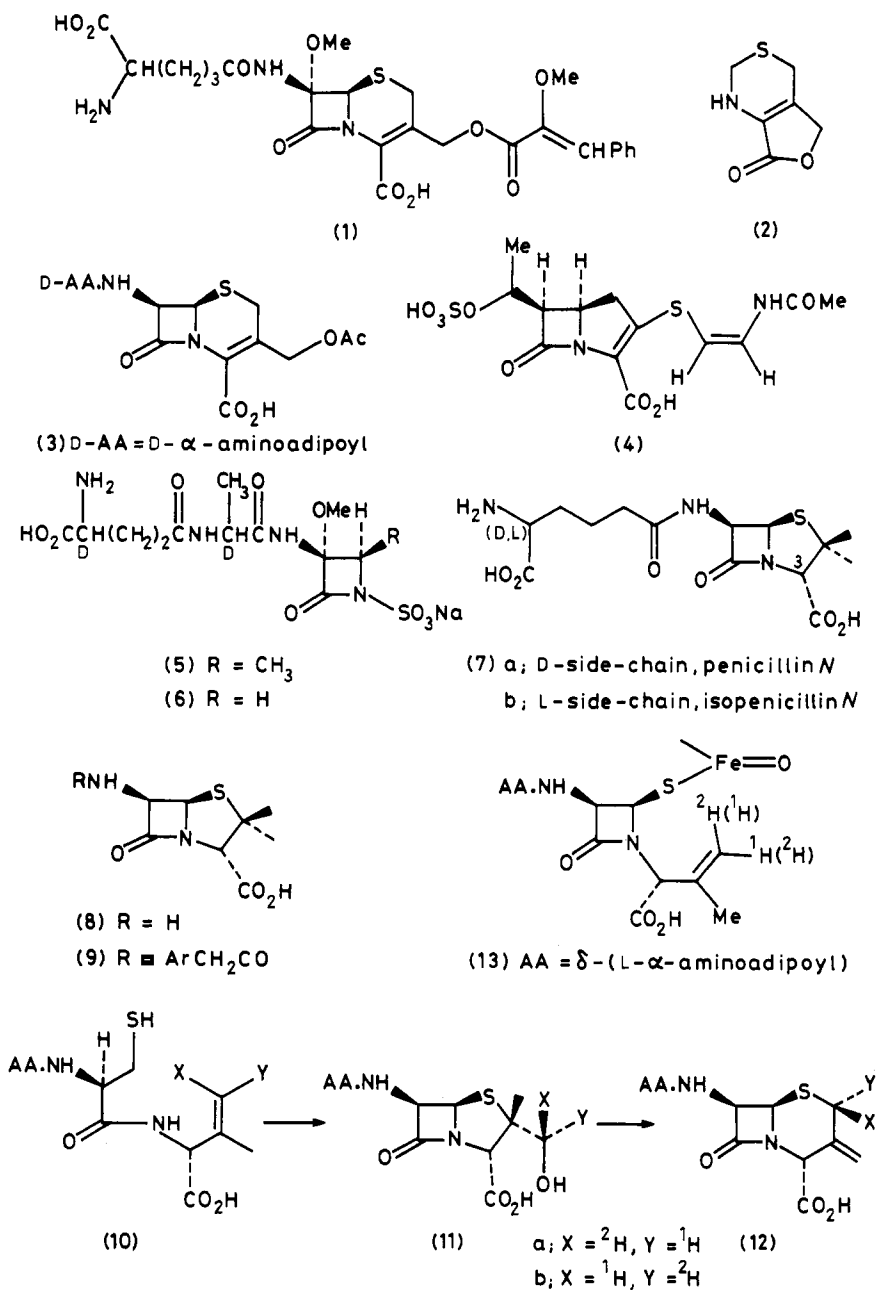
## 2. New Natural Products

Another 7 $\alpha$ -methoxy cephalosporin, namely deoxycephamycin B (1), has been isolated from actinomycetes present in Australian soils.<sup>10</sup> The dihydro-[1,3]-thiazine lactone (2) was isolated from a strain of Cephalosporium acremonium producing cephalosporin C (3);<sup>11</sup> the degradation (3)  $\rightarrow$  (2) involves lactonisation,  $\beta$ -lactam opening, fragmentation of the side-chain and finally reduction.

A new naturally occurring carbapenem AB-100-D produced by the actinomycete strain Kitasatosporia papulosa was strongly antimicrobially active against Gram-positive and Gram-negative bacteria;<sup>12</sup> comparative spectral data established its structure as (4), viz. the (Z) side-chain isomer of the known epithienamycin E. A novel monobactam antibiotic MM42842, produced by a culture of Pseudomonas cocovenenans, proved to have structure (5) after comparison with synthetic monobactams.<sup>13</sup> The similarity to sulfazecin (6) was clear, but (5) exhibited only some weak activity against Gram-positive bacteria. Interestingly, traces of L-amino-acids formed on hydrolysis suggested the presence of further unidentified monobactams.

## 3. Biosynthesis

Although the main features of penicillin and cephalosporin biosynthesis are now fairly well understood, interesting reports continue to appear on some aspects of the pathway. Cell-free extracts of S.clavuligerus contain an enzyme activity (ACV synthetase) which synthesises L- $\alpha$ -aminoacyl-L-cysteinyl-D-valine (LLD-ACV), the biosynthetic precursor of penicillins, from the constituent L-amino-acids.<sup>14</sup> Simple variations of the first and third residues are tolerated. Penicillin N (7a) and isopenicillin N (7b) are frequently required for biosynthetic studies; improved preparations of these materials



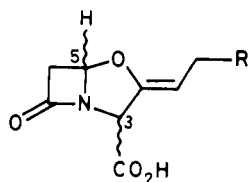
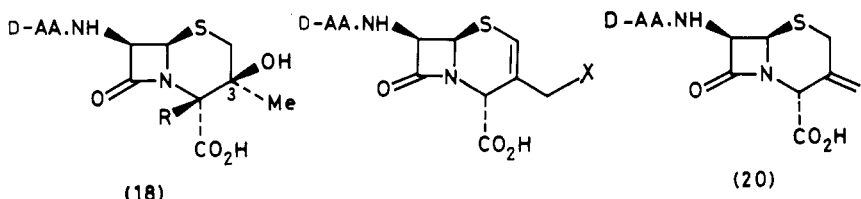
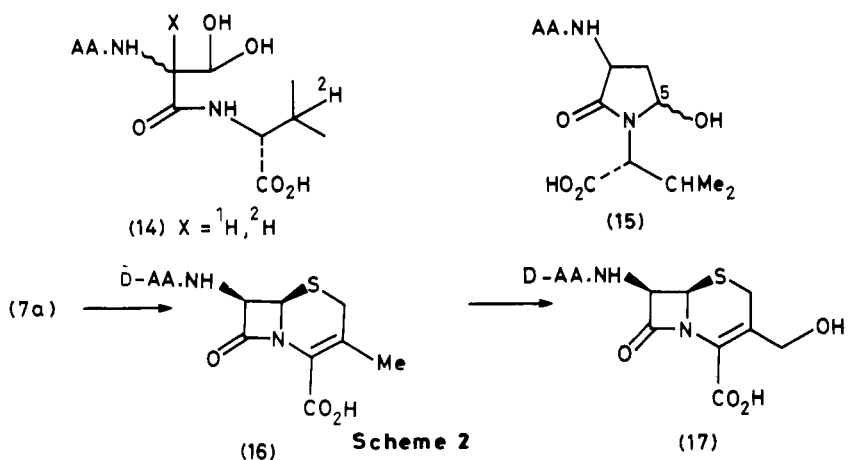
Scheme 1



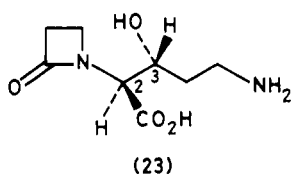
employing 4-nitrobenzyl based protecting groups have been reported<sup>15</sup> from the enantiomers of  $\alpha$ -amino adipic acid. The enzyme acyl-CoA: 6-APA acyltransferase, which in nature transforms (7b) to other penicillins such as benzylpenicillin, has now been isolated and purified<sup>16</sup> thus allowing the preparation of various penicillins of type (9) by acylation of 6-aminopenicillanic acid (6-APA)(8); cf. vol.19.

When the isodehydrovaline tripeptides (10a,b) with stereospecific deuterium labelling were incubated with isopenicillin N synthetase (IPNS), mixtures of the 2 $\alpha$ -hydroxy-methyl penams (11a,b) and exomethylene cepams (12a,b) were produced stereospecifically [*viz.* (10a)  $\rightarrow$  (11a) + (12a), (10b)  $\rightarrow$  (11b) + (12b)],<sup>17</sup> Scheme 1. The result for the unlabelled case (X=Y=H) had been reported previously (vol.19); an iron-oxo intermediate (13) was postulated which could react by *syn* [ $2\pi+2\pi$ ]-cyclo-addition ('hydroxylative' pathway) to the penam or by concerted 'ene' reaction ('desaturative' pathway) to the cepham. Other variations in the cysteinyl and valyl residues of LLD-ACV may also give unusual products derived from monocyclic iron-oxo intermediates like (13). Thus ACV analogues bearing [3-<sup>2</sup>H] Val were found<sup>18</sup> to yield small quantities of the hydrated aldehyde (14), perhaps by elimination of the sulphur moiety from a structure corresponding to (13) followed by addition of water. The 'normal' formation of (7b) was made less efficient by the primary isotope effect at the Val C-3 position. Replacement of cysteine by homocysteine in LLD-ACV gave on IPNS incubation only the 5-hydroxy  $\gamma$ -lactams (15) with no bicyclic products; an intermediate iron-oxo species was again invoked to account for this result.<sup>19</sup>

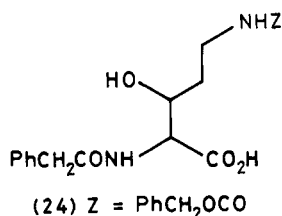
The subsequent biosynthesis of cephalosporins from penicillin N (7a) produces first deacetoxycephalosporin C (DAOC,16) then deacetylcephalosporin C (DAC,17) (Scheme 2). The bifunctional enzyme responsible, DAOC/DAC synthetase, is now available in purified form and its recombinant form has been shown to exhibit the same substrate specificity<sup>20</sup> and kinetics. Thus 3-<sup>2</sup>H penicillin N (7a) incubated with DAOC/DAC synthetase (cloned) gave some of the 3 $\beta$ -hydroxy cepham (18) as observed for the 'wild type' enzyme, and interestingly the  $\Delta^2$ -cephem (19a) was also a satisfactory substrate, giving the hydroxylated  $\Delta^2$ -product (19b).<sup>20</sup> Full details of the conversion of the exomethylene cepham (20) into DAC (17) by DAOC/DAC synthetase, including <sup>18</sup>O labelling in the



(21)  $3\alpha\text{-CO}_2\text{H}$ ,  $5\alpha\text{-H}$ ;  $R = \text{OH}$



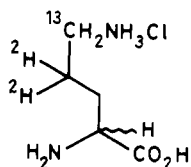
(23)



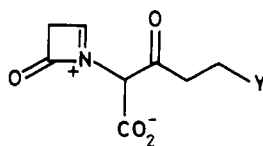
(24)  $Z = \text{PhCH}_2\text{OCO}$

(22)  $\alpha$ ;  $3\beta\text{-CO}_2\text{H}$ ,  $5\beta\text{-H}$ ;  $R = \text{NH}_2$

$b$ ;  $3\beta\text{-CO}_2\text{H}$ ,  $5\beta\text{-H}$ ;  $R = \text{NHCOX}$



(25)



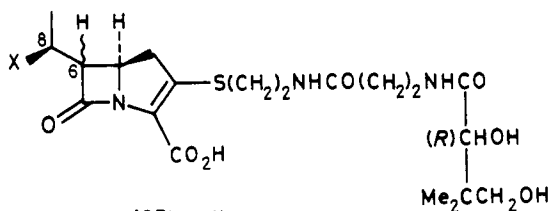
(26)

hydroxy group<sup>21</sup> (vol.20) have been published; DAOC was not an intermediate and again the cloned enzyme was effective.<sup>20</sup> Iron-oxo species were again proposed for the conversion of (16) to (17).

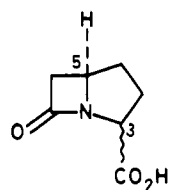
Considerable work is still being done to elucidate the biosynthesis of clavulanic acid (21). The absolute stereochemistry of proclavaminic acid (23), the biosynthetic precursor of clavaminic acid (22a) and hence of (21) (see vol.20) has been established as (2S,3R).<sup>22</sup> Following enzymic deacylation of the threo diastereoisomer of acid (24), a single enantiomeric amino-acid resulted and was transformed chemically into (23), thence by the synthetase into (22a). A blocked mutant of the enzyme, S.clavuligerus dcl-8, was incapable of clavulanic acid production, but three N-acyl clavaminates of type (22b) (X=CH<sub>3</sub>, CH<sub>2</sub>NH<sub>2</sub>, CH<sub>2</sub>NHCOME) were isolated from the culture broth.<sup>23</sup> Further investigation<sup>24</sup> revealed that the multiply labelled DL-ornithine derivative (25) yielded (22b), X=CH<sub>2</sub>NHCOME with both <sup>2</sup>H and <sup>13</sup>C labelling from the mutant but clavulanic acid containing only <sup>13</sup>C from the 'normal' enzyme. An enolisable intermediate (26) was proposed to account for the deuterium loss. When 'normal' S.clavuligerus cultures were grown in an <sup>18</sup>O<sub>2</sub> atmosphere, equally efficient <sup>18</sup>O incorporation into both the 1-position and the side-chain OH of (21) occurred.<sup>25</sup> It was argued that this demonstrated an oxidative deamination in the transformation (22a)  $\rightarrow$  (21), perhaps via a betaine like (26).

The use of blocked mutants has also been exploited in the carbapenem field. Streptomyces fulvoviridis A933 17M91501, an acylase-deficient mutant, produces carbapenems (27a-d) of the OA-6129 group. Further mutation produced strains incapable of the 'normal' later biosynthetic steps, hydroxylation (27b), C-6 isomerisation (27c) and O-sulphation at C-8 hydroxyl (27d).<sup>26</sup> It was concluded that the bioconversion of carbapenems was in the order (27a  $\rightarrow$  b  $\rightarrow$  c  $\rightarrow$  d). The previously-reported carbapenem-3-carboxylates (28a,b) have been found to be derived, together with the corresponding  $\Delta^2$ -carbapenem, from glutamate and acetate.<sup>27</sup> Possible biosynthetic pathways via the tricarboxylic acid cycle, then a pyrrolidine intermediate, were discussed.

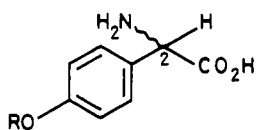
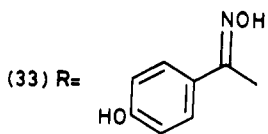
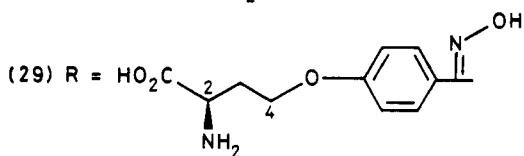
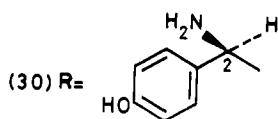
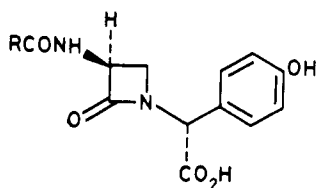
Nocardicin biosynthesis has also received more attention. Both C-4 epimers of [4-<sup>2</sup>H]methionine, obtained from aspartic acid, were incorporated into nocardicin A (29) using Nocardia uniformis with inversion of configuration<sup>28</sup> at C-4



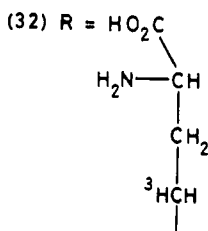
- (27) a;  $\text{X} = \text{H}$ , (6*R*)  
 b;  $\text{X} = \text{OH}$ , (6*R*)  
 c;  $\text{X} = \text{OH}$ , (6*S*)  
 d;  $\text{X} = \text{OSO}_3\text{H}$ , (6*R*)



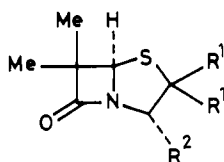
- (28) a;  $3\alpha\text{-CO}_2\text{H}$   
 b;  $3\beta\text{-CO}_2\text{H}$



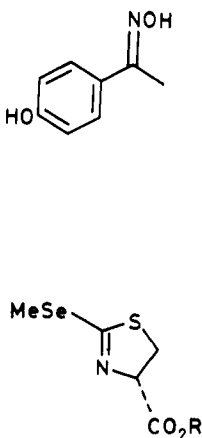
- (31)  $\text{R} = \text{H}$



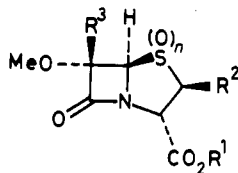
- (32)  $\text{R} = \text{HO}_2\text{C}$



- (34)



- (35)



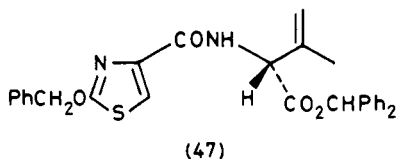
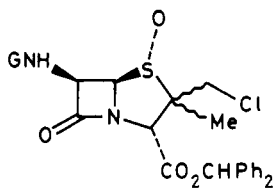
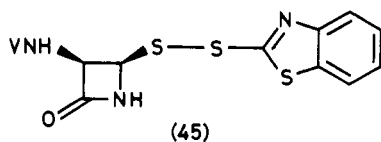
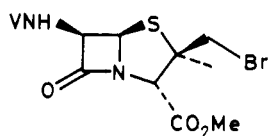
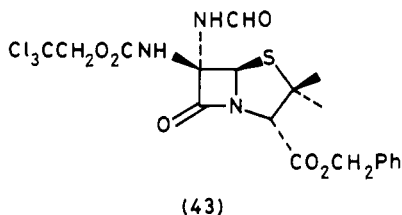
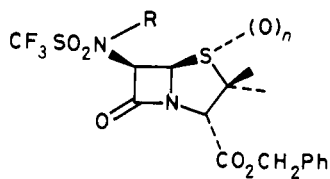
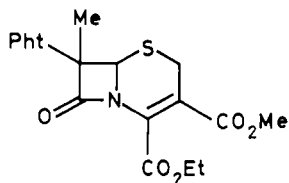
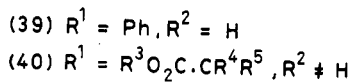
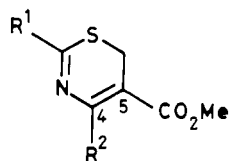
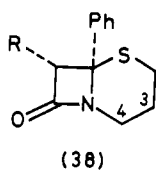
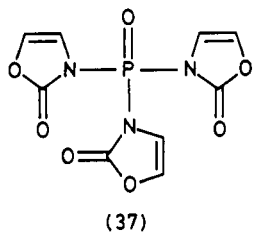
- (36)

(homoseryl portion). A feeding experiment with doubly labelled nocardicin G (30), synthesized from the  $[2-^{13}\text{C},^{15}\text{N}]$ amino-acid (31), showed intact incorporation of (30) into (29);<sup>29</sup> on the other hand, the di-amino-acid ether (32) was not incorporated, implying that (30) is a key precursor of (29). Nocardicin E (33) has been shown to be a later intermediate; in the presence of a partially purified cell-free system from *N. uniformis*, (33) was first acylated to the homoseryl C-2 epimer of nocardicin A (29), then epimerisation gave (29) itself,<sup>30</sup> i.e. there are two enzymic activities.

#### 4. Penicillins and Cephalosporins

Syntheses of these bicyclic  $\beta$ -lactams, including the parent penam and cepham rings, will be summarised first. Barrett has published further details on the synthesis of penams of type (34) from cationic iron (II) vinylidene complexes.<sup>31</sup> Addition of the complex to the appropriate thiazoline was followed by oxidation using, e.g.  $\text{Br}_2$  or iodosobenzene. Ketene addition to a 2-(methyl-seleno)- $\Delta^2$ -thiazoline like (35) followed by reductive dealkyl-selenation is also known (vol.19); some novel penamcarboxylic acids (36) were made in this way.<sup>32</sup> Certain oxazolinyl phosphonates, and phosphine oxide (37), were efficient in cyclising 2-thiazolidine-acetic acid to the penam.<sup>33</sup>

The analogous annulation of appropriate thiazine derivatives to cephams is also well known; some 6-phenyl cephams (38) were made in this way<sup>34</sup> and, in the case  $\text{R}=\text{protected amino}$ , elaborated to a novel di- $\beta$ -lactam ( $\text{R}=\text{azetidin-2-on-1-yl}$ ). Similarly, addition of diazomethane to the 4,5-bond of thiazine (39) followed by ketene addition and oxidative cleavage of the fused pyrazoline<sup>35</sup> afforded a 3-methoxycarbonyl, 4-formylcepham like (38). A modification of this route calls for the assembly of 2-(alkoxycarbonyl)methyl-1,3-thiazines of type (40); addition of HCN across the imine, followed by ester hydrolysis and closure of the  $\beta$ -lactam, led finally to a 6-cyanocephem<sup>36</sup> and a 7,7-dimethylcephem was similarly prepared following reduction of an imine<sup>37</sup> intermediate. Fuller details of the preparation of the 7,7-dimethyl compound and the phthalimido derivative (41) by a similar method<sup>38</sup> revealed that the  $\beta$ -lactam closure was best performed using a methanesulphonyl chloride- two-phase acylation, but (41)

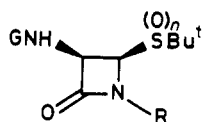


was contaminated with its  $\Delta^2$ -isomer. Methods for obtaining the requisite dihydro- and tetrahydro-1,3-thiazines include cyclocondensation between  $\alpha$ -thioxocarbamoylglycinates and vinylketoesters<sup>39,40</sup> and the cycloaddition of 1-thia-3-azabutadienes to appropriate olefins.<sup>40</sup>

Only one substantive account of 6(7)-substituted penicillins and cephalosporins appeared during the year, concerning 6 $\alpha$ (7 $\alpha$ )-formamido derivatives.<sup>41</sup> Thus further acylation of a 6 $\beta$ -trifluoromethanesulphonamide (42a) afforded the 2,2,2,-trichloroethoxycarbonyl derivative (42b); interestingly, this material gave solely the  $\alpha$ -sulphoxide (42c) on peracid oxidation. Treatment of (42b) with triethylamine and N,N-bis(trimethylsilyl)formamide gave the known carbamate (43); similar transformations were performed in the cephalosporin series and some other halogenated sulphonamides were effective. The biological activities of some 6 $\alpha$ -formamido penicillin sulphoxides- less than the parent sulphides- were also reported.

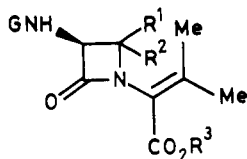
A very large crop of papers on various penicillin transformations appeared this year, though many were variations on old themes. Cases where the 6-amino group was retained, including penam to cephem conversions, will be considered first. The 2 $\beta$ -bromomethyl penam (44), on treatment with triphenyltin hydride, gave a mixture of 3 $\alpha$ - and 3 $\beta$ - methyl cephams;<sup>42</sup> trapping with an allyl stannane produced an S-allyl derivative, strongly implying a radical intermediate such as may be involved in the ring expansion of (7a) to (16).

Penicillin sulphoxides continue to be extensively used; the use of a tungsten or molybdenum catalyst with H<sub>2</sub>O<sub>2</sub> to prepare them<sup>43</sup> (and cephalosporin sulphoxides) has been mentioned. The 'Kamiya disulphides' generated from penicillin sulphoxides by thermolysis and trapping the sulphenic acid intermediate with an appropriate thiol are also well known; in one recent report<sup>44</sup> subsequent base-catalysed isomerization and ozonolysis afforded the azetidinone (45). It has been found that thermolysis of the 2 $\beta$ -chloromethyl derivative (46a) leads to the 'more stable' 2 $\alpha$ -chloromethyl compound (46b).<sup>45</sup> The sulphoxide of an N-benzyloxycarbonyl penicillin ester gave a normal Kamiya disulphide, but heating with trimethyl phosphite led to the unexpected thiazole (47) rather than an azetidinone.<sup>46</sup> The known (see vol.20) azetidinone-4-sulphide (48a) was converted to the sulphoxide (48b) which on thermolysis



(48) a;  $R = H, n = 0$

b;  $R = CH_2C(=CH_2)Me$ ,  
 $n = 1$

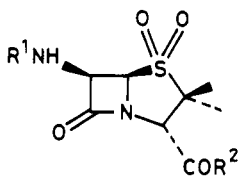


(49) a;  $R^1 = \alpha - OCOCOMe$ ,

$R^2 = \beta - H, R^3 = Me$

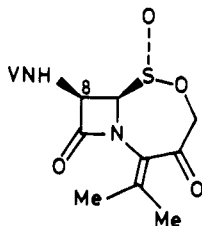
b;  $R^1, R^2 = O, R^3 = Me$

c;  $R^1, R^2 = O, R^3 = H$

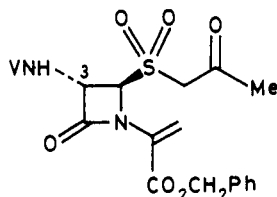


(50)  $R^1 = G, R^2 = OPNB$

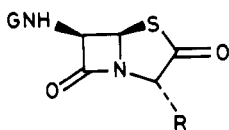
(51)  $R^1 = V, R^2 = CH_2Cl$



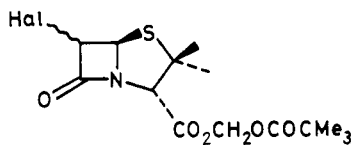
(52)



(53)

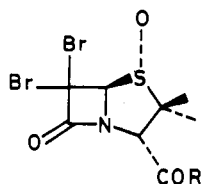


(54)



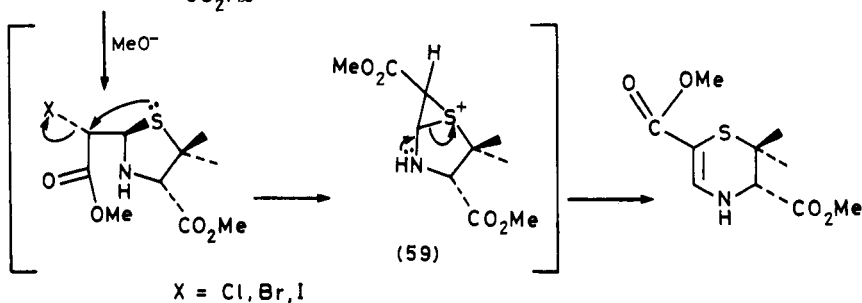
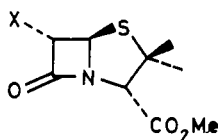
(55)  $Hal = \alpha - F$

(56)  $Hal = \alpha - or \beta - Br$



(57)  $R = OCH_2OCOCMe_3$

(58)  $R = NH_2$  or  $NHCH_2Ph$



Scheme 3



generated a penam-1-oxide via a presumed sulphenic acid intermediate, reversing the usual process; a C(3)-tritylamino version of (48b) also afforded a penam.<sup>47</sup> 1-Propynyl versions of (48b), however, failed to cyclize. Two procedures were employed<sup>48</sup> for the conversion of penicillins to 3-amidoazetidin-2,4-diones, using intermediates ultimately derived from sulphoxides; the key step was a Norrish type II photoreaction of pyruvates of type (49a) to give diones (49b) in up to 80% yield. The acid (49c) proved antibacterially inactive.

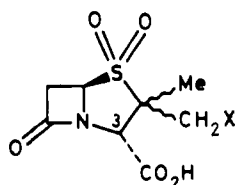
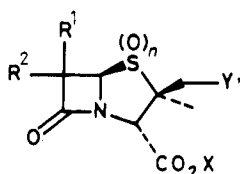
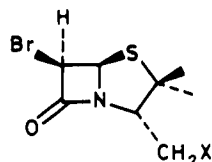
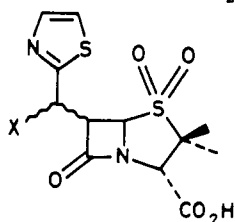
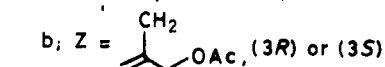
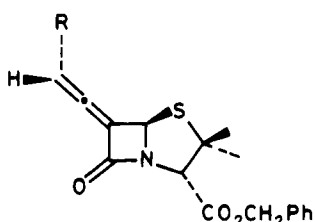
Moving to the sulphone oxidation level, it has been found that treatment of the sulphone (50) with potassium acetate in DMF gives the ring-opened sulphinic acid in about 20% yield without C(6)-epimerisation.<sup>49</sup> It was known that stronger bases, e.g. DBN, gave inversion at C(6) in this reaction. When the chloromethyl ketone (51) was treated with DBN, the novel bicyclic system (52) resulted<sup>50</sup> via elimination of sulphinic acid, then alkylation at oxygen. If instead the diazoketone precursor of (51) reacted with DBN then HCl, some C(8)-epimerisation was observed; other 6-substituted penams behaved similarly. In contrast to a 3 $\alpha$ -chloro or 3,3-H<sub>2</sub> analogue, the sulphone-derived oxalimide (53) did not yield a cephem sulphone on trimethyl phosphite treatment<sup>51</sup> but its 3 $\beta$ -epimer did cyclize.

The unusual 2-oxopenam (54) was obtained<sup>52</sup> by carbodiimide closure of a thiol acid. A new ester, phenylacetoxy-methylene, has been used for penicillin C(3)-carboxy protection;<sup>53</sup> it is removable with immobilised penicillin acylase and has been employed during a conversion of penicillin G to cephalosporins.

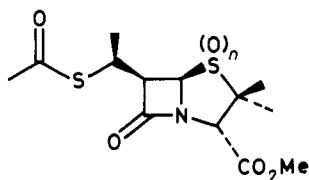
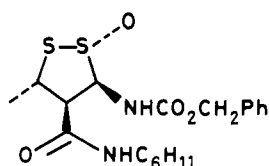
Penams lacking the 6-amino group continue to be much explored, particularly as potential  $\beta$ -lactamase inhibitors. Considering first 6-halopenams, the 6 $\alpha$ -fluoropenicillanate (55) was obtained in low yield by the action of pyridine-HF on the 6-diazoester.<sup>54</sup> Chlorination of either isomer of 6-bromopenicillanate (56) led to C(5)-S cleavage and 3,4-dihaloazetidin-2-ones were formed;<sup>55</sup> t-butyl hypochlorite effected a similar ring opening on a 6,6-dibromo compound. Many methods have been used for the reduction of 6-halo penams to the 6-unsubstituted compounds; two reports on electroreduction have appeared, of both 6-mono and dihalopenicillins as either sulphides or sulphones.<sup>56,57</sup> Yields were moderate to good. Tri-n-butyl phosphine<sup>58</sup> was also effective in mono-debromination and both hydrogenolysis over platinum and

dissolving metal reduction were used in converting a 6,6-dibromosulphone to sulbactam.<sup>59</sup> The dehalogenation of (pivaloyloxy)methyl 6,6-dihalogenopenicillanates and their sulphones [cf. (56)] using tributyl tin hydride gave largely 6 $\beta$ -halo products, while the (1R)-sulphoxides, e.g. (57), were reduced exclusively to 6 $\alpha$ -halo compounds.<sup>60</sup> Interestingly, peracid oxidation of 3 $\alpha$ -amido-6,6-dibromopenicillanates gave (1R)-sulphoxides (58) as sole products,<sup>61</sup> probably by a directing effect of the amide as related 3 $\alpha$ -cyanopenams gave (1R,1S) mixtures. A detailed mechanistic investigation<sup>62</sup> of the rearrangement of 6-halopenicilloates to 2,3-dihydro-1,4-thiazines (Scheme 3) concluded that an episulfonium ion intermediate (59) was probably involved.

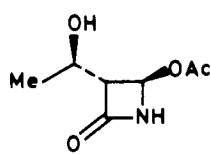
Analogues of sulbactam (60) continue to be actively explored as  $\beta$ -lactamase inhibitors. The above mentioned 2 $\alpha$ -chloromethyl compound (46b)<sup>45</sup> was transformed via a 6-bromo intermediate to the sulphone (61) which was less effective than the 2 $\beta$ -isomer. Two 2 $\beta$ -(thio-substituted)methyl analogues (62) were prepared by displacement on a 2 $\beta$ -chloromethyl penam, followed by oxidation;<sup>63</sup> some disulphide by-products but no cephams resulted. A new route to the 2 $\beta$ -methyl substituted compounds (63a) called for electrolysis of a disulphide akin to (45) in a two-phase system containing KSCN or KSeCN; sulphones like (63b) were already known.<sup>64</sup> On the other hand, electrolysis with NaN<sub>3</sub> present gave 2 $\alpha$ /2 $\beta$  mixtures. Analogues of (60) bearing a 3 $\alpha$ -hydroxy-or-fluoromethyl group (BH<sub>3</sub> reduction, then DAST treatment) or a 3 $\alpha$ -cyano group (dehydration of a 3 $\alpha$ -amido group) gave compounds which were not  $\beta$ -lactamase inhibitors; nor were the 6 $\beta$ -bromo compounds (64a,b).<sup>65</sup> (6-Methylene)penam sulphones were known to be  $\beta$ -lactamase inhibitors (see vol.19); a series of 6 $\alpha$ -and 6 $\beta$ -thiazolyl penam sulphones (65) was prepared from a C(6)-Grignard reagent and also showed inhibitory properties.<sup>66</sup> When 6-diazopenicillanate esters were allowed to react with oxalyl halides, then various nucleophiles, the spiro-epoxy derivatives (66a) resulted; the 3-epimers were separable and, in the cases X=Br, reduction to X=H was possible using Zn-Cu.<sup>67</sup> The series exhibited some antibacterial activity and  $\beta$ -lactamase inhibition and the related cephalosporins (66b) were similarly obtained;<sup>68</sup> here the 3-configuration was important. It has been found that bis (tributyltin)oxide is effective<sup>69</sup> in the deprotection of

(60)  $X = H$ (61)  $X = Cl, \alpha\text{-CH}_2Cl$ (62)  $X = SR, \beta\text{-CH}_2SR$ (63) a;  $Y = SCN$  or  $SeCN, n = 0$ b;  $Y = SCN$  or  $SeCN, n = 2$ (64) a;  $X = OH$ b;  $X = F$ (65)  $X = OCOR$  or  $F$  (66) a;  $Z = Pr^i, (3R)$  or  $(3S)$ b;  $Z = \text{CH}_2\text{OAc}, (3R)$  or  $(3S)$ 

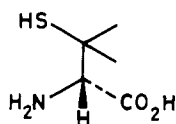
(67)

(68) a;  $n = 0$ b;  $n = 1$ 

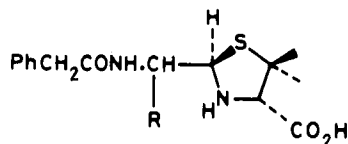
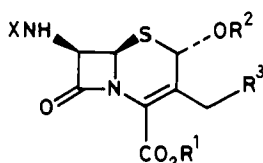
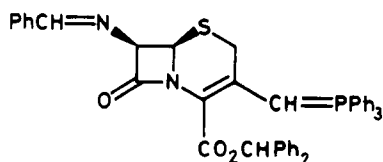
(69)



(70)



(71)

(72) a;  $R = H$ b;  $R = CONHR^1$ (73)  $X = G, V$  etc.;  $R^2 = Me, Et, Pr^i, CH_2Ph$ ;  $R^3 = H, OAc, etc.$ 

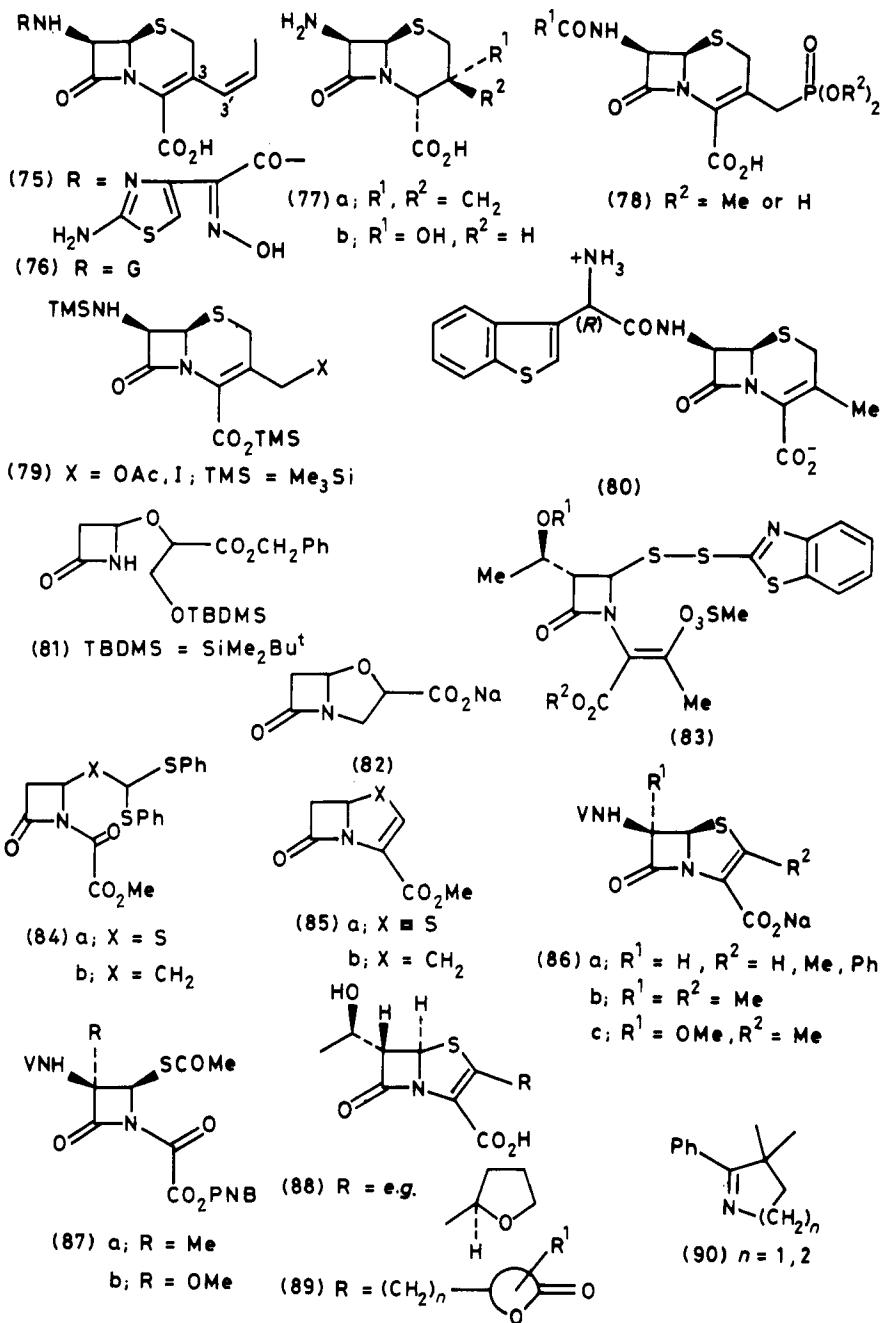
(74)

(pivaloyloxy)methyl esters used in the synthesis of 6-halo penams or penam sulphones; an organotin ester intermediate is proposed; yields range from 43-61%.

The novel vinylidene penams (67) have been prepared; addition of a metal acetylide to the 6-oxopenam was followed by conversion to a triflate and vinylogous displacement with an organocuprate.<sup>70</sup> A 6 $\beta$ -(1-hydroxy)ethyl penam was transformed into the acetylthio compound (68a) using Mitsunobu's procedure; the derived sulphoxide (68b) rearranged thermally to a disulphide, thence to the asparagusic acid analogue (69).<sup>71</sup> Another report on the conversion of 6-aminopenicillanic acid to the popular penem/carbapenem intermediate (70) has appeared.<sup>72</sup>

D-Penicillamine (71) is a well-known breakdown product from many penicillins and has various clinical applications. A series of papers<sup>73-75</sup> has described the preparation of (71) by the action of arylamines on benzylpenicilloic acid (72a) or the penicilloic acid amide (72b) or of N,N'-diphenylethylene diamine on (72a), (72b) or benzylpenicillin itself; yields up to 87% are obtained.

Moving on to cephalosporin chemistry, novel syntheses of 2-alkoxy cephalosporins of type (73) have been found, either by direct oxidation of the parents with cerium (IV) ammonium nitrate in alcohol-THF mixtures or by electro-oxidation in the same solvents.<sup>76</sup> As ever, new methodology for C(3')-substitution of natural cephalosporins continues to be sought. The stabilised phosphorane (74) has been used as an intermediate in the preparation of the C(3)-[(Z)-1-propenyl] compound (75) by Wittig methodology (cf. vol.20);<sup>77</sup> a pro-drug form of (75) was orally absorbed. Another route to C(3)-vinyl products of type (76) called for reaction of a C(3)-triflate with (Z-propenyl)tributyl-n-stannane; high stereospecificity and good yields were obtained in N-methylpyrrolidinone using ZnCl<sub>2</sub> with a Pd(O) catalyst and added tri-(2-furyl)phosphine ligand.<sup>78</sup> Similarly were obtained C(3)-aryl, alkynyl and H compounds. Palladium-catalysed coupling also featured in displacement of a C(3')-chloroceph-3-em by unsaturated stannanes, using the same ligand; C(3')-alkenyl, allyl, and aryl ceph-3-ems resulted.<sup>79</sup> Zinc-mediated reduction of a C(3')-(heteroaryl)thio derivative afforded exomethylene cepham (77a) with no need for amino or carboxy protection; ozonolysis in methanol at -65°C and subsequent borohydride reduction led to the



3 $\alpha$ -hydroxycepham (77b),<sup>80</sup> a ceftizoxime intermediate. A 3-chloromethylceph-3-em was again the intermediate for the synthesis of 3-phosphinylmethyl compounds (78) by a Michaelis-Arbuzov type reaction; such cepheems (R<sup>2</sup>=Me or H) offered no advantage in antibacterial activity over C(3')-acetoxy compounds.<sup>81</sup> The use of N-,O-bis(trimethylsilyl)derivatives such as (79) for improved C(3')-displacement by N and S nucleophiles has been advocated<sup>82</sup> and silylation also figured in an improved preparation of the oral antibiotic (80) on a large scale.<sup>83</sup>

### 5. Clavulanic Acid and Oxapenams

Displacement of 4-acetoxazetidinone with the appropriate hydroxyester produced intermediate (81) which was transformed by desilylation, alcohol activation, cyclisation, and finally hydrogenolysis to the ( $\pm$ )-clavam-2-carboxylate (82).<sup>84</sup> For some partial synthetic clavulanates, see the Appendix.

### 6. Penems

Two mechanistic investigations on commonly-used penem syntheses have appeared. Displacement of the sulphonate in (83) using Na<sub>2</sub><sup>35</sup>S gave a 2-thiacephem; treatment with triphenylphosphine or peracid then led to a (5R,5S)-mixture of penems which had much the same specific <sup>35</sup>S activity.<sup>85</sup> A re-investigation of the Schering penem synthesis via oxalimide cyclisation, thought to proceed via a carbene, has led to a new penem synthesis. Thus the dithioacetal (84a) on treatment with triethyl phosphite afforded a 2,3-bis(phenylthio)penam which underwent elimination with tri-*n*-butyltin hydride to the penem (85a).<sup>86</sup>

Woodward's original 'intramolecular Wittig' synthesis of penems continues to be well employed, and was used in a reinvestigation of 6 $\beta$ -acylaminopenems.<sup>87</sup> Penems of type (86a) were already known to be unstable and showed little antibacterial activity. The appropriate 6 $\alpha$ -substituted penams were transformed via sulfoxides to oxalimides (87a,b) and cyclised to penems (86b,c) via the derived phosphoranes; both showed only weak activity, though (86b) was more stable than (86a). A related

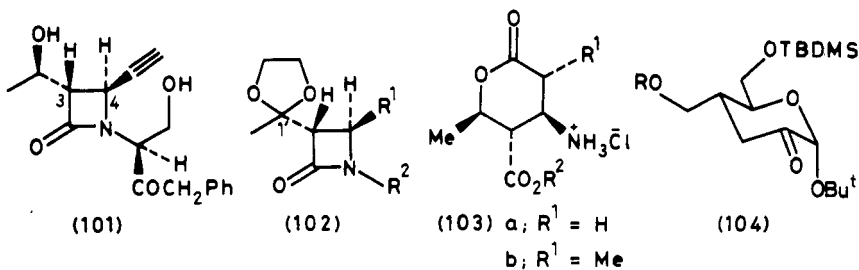
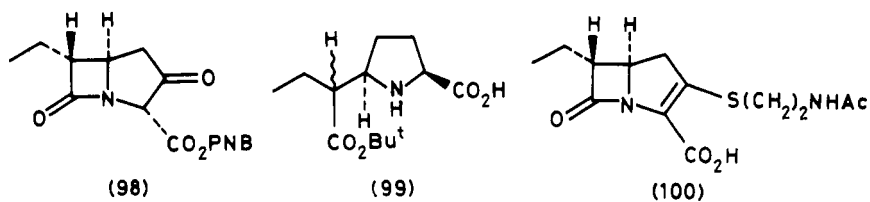
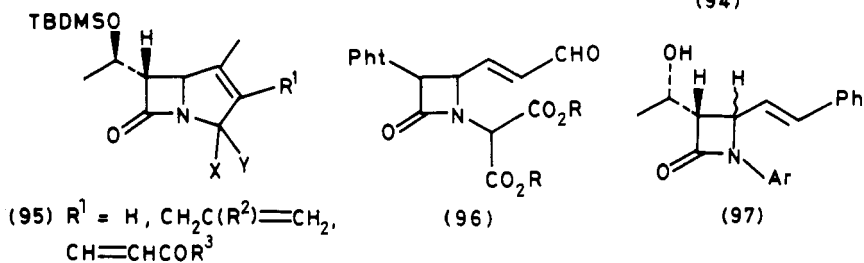
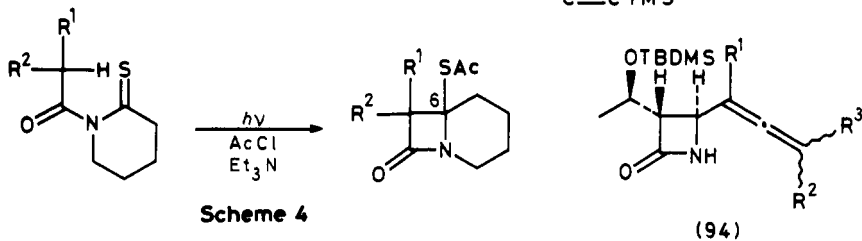
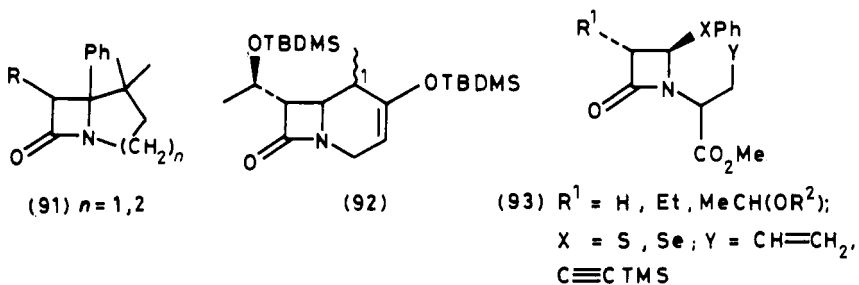
6 $\beta$ -(malonylamido)penem offered no advantages. A number of 6 $\alpha$ -(1-hydroxyethyl)penems (88) where most C(2)-substituents were chiral 5 or 6-membered oxygen rings were prepared by the intramolecular Wittig or oxalimide-phosphite routes.<sup>88</sup> The C(2)-lactonyl penems (89) were also obtained by Wittig methodology, together with related 6 $\alpha$ -(hydroxymethyl)penems; most had potent antibacterial activity.<sup>89</sup>

A series of penems like (88) with R=CH<sub>2</sub>-imidoyl or other N-substituent was generally prepared by Mitsunobu-type modification of (88), R=CH<sub>2</sub>OH or quaternisation of R=CH<sub>2</sub>OSO<sub>2</sub>CF<sub>3</sub>; the Wittig route and the ring-contraction of a 2-thiacephem were employed in some cases.<sup>90</sup> See the Appendix for further analogues of type (88).

## 7. Carbapenems and Related Systems

For the scope of this section, see vol.19; section 8 should be consulted for azetidinone precursors of carbapenems. Considering first the application of cycloaddition methodology, two reports<sup>91,92</sup> have discussed the addition of ketenes to cyclic imines of type (90), generating carbapenams and carbacephams of type (91) in yields up to 65%. Meyers has adapted a previous synthesis (see vol.20) by treating intermediate (70), R=TBDS, with a silyloxypentadiene and ZnCl<sub>2</sub> catalysis to give carbacephem (92),<sup>93</sup> 1 $\beta$ :1 $\alpha$  ca.70:30. Further manipulation afforded the desired 1 $\beta$ -methylcarbapenem.

Two reports featured the radical cyclisation of 4-(phenylthio) or -(phenylseleno)azetidinone precursors. Thus alkylation of N-[(methoxycarbonyl)methyl]-4-(phenylthio)azetidin-2-one with allyl bromide afforded (93), R=H, X=S, and Y=CH=CH<sub>2</sub>. Cyclisation using tri-n-butyltin hydride produced a carbacephem (and no carbapenem); where R=Et or MeCH(OR<sup>1</sup>), 6,7-cis/trans mixtures resulted.<sup>94,95</sup> The selenyl precursor, (93), X=Se, gave superior cyclisation yields of up to 59%, and the acetylenic derivative (93), Y=C CTMS, led to a  $\Delta^1$ -carbacephem. The above mentioned oxalimide-dithioacetal condensation<sup>86</sup> led in analogous manner in the carbon series from (84b) to carbapenem (85b). An interesting photochemical synthesis involved the irradiation of monothioimides to give, with subsequent acetylation, a 6-S-acetylcarbacephem (Scheme 4)<sup>96</sup> but the corresponding





5-membered ring gave no carbapenam; a homo-carba-cepham was similarly prepared, however.

Other new approaches included two papers on metal-induced cyclization of appropriate azetidinones. 4-Allenylazetidin-2-ones of type (94), available from (70), were successfully cyclized to  $\Delta^1$ -carbapenems using silver (I) salts (especially the tetrafluoroborate)<sup>97</sup> or palladium (II) reagents in conjunction with allyl halides or activated olefins,<sup>98</sup> giving products of type (95). The same reagents could effect the conversion of some related 4-(2-propynyl)azetidinones to  $\Delta^2$ -carbapenems but in lower yields. A new synthesis of carbaceph-3-ems involved hydrogenation of the azetidinone (96);<sup>99</sup> the product cyclized spontaneously and was converted by decarbalkoxylation-elimination to the desired product; a 2 $\alpha$ -OH analogue was prepared similarly.

Alternative syntheses of naturally occurring carbapenems of the PS-5 and thienamycin series continue to appear. The azetidinone (97) was obtained from (S)-ethyl 3-hydroxybutanoate, giving the required absolute configuration at C(3); deoxygenation to the PS-5 series was achieved by reduction of either the derived iodide or N-thiocarbonylimidazolidine (NaBH<sub>4</sub>). The Merck carbene insertion was finally employed to deliver the bicyclic ketone (98).<sup>100</sup> In another approach, an L-pyroglutamate ester was opened with an ester enolate and the product deprotected and recycled to the 2,5-cis-pyrrolidine (99); after closure of the  $\beta$ -lactam either 5,6-cis-or trans-products were obtainable and the latter was converted to (+)- PS-5 (100).<sup>101</sup> In the thienamycin series, the azetidinone (101) was derived ultimately from L-serine and diketene with correct absolute 3,4-stereochemistry; eventual cyclization by the oxalimide route gave a (2-phenylthio)carbapenem.<sup>102</sup> A further report on the use of 1,3-dioxolane intermediates such as (102) in the synthesis of thienamycin analogues has appeared; acid hydrolysis then borohydride reduction gave 1'-(hydroxyethyl)epimers which were converted to 1'-epimeric thienamycin analogues by the Merck route.<sup>103</sup>

A common means of achieving the desired stereochemistries at positions 5,6, and 8 in thienamycins (also position 1 in 1 $\beta$ -methyl carbapenems) is the use of a 'Melillo's lactone' (effectively a masked  $\beta$ -amino-acid) like (103a,b); another preparation of optically active (103a) or (103b) from (R)-3-hydroxybutyrate has appeared.<sup>104</sup> A novel approach to (103b)

involved electrophilic amination of the sugar-derived ketone (104), reduction of the carbonyl group to methyl and further functional group adjustment.<sup>105</sup> Intermediate (105) could be generated with high enantiomeric excess by addition of a chiral oxazoline anion to an  $\alpha$ -methoxycarbamate; acidolysis gave a simple analogue of (103a).<sup>106</sup>

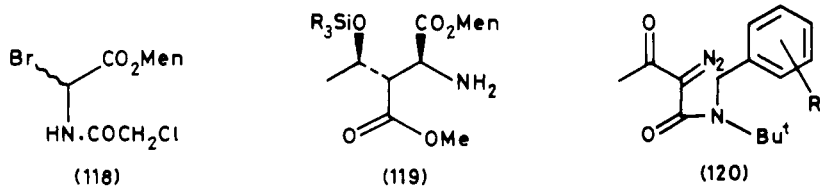
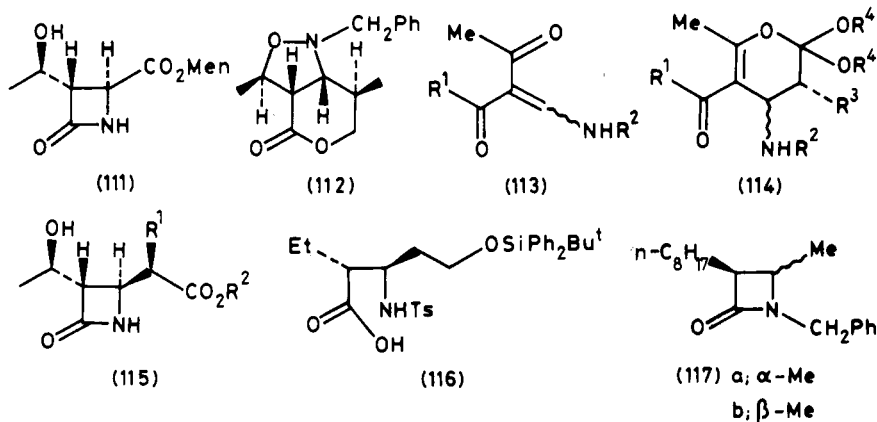
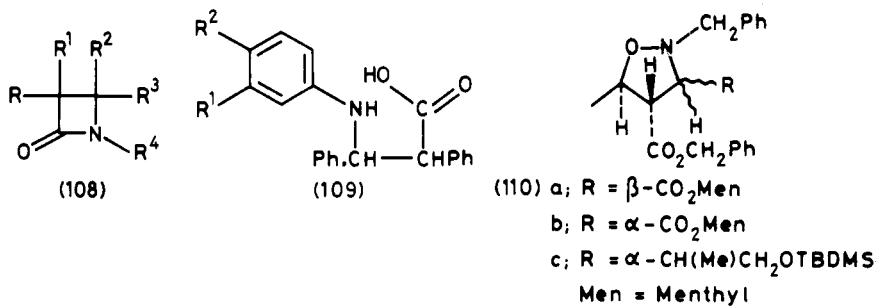
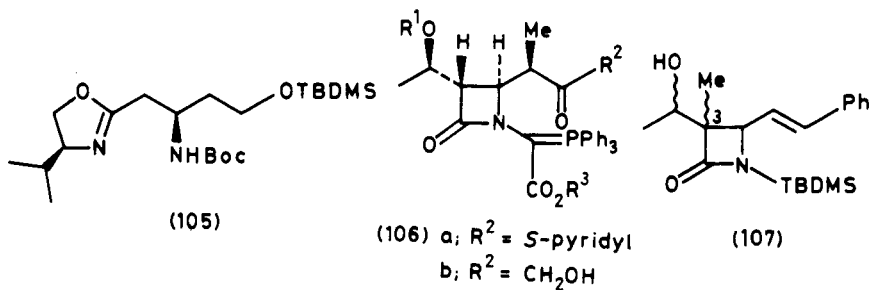
Analogues of 1 $\beta$ -methylcarbapenems bearing a C(2)-functionalised methyl group were prepared from thioester (106a); the derived hydroxymethyl ketone (106b) was cyclized to the 2-(hydroxymethyl)carbapenem which was transformed to several analogues,<sup>107</sup> which had variable dehydropeptidase susceptibilities. The concept of 6-substitution for possible enhancement of enzymic stability [*cf.* penems (86b,c) above] has been examined. Azetidinone (107) was obtained by aldol condensation of the 3-methyl precursor with acetaldehyde; following Merck cyclization and C(8)-isomer separation, ( $\pm$ )-6 $\beta$ -methylthienamycins were obtained<sup>108</sup> which had superior chemical stability but poorer antibacterial activity.

## 8. Azetidinones

Syntheses are listed first, according to the bond(s) created in the ring-forming step.

### Reactions in which one bond is formed

1-2 bond forming reactions.- This section includes 'two-step' [2+2] additions where an intermediate  $\beta$ -amino-acid or-ester was isolated. New reagents for closing  $\beta$ -amino-acids to azetidinones continue to be sought; some benzoxazoliny phosphorus compounds very similar to (37)<sup>33</sup> gave up to 88% of azetidinone<sup>109</sup> from a range of substituted  $\beta$ -alanines. Other reagents used similarly to prepare azetidinones of type (108) were (benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium hexafluorophosphate (BOP),<sup>110</sup> diphenylphosphonic chloride-triethylamine<sup>111</sup> and triphenylphosphine with carbon tetrachloride or *N*-bromosuccinimide.<sup>112</sup> Acetonitrile was the best solvent for the latter two procedures (markedly improving earlier  $\text{Ph}_3\text{P}\cdot\text{CCl}_4$  condensations); products with  $\text{R}^4=\text{H}$  were formed in lower yields. Polyphosphoric acid-mediated cyclization of



(3-arylamino)propenoic acids of type (109) led to up to 11% of 1,3,4-triarylazetidin-2-ones in addition to quinolin-4(1H)-ones; ring fission to imines, then recyclization, was shown to occur.

Three papers explored the use of 1,3-dipolar cycloadditions of nitrones in the highly stereocontrolled synthesis of  $\beta$ -amino-acids. Thus aldehyde-derived nitrones were added to appropriate crotonates to give isoxazolidines (110a,b)<sup>114</sup> and (110c).<sup>115</sup> Isomer (110b) was separated; hydrogenolysis, then DCC-mediated closure of the resulting  $\beta$ -amino-acid, gave an azetidinone (111) convertible to the penem and carbapenem intermediate (70), R=H, with correct absolute 3,4-stereochemistry. Similarly (110c) was converted to an azetidinone intermediate for  $\beta$ -methylcarbapenems. An intramolecular version led to bicyclic isoxazolidine (112) as a single isomer<sup>116</sup> ultimately from methyl (R)-3-hydroxy-2-methylpropionate; the hydrogenolysis-DCC sequence again afforded a  $\beta$ -methylcarbapenem intermediate. Another cycloaddition reaction employed was the addition of  $\alpha$ -enamino,  $\beta$ -ketoesters (113) to ketene acetals, generating dihydropyrans (114) in high yields; the 4-epimers were separable.<sup>117</sup> Hydrolysis of (114) with HCl, then triethylsilane reduction, gave lactones like Melillo's (103a,b)<sup>104-106</sup> with high stereocontrol.<sup>118</sup> Deprotection, ring-opening and DCC closure generated the azetidinones (115), again en route to  $\beta$ -alkylcarbapenems. The synthesis of  $\beta$ -sulphonamido acids of type (116) from aziridines has been described before (see vol.20); a combination of DCC and 4-pyrrolidinopyridine effected closure to the  $\beta$ -lactam in 83% yield (a general preparation of N-tosyl amides and lactams).<sup>119</sup> The product had the correct absolute stereochemistry for progression, by N-deprotection (sodium naphthalide), acidolysis and oxidation<sup>120</sup> to a known precursor of (+)-PS-5(100). It was found that lithium N-benzyltrimethylsilylamide would add in Michael fashion in high yield to crotonates;<sup>121</sup> trapping the enolates resulting with electrophiles, e.g. alkyl halides, gave intermediates which were hydrolysed to  $\beta$ -amino-acids and closed to cis or trans- $\beta$ -lactams (117a,b) by the Mukaiyama-Ohno procedure.

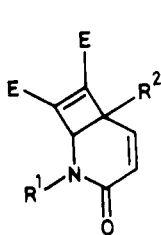
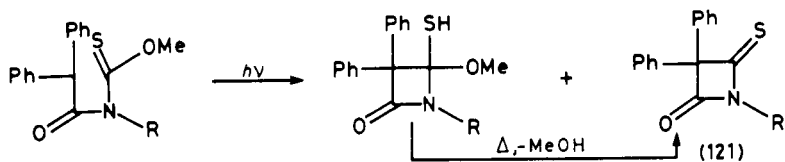
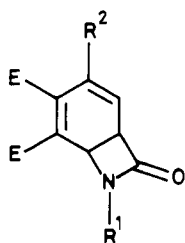
A conceptually quite different approach began with the  $\alpha$ -bromoglycine menthyl ester (118).<sup>122</sup> Displacement of Br with methyl acetoacetate and borane reduction gave the product of

desired stereochemistry as the major and separable one; subsequently ester (119) was cyclised to the azetidinone using  $\text{Pr}^i\text{MgBr}$  in 91% yield en route to (70).

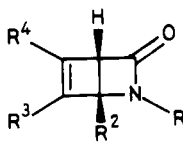
3,4 bond-forming reactions.— A reinvestigation of the use of carbenoid methodology in azetidinone synthesis has shown that diazo-(*N*-*t*-butyl)amides (120) were cyclized using rhodium (II) acetate in benzene to the 3,4-*trans*- $\beta$ -lactam in 98% yield.<sup>123</sup> Mixtures resulted if other C-H bonds were available for carbenoid insertion, but other azetidinones and a carbacepham were also prepared by judicious choice of substituents.

Photochemical methods are well-established for this mode of ring formation, including the above mentioned photolysis of monothioimides (*cf.* Scheme 4).<sup>96</sup> Similarly, irradiation of some acetylthiocarbamates led, again by Norrish type II reaction, to 4-thioxoazetidin-2-ones (121) (Scheme 5) in up to 65% yield after brief heating;<sup>124</sup> alternative preparations and the photochemistry of (121) were given. Photocycloaddition of dimethyl acetylenedicarboxylate to 2-pyridones gave, among other products, [2+2] adducts (122) which rearranged thermally to fused bicyclic  $\beta$ -lactams (123).<sup>125</sup> The well-known photoconversion of 2-pyridones to azabicyclo [2.2.0] hexanes (124) (*cf.* vol.19) may give products of high enantiomeric excess on using appropriate chiral hosts.<sup>126</sup> A further report on the photolysis of pyrimidin-4-ones has appeared; in acetic acid-acetonitrile mixtures, the intermediate 'Dewar pyrimidinone' [*cf.* (124)] led to 4-acetoxiazetidinones (125); where  $\text{R}^2=\text{Me}$ , 4-vinylazetidinones also resulted.<sup>127</sup> Other products were examined in detail and mechanisms discussed.

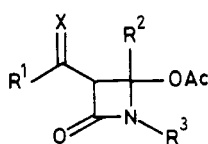
Cyclisation of epoxides is another popular means of effecting this mode. Thus the threonine-derived epoxyamide (126) with lithium hexamethyldisilazide gave only the *trans*-azetidinone product in 71% yield;<sup>128</sup> the *N*-phenylalkoxymethyl group was removed by dilute acid. In another report a chiral 'Sharpless' epoxy alcohol was transformed to an intermediate of type (126) and cyclized to (127) with the same base. An interesting radical elimination of the ditosylated C(3)-side-chain of (127) ( $\text{NaI}$ , tri-*n*-butyl tin hydride) led to C(3)-vinyl, thence the PS-5 ethyl group.<sup>129</sup> The racemic epoxy mono-acid (128), obtained by enzymic hydrolysis, was progressed to a  $\beta$ -lactam like (127), but with a (1*S*)-1-hydroxymethyl-1-[(1*S*)-1-phenylethylaminocarbonyl]substituent

(122) E = CO<sub>2</sub>Me

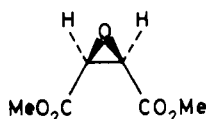
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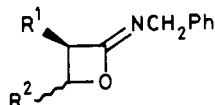
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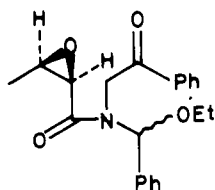
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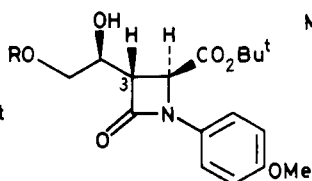
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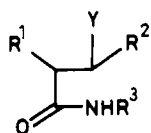
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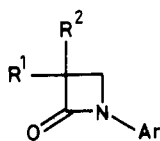
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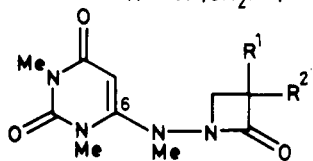
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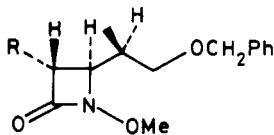
(130) Y = Br, OSO<sub>2</sub>Me;  
R<sup>3</sup> = Ar, CH<sub>2</sub>Ph, OCH<sub>2</sub>Ph

(131) R<sup>1</sup> = Me, R<sup>2</sup> = Br

(132) R<sup>1</sup>, R<sup>2</sup> = H, F, Br; Ar =

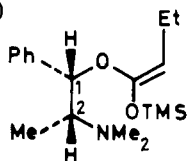


(133)

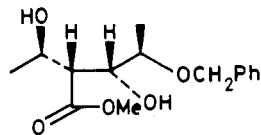


(135) R = α-Et

(137) R = H



(134)



(136)

at C(3); here the base employed was tetra-*n*-butylammonium fluoride.<sup>130</sup>

4-1 bond forming reactions.- Almost invariably this mode involves nucleophilic displacement by N(1) at C(4). The previously reported 2-iminooxetane to azetidinone rearrangement (see vol.20) is an exception; a full account of the lanthanide-catalysed addition of ketimines to aldehydes, including cis-trans ratios of the products, e.g. (129), has appeared.<sup>131</sup>

An electrochemical procedure, using tetramethylammonium perchlorate as supporting electrolyte, was used to close propionamides or -hydroxamates (130) to β-lactams.<sup>132</sup> High yields and good stereochemical control were obtainable, especially using bromomalonate ester as the 'pro-base' whose anion then generates the N-anion. Other more conventional β-bromopropionamide cyclizations featured syntheses of azetidinones (131) in a two-phase basic system using a phase-transfer catalyst<sup>133</sup> from 2,3-dibromo precursors and of a series of N-(carboxyaryl)-azetidinones (132) using sodium hydride in CH<sub>2</sub>Cl<sub>2</sub>-DMF.<sup>134</sup> Compounds (132) were competitive inhibitors but not substrates for a β-lactamase. Some novel uracils (133) (and cognate γ- and δ-lactams) were prepared from the 6-(methylhydrazino)uracils by acylation with haloacyl halides and phase-transfer base cyclization.<sup>135</sup> As an instance of a general iodolactam synthesis (four- to six-membered rings), treatment of the N-,O-bis(trimethylsilyl)imidate of 3-butenamide with iodine in THF gave<sup>136</sup> 4-(iodomethyl)azetidin-2-one in 35% yield. Crotonamide was a major by-product.

Cyclizations of hydroxamates, sulphamates etc. by intramolecular alkylation or Mitsunobu-Miller condensation continue to be much utilised. Aldol condensation of chiral (1*S*,2*R*) silyl ketene acetal (134) with (*S*)-3-(benzyloxy)-2-methylpropionaldehyde gave a single aldol isomer in 70% yield which was converted into β-lactam (135) by hydroxamate-mesylate alkylation;<sup>137</sup> this β-methylcarbapenem precursor was also obtained from similar intermediates using a thiolester. A double asymmetric aldol reaction of a silyl ketene acetal with (2*S*)-2-(benzyloxy-methyl)propionaldehyde led to the aldol (136), bearing four chiral centres, in 97% diastereomeric purity; closure to the β-lactam was again effected via a hydroxamate-mesylate.<sup>138</sup> Using similar

methodology, the 3-unsubstituted  $\beta$ -lactam (137) was obtained ultimately from a silylthioester enolate-aldehyde chelation-controlled addition.<sup>139</sup> Two reports featured syntheses of monobactams [3 $\beta$ -(acylamino)azetidin-2-one-1-sulphonates] by this route. In one, D-glyceraldehyde was converted (with one isomer separation) into acylsulphamate (138) which was closed to the 3,4-trans- $\beta$ -lactam with base.<sup>140</sup> In the other, (2R,3S) epoxides (139), from calcium L-threonate or L-tartaric acid, were progressed by ammonolysis (on the sodium salt) and further steps to the epimeric acylsulphamate (140) which afforded a 3,4-cis-monobactam (carumonam precursor) on basification.<sup>141</sup>

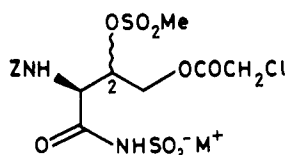
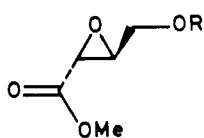
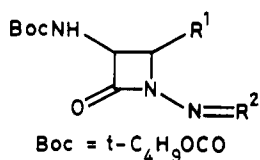
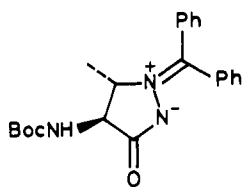
Uses of the Mitsunobu-type procedure included a synthesis of novel N-azamonobactams (141) by cyclization of hydrazides of ( $\alpha$ -amino, $\beta$ -hydroxy)acids.<sup>142</sup> Here the mesylation-base treatment sequence gave mostly the ylid (142). Arylamides of Boc-L-serine were also sufficiently acidic to undergo Mitsunobu closure to protected N-(hydroxyaryl)azetidinones (143).<sup>143</sup> Derivatives of (141) and (143) were antibacterially inactive. The above mentioned silyl ketene acetal (134) was also condensed with glyoxylate or cinnamaldehyde; the aldol products were cyclised (Mitsunobu) to separable  $\beta$ -lactams (144), R=OCH<sub>2</sub>Ph. Similarly, addition of (134) to suitable imines, followed by  $\beta$ -amino-acid or-ester closure, gave (144), R=H,Ph.<sup>144</sup> The propionaldehyde-derived acid (145a) was transformed to hydroxamate (145b); after Mitsunobu cyclisation, the  $\beta$ -lactams were transformed to cis- and trans-4-vinyloxamazins which were weak antibacterials.<sup>145</sup>

### Reactions in which two bonds are formed

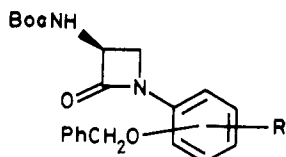
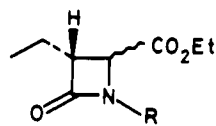
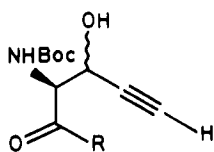
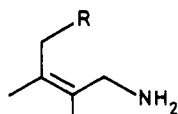
This sub-section includes formal [3+1] or [2+2] additions which may be concerted or stepwise under the conditions used.

[3+1]additions: 1-2 and 2-3 bond formation.- The bromoallylamine (146) gave, stereoselectively, the  $\beta$ -lactam (147) on treatment with carbon monoxide and tetrakis(triphenylphosphinyl)palladium (0).<sup>146</sup> Conversion of aziridines (148) to  $\beta$ -lactams was achieved with iodide ion-nickel tetracarbonyl; the less substituted C-N bond was carbonylated.<sup>147</sup>

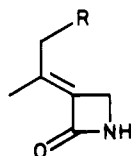


(138) 2  $\alpha$ - $\text{OSO}_2\text{Me}$ (140) 2  $\beta$ - $\text{OSO}_2\text{Me}$ (139)  $\text{R} = \text{H}, \text{Ac}$ (141)  $\text{R}^1 = \text{H}, \text{Me};$  $\text{R}^2 = \text{CPh}_2, \text{H}_2$ 

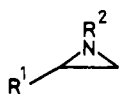
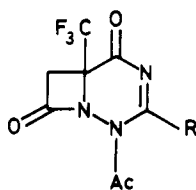
(142)

(143)  $\text{R} = \text{H}, \text{CF}_3$ (144)  $\text{R} = \text{OCH}_2\text{Ph},$  $\text{Ph}, \text{H}$ (145) a;  $\text{R} = \text{OH}$ b;  $\text{R} = \text{NHCH}_2\text{CO}_2(\text{CH}_2)_2\text{TMS}$ 

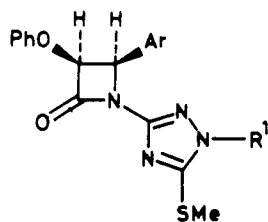
(146)



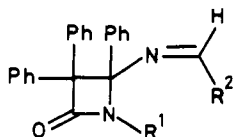
(147)

(148)  $\text{R}^2 = \text{CH}_2\text{Ph},$  $\text{SO}_2\text{Me}$ 

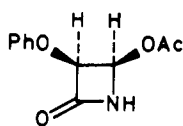
(149)



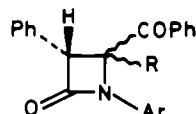
(150)



(151)



(152)



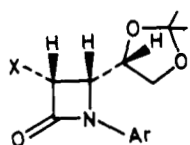
(153)

[2+2]additions

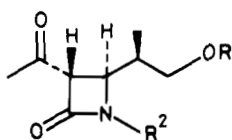
1-2 and 3-4 bond formation.- As in vols.19 and 20, detailed mention of condensations of this class will be made only where new chemical features were present; see also the Appendix. A fuller report has appeared on the photolytic addition of chromium carbene complexes (cf.vol.20) to imines, or s-1,3,5-triazines, generating azetidin-2-ones as well as a carbapenam.<sup>148</sup> Photolytic addition of ketene (or of allene, followed by ozonolysis) to 6-trifluoromethyl 1,2,4-triazines and their 5-ones gave fused  $\beta$ -lactams, e.g. (149).<sup>149</sup>

Cycloaddition of thioketenes to isocyanates gave mainly 4-thioxoazetidinones like (121); azetidin-2,4-diones were among the by-products.<sup>150</sup> Schiff's bases derived from 2-substituted-5-amino-1,2,4-triazoles underwent addition to ketenes, giving  $\beta$ -lactams (150), but 1-substituted analogues gave other products.<sup>151</sup> Cycloaddition of 1,3-diazabutadienes to diphenylketene could give the [2+2] products (151) depending on substitution.<sup>152</sup> 2-Aza-1,3-dienes (from aldehydes plus allylamine) similarly underwent the acid chloride-imine reaction, giving N-(1-propenyl)  $\beta$ -lactams, one of which was transformed to a 4-acetoxy compound (152)<sup>153</sup> (34% overall). A reinvestigation of 4-acyl  $\beta$ -lactam formation from 1,2-iminoketones (cf.vol.19) showed the same product stereochemistry [in, e.g. (153)] using ketene or acid chloride with or without base.<sup>154</sup> An intermediate zwitterion is implicated.

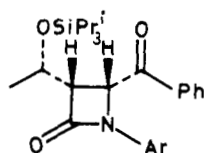
Two further reports have appeared on the use of D-glyceraldehyde-derived imines in condensation with acid chlorides. The optically active cis-products, e.g. (154) could be inverted at C(3) by mesylate displacement or base epimerisation (Z=Pht) and at C(4) by prolonged lead (IV) acetate oxidation of the derived diol.<sup>155,156</sup> Applications to syntheses of carbapenam intermediates involved cycloaddition of diketene to a chiral imine to give 3-acetyl derivative (155), with its 3,4-di-epimer, in fair yield<sup>157</sup> and of a ketene derived from (3S)-3-hydroxybutanoic acid to an  $\alpha$ -iminoketone, generating the cis-3,4  $\beta$ -lactam (156) as the major product,<sup>158</sup> epimerised by fluoride at C(4). Moving to some 3-amino examples, the chiral imines (157), derived from 'Sharpless' epoxyalcohols, generated very largely cis- $\beta$  lactams of type (158) on adding to glycine acid chlorides.<sup>159</sup> The nocardicin series was entered using a phenylglycine-cinnamaldehyde imine; [2+2] addition,



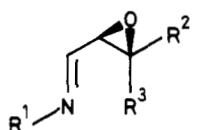
(154)  $X = N_3, \text{Pht}, \text{OSO}_2\text{Me}, \text{etc.}$



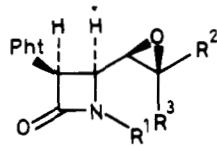
(155)



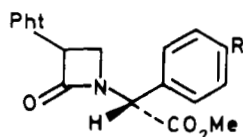
(156)



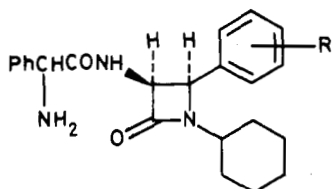
(157)



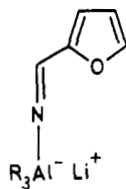
(158)



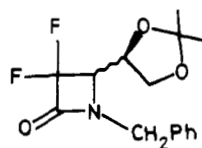
(159)  $R = H, \text{OAc}$



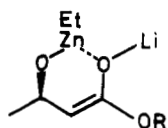
(160)



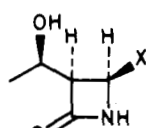
(161)



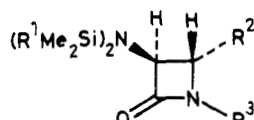
(162)



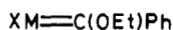
(163)  $R = \text{Me}, \text{Pr}^i$



(164) a;  $X = \text{C}\equiv\text{C}\cdot\text{Ph}$   
b;  $X = \text{CH}=\text{CHPh}(E)$

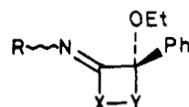


(165)



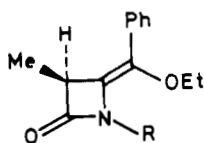
(166)  $X = (\text{CO})_2(\text{MeC}_5\text{H}_4)\text{Mn}$

(168)  $X = (\text{CO})_5\text{Cr}$  or  $(\text{CO})_5\text{W}$

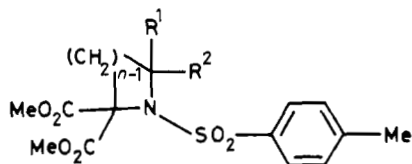


(167) a;  $X = \text{C}=\text{O}, Y = \text{NR}$

b;  $X = \text{NR}, Y = \text{C}=\text{O}$



(169)  $R = \text{Me}, \text{c-C}_6\text{H}_{11}$



(170)  $n = 1$  to  $3$

oxidative cleavage of the C(4)-group and modified silane reduction (vol.20) led to racemic (159).<sup>160</sup> Other examples were a series of 4 $\beta$ -aryl nocardicins, some of which were  $\beta$ -lactamase inhibitors,<sup>161</sup> and some 3 $\beta$ -( $\alpha$ -amino)phenylacetamido azetidinones (160)<sup>162</sup> which also had antibacterial interest.

Considering now the ester enolate plus imine variant, three further reports discussed the use of silylimines (see vol.20). In one, an *N*-trimethylsilyl imine from (S)-lactic aldehyde reacted directly with a lithium enolate to give primarily (96:4) a 3,4-trans- $\beta$ -lactam readily converted to a (+)-PS-5 precursor.<sup>163</sup> Another approach employed nitrile reduction with a metal aluminium hydride; the imino-aluminate, e.g. (161), was treated with trimethylsilyl chloride and a lithium enolate, giving several 3,4-disubstituted  $\beta$ -lactams in fair yield<sup>164</sup> (free-NH compounds resulted on workup). A related procedure utilised ZnI<sub>2</sub>-catalysed addition of non-enolizable silyl aldimines to silyl ketene acetals;<sup>165</sup> addition of MeMgBr completed the synthesis of predominantly 3,4-trans- $\beta$ -lactams. The Reformatsky reaction is often used in this way; for instance, addition of Zn-HgCl<sub>2</sub>, then an *N*-arylcinnamaldimine, to  $\alpha$ -bromobutyrate led again to a precursor of ( $\pm$ )-PS-5.<sup>166</sup> Some 3,3-difluoro-4-substituted azetidinones, e.g. (162) were similarly prepared.<sup>167</sup> A related concept was the addition of the strongly chelated zinc-lithium enolate (163), from (*R*)-3-hydroxybutyrate, to imines generating single cis-isomers (164a,b) in high yield.<sup>168</sup> The use of zinc enolates of  $\alpha$ -amino-acid esters (see vol.20) has been extended to the synthesis of trans-3-amino- $\beta$ -lactams (165) with high trans preference.<sup>169</sup>

### Reactions in which three bonds are formed

#### [1+1+2] additions

Another example of the addition of a carbene complex, viz. (166) with two isocyanide molecules has been reported; after oxidative hydrolysis both azetidinone isomers (167a,b) were isolable.<sup>170</sup> Apparently the first-formed ketenimine complex may react in a [3+1] or [2+2] mode with the second isocyanide. One of the products from the reaction of the similar complexes (168) with an isocyanide-ynamine mixture was the 4-vinylidene azetidinone (169).<sup>171</sup>

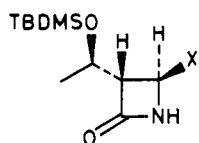
Chemistry of azetidinones

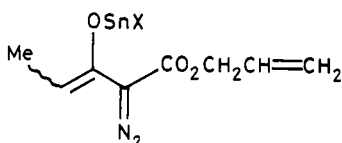
A new  $\beta$ -lactam synthesis began with the electro-oxidation of  $\omega$ -(tosylamino)malonates to diesters (170). In the case  $n=2$ , ester hydrolysis followed by a second anodic oxidation afforded N-tosyl  $\beta$ -lactams.<sup>172</sup>

As usual, the year delivered a fair crop of C(4)-displacements. An example of a general amidoalkynylation reaction was the treatment of a C(4)-phenylthio  $\beta$ -lactam using a 'neutral' (trimethylsilyl)alkynylzinc reagent, giving 80-90% of (171).<sup>173</sup> Displacement of the C(4)-acetoxy group in (70),  $R=\text{TBDMS}$ , was efficiently achieved using an O-silyl enol ether of a thiopropionate, especially the (2-picoyl)thiopropionate, which gave products like (115) with excellent (3S,4S)-selectivity.<sup>174</sup> Other enolates used for displacement of C(4)-acetoxyazetidinones en route to 1 $\beta$ -methylcarbapenem precursors were the di-anion or (bis-silyl)enol ether of methyl 3-oxopentanoate, giving (172) and its 1'- $\alpha$ -Me epimer in a ratio dependent on the metal catalyst used,<sup>175</sup> and the tin enolate (173).<sup>176</sup> (cf. vol.20). The latter delivered >98% of a single isomer on reaction with (70),  $R=\text{TBDMS}$ , in 45% yield. Enolate displacements of C(4)-OAc on N-substituted azetidinones required trimethylsilyl triflate catalysis; N-(trimethylsilyl)methyl examples were more reactive, and (4-phenacyl)  $\beta$ -lactams (174) were formed in up to 70% yield.<sup>177</sup> Another kind of C(4)-modification was the reaction of epoxide (175a), itself available from (70),  $R=\text{TBDMS}$ , by epoxide opening with  $\text{MgBr}_2$  followed by oxidation to  $\alpha$ -bromoketone (175b).<sup>178</sup>

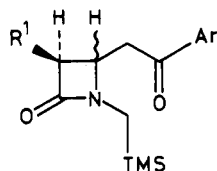
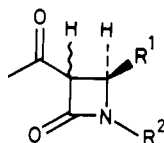
Modification at C(3) was achieved via an azetidin-2,3-dione,<sup>179</sup> itself accessible by oxidation of a 3 $\beta$ -hydroxy  $\beta$ -lactam ( $\text{Me}_2\text{SBr}_2, \text{NEt}_3$ ); condensation with a nitroalkane, then treatment with tri-*n*-butyl tin hydride and ozone, gave a 3-acetyl product (176). Azetidinone (177a) was available as a single enantiomer; it was transformed by a sequence of steps including oximation and reduction to monobactam precursor (177b).<sup>180</sup> Simultaneous C(3)- and C(4)-modification were performed on bicyclic azetidinone (178), also a single enantiomer. A sequence of steps including allylic selenoxide rearrangement led to lactone (179), thence by reduction and C(3)-epimerisation to a thienamycin precursor.<sup>181</sup>

Oxidative modifications were mentioned in several papers. Electrochemical oxidation of N-(4-methoxybenzyl)- $\beta$ -lactams

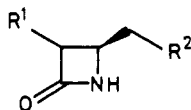
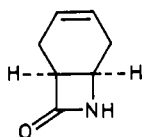
(171)  $X = C \equiv C TMS$ (172)  $X = (R) - CH(Me)COCH_2CO_2Me$ (175) a;  $X = CH_2CH-CH_2$   

b;  $X = CH_2CO_2CH_2Br$ 

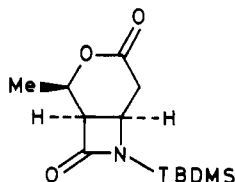
(173)

(174)  $R^1 = Ph, PhO$ 

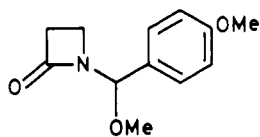
(176)

(177) a;  $R^1 = H, R^2 = CO_2Me$ b;  $R^1 = \beta-ZNH,$   
 $R^2 = CH_2OH$  or  $CH_2OMe$ 

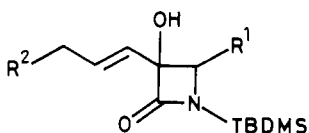
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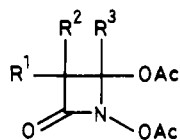
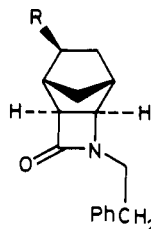
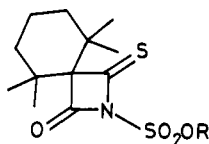
(179)



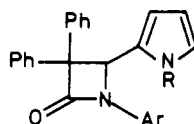
(180)



(181)

(182)  $R^3 = H$  or  $OAc$ (183) a;  $R = H$ b;  $R = OH$ 

(184)

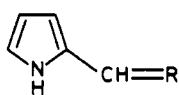
(185)  $R = H, Me$

in methanol gave 'exo' products, e.g. (180); acid cleavage to the free-NH-compound, then electrolysis in acetonitrile-acetic acid, gave 4-acetoxy- $\beta$ -lactams. In a similar vein, 'Kolbe' electrolysis of 4-carboxy- $\beta$ -lactams in MeCN-AcOH produced 4-acetoxy products, e.g. (70), R=TBDMs, in about 80% yield.<sup>182,183</sup> Anodic N-dearylation of N-(methoxyphenyl)azetidin-2-ones in aqueous MeCN led to a series of 3,4-disubstituted products in 59-95% yield.<sup>184</sup> A series of 3-alkenylazetidin-2-ones, available by Peterson olefination, afforded 3-alkenyl-3-hydroxy products (181) in 31-53% yield on treatment with LDA and oxodiperoxymolybdenum(pyridine)-hexamethyl phosphoramidate (MoOPH).<sup>185</sup> Potassium peroxomonosulphate effected oxidation of a 4-benzoyl- $\beta$ -lactam to the corresponding benzoyloxy derivative; a 3-(1-hydroxyethyl) group was unaffected.<sup>186</sup> Further details have appeared (cf. vol.20) of the oxidation of N-hydroxyazetidines with Pb(OAc)<sub>4</sub> to di- or tri-acetoxy- $\beta$ -lactams (182); similarly a bicyclic N-hydroxyazetidine gave a diacetoxy carbacepham.<sup>187</sup>

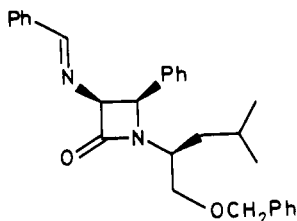
Biohydroxylation of several mono and polycyclic  $\beta$ -lactams was achieved<sup>188</sup> by the fungus *Beauveria sulfurescens*; thus (183a) gave (183b) (65%), regio- and stereospecifically. Treatment of N-sulphonylthioxoazetidinones (184) with alcohol and base led to N-S bond fission; alkylation of the liberated anion by sulphonate then gave mainly N-rather than S-attack.<sup>189</sup> Further chemistry of (184) was discussed. Two relevant mechanistic papers discussed the Mitsunobu esterification-inversion of the hydroxy group in 3-(1-hydroxyethyl)azetidin-2-ones<sup>190</sup> and the alkaline hydrolysis of 4-(pyrrol-2-yl)azetidin-2-ones (185).<sup>191</sup> Alternative cleavages of C(3)-C(4) or C(4)-N(1) led to both olefins (186a) and imines (186b).

#### Further uses of azetidinones

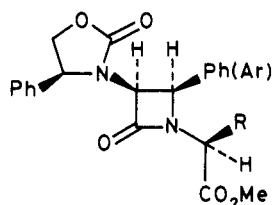
For the scope of this sub-section, see vol.20. Considering first applications to amino-acids and peptides, stereospecific 3 $\alpha$ -alkylation of chiral imine (187) followed by reductive N(1)-C(4) cleavage led to ( $\alpha$ -alkyl)phenylalanines<sup>192</sup> (cf. vol.20). A full account of a related synthesis of phenylalanine dipeptides via highly chiral  $\beta$ -lactam (188) has appeared.<sup>193</sup> A novel route to  $\alpha$ -amino-acid N-carboxy-anhydrides,



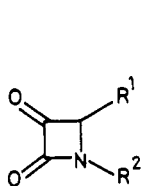
(186) a; R = CPh<sub>2</sub>  
b; R = NAr



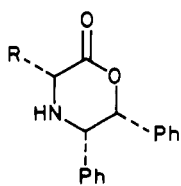
(187)



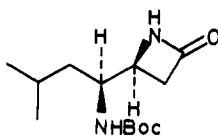
(188)



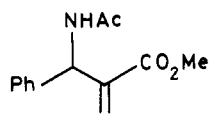
(189)



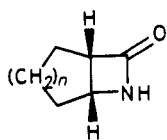
(190)



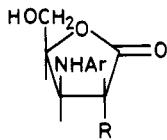
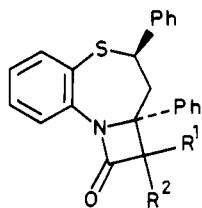
(191)



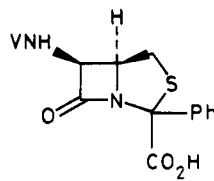
(192)



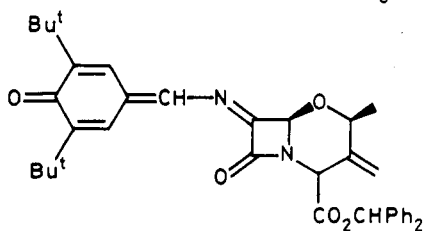
(193)

(194) R = OMe, N<sub>3</sub>

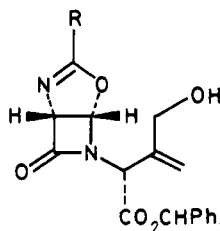
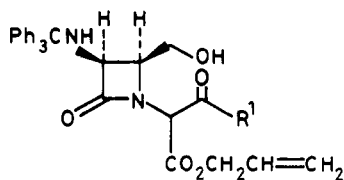
(195)



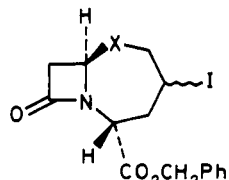
(196)



(197)

(198) R = Ph or PhOCH<sub>2</sub>

(199)

(200) X = O, CH<sub>2</sub>



and thence various amino-acid derivatives, was the Baeyer-Villiger oxidation of azetidine-2,3-diones (189).<sup>179</sup> Consideration of the likely mechanism of reaction of chromium-carbene complexes with imines, leading to  $\beta$ -lactams, led to the conclusion that metal-ketene complexes were involved; intramolecular trapping with amino alcohols led to lactones (190) and thence amino-acids.<sup>194</sup>

A stereospecific synthesis of 3-aminodeoxystatine called for the hydrolysis of  $\beta$ -lactam (191), formed by a Mitsunobu cyclization.<sup>195</sup> The diastereoselection achieved by some Ru(II) and Rh (I) complexes in hydrogenation of acrylates (192) was assessed in some cases by cyclizing the products to  $\beta$ -lactams (characteristic 3-H to 4-H coupling).<sup>196</sup> Reduction of bicyclic  $\beta$ -lactams (193) afforded either  $\gamma$ -amino alcohols (Raney Ni), carboxamides (Ni on Cab-O-Sil®) or azetidines ( $\text{LiAlH}_4$ ).<sup>197</sup> The above mentioned<sup>156</sup> glyceraldehyde-derived  $\beta$ -lactams (154) were convertible to aminoglycoside-like species (194) and the 3,3-difluorocompound (162)<sup>167</sup> could be converted to either daunosamine or difluorogulopyranoside derivatives. The fused 1,5-benzothiazepine- $\beta$ -lactams (195) were subjected to detailed spectroscopic study to determine the conformations of the seven-membered ring<sup>198</sup> (cf. the 1,4-analogues, vol.20).

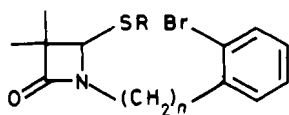
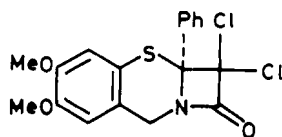
## 9. Major Structural Variants

As usual, systems which maintain a  $\beta$ -lactam element will be mentioned first. A synthesis of isopenam (196), which was antibacterially inactive, was achieved in three steps from a 4 $\beta$ -(hydroxymethyl)azetidinone precursor.<sup>199</sup> In a synthesis of a 2[3-methyl-1-oxa analogue of ceftazidime starting from a 7 $\alpha$ -acylamino precursor, the 7 $\beta$ -stereochemistry was obtained by borohydride reduction of quinomethine (197); the final product showed good activity.<sup>200</sup> On heating an exomethylenecepham 1[3-sulphoxide with  $\text{Ph}_3\text{P}$ , the oxazoline- $\beta$ -lactam (198) was formed directly; boron trifluoride treatment then gave a 1-oxacepham in 27% overall yield.<sup>201</sup> A series of orally absorbed 0-2-iso-cephems<sup>202</sup> were produced by Mitsunobu cyclization of hydroxy  $\beta$ -ketoesters (199) followed by transformation to 7[3-acylamido derivatives; they showed fair antibacterial potency.

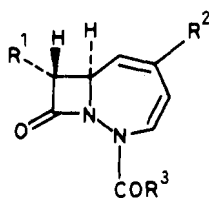
Further investigation (cf. vol.19) of the  $\gamma$ -elimination of hydrogen halide from the homo-carbacephams(-oxacephams) (200) has shown<sup>203</sup> that the fused cyclopropyl products have  $\alpha$ -or  $\beta$ -stereochemistry according to the iodo stereochemistry (W-elimination). A tricyclic benzopenam and a benzocepham were formed by radical substitution at sulphur<sup>204</sup> from bromoaryl precursors (201) together with other products. Treatment of the dichloro benzothiaoctem (202) with methanol in base<sup>205</sup> led to complex rearrangements; a 1,4-benzothiazepine, an isoquinoline, and a thiazole were isolated.

Some azetidinodiazepines (203) underwent cycloaddition to acylnitroso compounds to give regioisomeric [4+2] adducts on their convex face; osmium tetroxide treatment of the products led to rearrangement<sup>206</sup> and 3-oxazinyl- $\beta$ -lactams (204) resulted. The addition of isocyanates to diphenylcyclopropenone oxime afforded 1,3-diazetidinones (205).<sup>207</sup> The product from addition of ethyl isocyanate in acetone to tryptophan,<sup>208</sup> however, is now considered to be a 1,3-oxazolidin-5-one, not a 1,3-diazetidinone (cf. vol.20). Other modified monocyclic systems were the 1,3-thiazetidin-2-ones (206)<sup>209</sup> resulting from addition of phosgene to (acetylthio)-acetanilides in 40-65% yield.

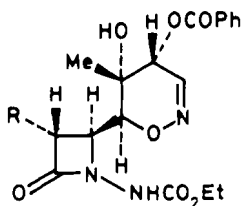
Analogues in which a  $\beta$ -lactam has been replaced by a five-membered ring continue to be explored. An interesting synthesis of a 'homopenicillin' (207) involved addition of a sulfoxonium ylid to a benzylpenicillin ester and u.v. irradiation of the product,<sup>210</sup> possibly via oxacarbene-ketene rearrangement. L-Aspartic acid was transformed to pyrrolidinone (208) in several steps<sup>211</sup> and a carbene-NH insertion then used to complete a  $\gamma$ -lactam-carbapenem synthesis; final products had slight activity. Three further reports from Lilly workers described bicyclic pyrazolidinones which can show good antibacterial activity (cf. vol.20). The key pyrazolidinium ylid (209) reacted with E-vinyl sulphones to give, after sulphinic acid elimination, very largely (86-100%) the desired regioisomer (210).<sup>212</sup> A nuclear analogue with sulphur at position 2 and no  $\Delta^2$ -double bond was obtained by cycloaddition of an in situ generated<sup>213</sup> thioaldehyde to (209); neither of the final 3 $\alpha$ -and 3 $\beta$ -carboxyepimers was active. The higher bicyclic [4.3.0] fused pyrazolidinones (211) prepared as in Scheme 6 also proved, after conversion of R to an ( $\alpha$ -oximino)aminothiazolyl side chain, to be antibacterially inactive.<sup>214</sup> A full report (cf. vol.19) has appeared on the

(201) R = alkyl, aryl;  $n = 0$  or 1

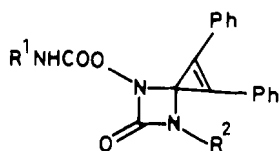
(202)



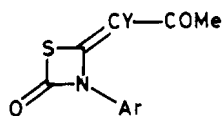
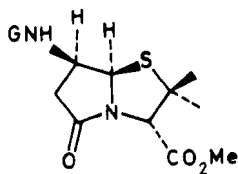
(203)



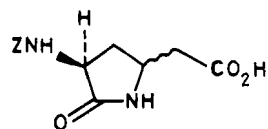
(204)



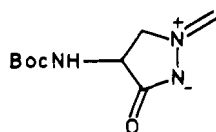
(205)

(206) Y = H, SCF<sub>3</sub>

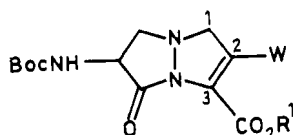
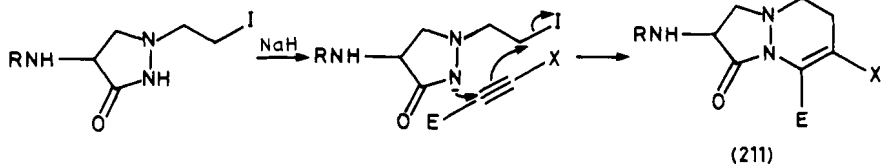
(207)



(208)



(209)

(210) W = COR<sup>2</sup>, CN, H

(211)

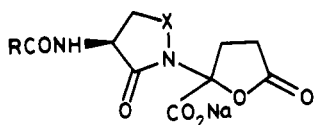
Scheme 6

isolation, structure and chemistry of lactivicin (212)<sup>215</sup> and, in a logical extension of this work, some aza-analogues such as (213) have been prepared, the key step being addition of hydrazine to a dehydroalanine derivative: (213) displayed weak activity.<sup>216</sup>

#### 10. Mechanistic Studies on Mode of Action and Degradation

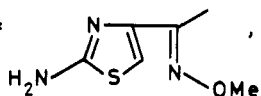
A number of papers have examined the interaction of penicillins and cephalosporins with  $\beta$ -lactamases. The above spiroepoxy penicillins (66a), where  $X = \text{PhNHCO}$  and  $Y = \text{Cl}$  or vice versa, proved to be irreversible inhibitors of  $\beta$ -lactamase I from Bacillus cereus,<sup>217</sup> both were turned over to the same hydrolysis product, dihydrothiazine (214), and a mechanism was given. Three reports examined the hydrolysis of 6-alkyl penicillins, 7-substituted cephalosporins, and 3-substituted cephalosporins by the same enzyme (I and II strains) and by hydroxide.<sup>218,219,220</sup> It was concluded, on kinetic grounds, that there was no evidence for an enzyme 'pocket' for recognising hydrophobic 6-acylamido side-chains of penicillins, or for recognising aromatic residues in 7-substituted cephalosporins. General acid catalysis by a carboxy group in the  $\beta$ -lactamase I was thought to facilitate  $\beta$ -lactam fission. Nor is there recognition of a cephalosporin C(3') substituent, and the previous contention that loss of a C(3') leaving group occurs after  $\beta$ -lactam opening (see. vol.19) seems justified.

A set of parameters 'MMPEN' has been developed which allows the application of Allinger's force field method to molecular mechanics of penicillins. Crystal structures of simple penicillins could be reproduced and differences between crystal and solution structures discussed.<sup>221</sup> In an accompanying study<sup>222</sup> the well-known suggestion (Tipper and Strominger, Hasan) that the active conformation of a penicillin corresponds to an acyl-D-Ala-D-Ala unit was examined. This appears to be valid for the pseudoaxial conformation of penicillin G, which is the global minimum. From a Hückel molecular orbital study, the electronic structure of some cephalosporins was correlated with antimicrobial activity;<sup>223</sup> orally-absorbed cephalosporins showed significantly greater stability to hydrolysis (frontier orbital coefficients).

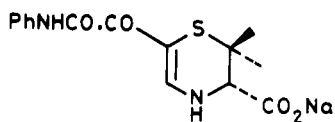


(212)  $R = \text{Me}$ ,  $X = \text{O}$

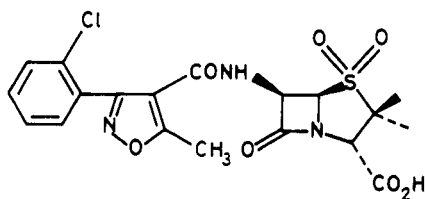
(213)  $R =$



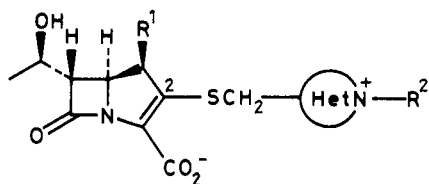
,  $X = \text{NH}$



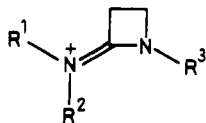
(214)



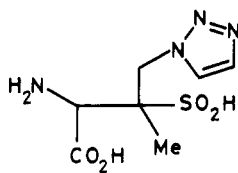
(215)



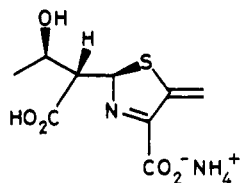
(216)



(217)



(218)



(219)

The cloxacillin sulphone (215) is not a cephalosporinase inhibitor but develops inhibitory activity on incubation with phosphate ions; however, this 'secondary inhibitor' has been shown not to be a  $\beta$ -lactam.<sup>224</sup> The ready  $\Delta^3 \rightarrow \Delta^2$  isomerisation of cefazolin methyl ester in pH7-8 buffer has been suggested as a possible reason for the failure to obtain more than modest enhancements of antibiotic concentrations by using cephalosporin esters.<sup>225</sup>

Moving to other  $\beta$ -lactams, a theoretical study of carbapenems of type (216) was made in an attempt to correlate the C(2)-side-chain with antibacterial activity;<sup>226</sup> the differential acidity of the  $-\text{SCH}_2$ -protons was spectroscopically studied and related to the quaternization site of the  $\text{N}$ -heterocycle. The acylating ability of  $\gamma$ -lactams of type (210) was compared with that of 'classical'  $\beta$ -lactams by measuring relative rates of lactam opening by hydroxide: this rate also correlated well with the  $\sigma_p$  value of the C(3)-substituent.<sup>227</sup> A theoretical study of  $\beta$ -lactam amide resonance in some 4-acylazetidiones has been made using infrared spectroscopy.<sup>228</sup> A fuller account (*cf.* vol.20) has appeared on the hydrolyses of azetidin-2-ylideneammonium salts of type (217) and their relevance to  $\beta$ -lactam action.<sup>229</sup>

The stability of the (acylamino)penicillins piperacillin, mezlocillin, and azlocillin was studied by liquid chromatography over the range pH3-10;<sup>230</sup> piperacillin was the most stable at pH3 (penilloic acid the main product) but much the least stable at pH10. Two papers discussed the degradation of a  $\beta$ -lactamase inhibitor, YTR-830H, *viz.* (60),  $\text{X}=\text{1H-1,2,3-triazol-1-yl}$  (B), both in solution<sup>231</sup> and the solid state.<sup>232</sup> In both cases the amino-acid (218) was isolable among a variety of products including formylacetic acid and the triazole. The aqueous degradation of sodium ceftriaxone has been studied;<sup>233</sup> products of both 7 $\beta$ -acylamino group hydrolysis and C(3')-substituent loss were found. When a 2-(carbamoyloxymethyl)penem FCE22101 was treated with renal dehydropeptidase, the imine metabolite (219) was isolable with careful buffering; it readily rearranged in base to a 5-methylthiazole.<sup>234</sup>

Appendix to Chapter 5:  $\beta$ -lactam Antibiotics Prepared for  
Structure-Activity Relationships and Miscellaneous  $\beta$ -Lactams

The  $\beta$ -lactams are arranged in the same sequence as the main sections of the report.

$\beta$ -Lactam

|                                                                                                              | Ref             |
|--------------------------------------------------------------------------------------------------------------|-----------------|
| 6-Fluoroquinolone carboxamidopenicillins                                                                     | 235,236         |
| 1,2,4-Triazino analogues of amoxycillin                                                                      | 237,238         |
| Oxaphosphorine derivatives of ampicillin                                                                     | 239             |
| C(6)-Vinylpenicillin polymers                                                                                | 240             |
| Penicillin V and cephalothin amino-acid coupling products                                                    | 241             |
| $^{14}\text{C}$ -labelled penicillins and cephalosporins                                                     | 242,243,244,245 |
| 7 $\beta$ -(4-Oxo-3,4-dihydrothieno[2,3-d]pyrimidyl-2-thioacetamido)cephalosporins                           | 246             |
| 7 $\beta$ -(Arylcycloalkyl)carboxamidocephalosporins                                                         | 247             |
| 6-Substituted-coumarin-3-formamidocephalosporins                                                             | 248             |
| 7 $\beta$ -[3-Chloro-2-(thiazol-4-yl)propenamido]cephalosporins                                              | 249             |
| Some[2-(heteroaryl methoxyimino)aminothiazolyl acetamido]cephalosporins                                      | 250             |
| FK 482, a new orally active 3-vinyl-3-cephem C(3')-[1-(Halophenyl)-1H-tetrazol-5-yl]thiomethylcephalosporins | 251             |
| Various 3-substituted 7 $\beta$ -(arylacetamido)cephalosporins                                               | 252,253         |
| 7 $\alpha$ -Methoxy-7 $\beta$ -(arylacetamido)-3-chloroceph-3-ems                                            | 254,255         |
| Cefpirome analogues with 7 $\beta$ -and C(3')-variation                                                      | 256             |
| 7 $\alpha$ -Methoxy and-formamido cefpirome analogues                                                        | 257,258         |
| A new C(3')-(triazolopyrimidinyl)thiomethyl 7 $\beta$ -aminothiazolyl cephalosporin                          | 259             |
| New 3-(O-substituted) cephalixin and cefixime analogues                                                      | 260             |
| 9-(2-Amidoethenylthio)clavulanic acid derivatives                                                            | 261,262         |
| 2-Sulphinyl-and-sulphonylmethylpenems                                                                        | 263             |
| $^{14}\text{C}$ -labelled penems and carbapenems                                                             | 264             |
| 1,4-Disubstituted monobactams                                                                                | 265,266         |
|                                                                                                              | 267,268         |

|                                                          |                 |
|----------------------------------------------------------|-----------------|
| 1,4-Disubstituted azetidinones from $\beta$ -amino-acids | 269             |
| 1-Aryl or heteroaryl-3-(or 4-)                           | 270,271,272,273 |
| chloroazetidinones                                       | 274             |
| 3-Chloroazetidinone-quinazolinones                       | 275             |
| 1,3,4-trisubstituted azetidinones                        | 276             |
| A $^{14}\text{C}$ -labelled oxacephem                    | 277             |
| 7 $\beta$ -Halovinylthioacetamido-7 $\alpha$ -methoxy-   | 278,279         |
| 1-oxacephems                                             |                 |

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# 6

## Metal Complexes of Amino Acids and Peptides

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BY R. W. HAY AND K. B. NOLAN

### 1 Introduction

This chapter deals with the synthesis, structures, reactions and applications of metal-amino acid and metal peptide complexes, and with a few exceptions reviews material published during 1988. A new journal "Biology of Metals" has appeared,<sup>1</sup> which covers some of the topics discussed in this review. Metal clusters in proteins are reviewed in a recent book,<sup>2</sup> as is the topic of amino acids, peptides and proteins as ligands.<sup>3</sup> The role of amino acid residues at the active sites of metalloproteins has been reviewed,<sup>4</sup> and dinuclear active sites of copper proteins discussed as stimulants for the development of new copper coordination chemistry.<sup>5</sup> There have been seven major reviews covering various aspects of the chemistry of metal-peptide complexes. One of these deals with electron transfer in metal complexes containing oligopeptides and proteins,<sup>6</sup> while another reviews the effect of peptide environments on electron transfer in Fe-S clusters which occur in proteins and in oligopeptide model complexes.<sup>7</sup> The synthesis and reactivity of platinum(II) and palladium(II) complexes of amino acids, peptides and their derivatives and the possible application of platinum(II) as an N-protecting group is the subject of another review.<sup>8</sup> Ligand-ligand interactions in ternary complexes of copper(II) and palladium(II) which contain amino acid or peptide ligands and their investigations by synthetic, potentiometric, spectroscopic and crystallographic methods are described.<sup>9</sup> The present status of high performance immobilised metal ion affinity chromatography is reviewed.<sup>10</sup> Using this technique high resolution of peptide and protein mixtures can be obtained and under specified conditions more than 1000 runs can be made repeatedly on a single column with up to a 10<sup>4</sup>-fold protein purification in a single run. The remaining reviews deal with the use of biscyclopentadienyltitanium polypeptides and bis(peptide)platinum complexes in the site specific delivery of drugs or as enzyme mimics,<sup>11</sup> and the use of n.m.r. studies to probe the role of zinc in the biological activity of thymulin, a thymic metallopeptide hormone.<sup>12</sup>

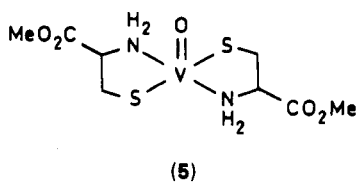
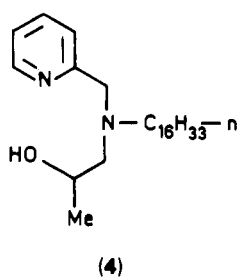
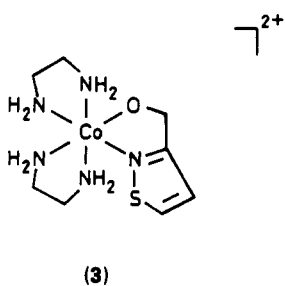
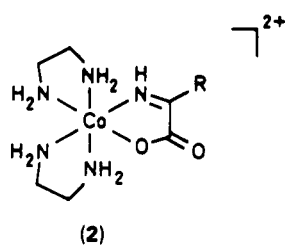
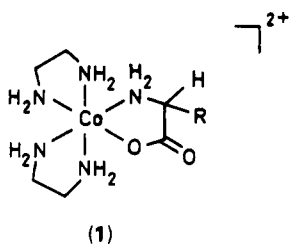
## 2 Amino Acids

**2.1 Synthesis of Amino Acids.**— A chiral Ni(II) complex of a Schiff's base derived from (*S*)-*O*-[(benzylpropyl)amino]benzophenone and  $\alpha$ -bromoglycine has been prepared and its stereoselective reaction with nucleophiles studied;<sup>13</sup> the synthesis of aspartic acid of 80% optical purity is described. A general approach to the synthesis of  $\gamma,\delta$ -unsaturated  $\alpha$ -amino acids has also been described.<sup>14</sup> Schiff bases derived from glycine and alanine esters were alkylated in the presence of palladium or molybdenum catalysts under neutral or basic conditions using allylic carbonates, esters or halides (20-95% yield). These less stabilised nucleophiles reacted with  $\eta^3$  allyl species on the opposite side from the palladium. The regioselectivity was studied with various unsymmetrical electrophiles. After hydrolysis, several functionalised  $\alpha$ -amino acids of biological interest (enzyme inhibitors) were obtained.

Sargeson has described a number of interesting reactions involving cobalt(III) complexes. Rapid oxidation of chelated amino acids in complex (1) to imines (2) and amides by thionyl chloride in DMF occurs.<sup>15</sup> Treatment of chelated threonine in complex (1) with  $\text{SOCl}_2$  in DMF gives complex (3) containing chelated 3-carboxyisothiazoline.<sup>16</sup> The mechanism of this transformation is discussed in detail. Oxidation of chelated hydroxy-L-proline by thionyl chloride gives substituted dihydropyrroles, which undergo subsequent base-induced elimination to give chelated pyrrole-2-carboxylate complexes.<sup>17</sup>

Complexes of Ni(II) Schiff bases of glycine and alanine with (*S*)-*N*-( $\alpha$ -naphthylmethyl)proline derivatives for the asymmetric synthesis of amino acids have been described.<sup>18</sup> An efficient synthesis of erythro- $\beta$ -hydroxy-L-histidine, the pivotal amino acid of the bleomycin-Fe(II)- $\text{O}_2$  complex has been reported.<sup>19</sup> Other studies of interest include a kinetic investigation of the catalysed oxidation of amino acids by *N*-bromosuccinimide in aqueous acetic acid,<sup>20</sup> and the role of the metal cation in the diastereoselective alkylation of glycinate.<sup>21</sup>

**2.2 Reactions in Solution.**— Some interesting kinetic and mechanistic studies have been described. Chiral Cu(II)-chelating



micelles of 2[hexadecyl- $\underline{N,N}$ -(2-hydroxypropyl)aminomethyl]pyridine, (4), are effective and remarkably enantioselective catalysts for the hydrolytic cleavage of *p*-nitrophenyl esters of  $\alpha$ -amino acids.<sup>22</sup> Thus in the presence of the ( $\underline{R}$ )-enantiomer of (4) ( $\underline{S}$ )-PhePNP is hydrolysed some 14 times faster than ( $\underline{R}$ )-PhePNP. In a further paper,<sup>23</sup> it is reported that the chiral ligand ( $\underline{R}$ )-(4) acts as a carrier for the Cu(II) mediated transport of  $\alpha$ -amino acids coupled with the counter-transport of protons. Amino acidate dechelation on hydroxo complex formation in mixed ligand metal complexes has been studied.<sup>24</sup> Transamination reactions of  $\alpha$ -amino acids and  $\alpha$ -keto acids have been investigated by 200 MHz  $^1\text{H}$  n.m.r. using 1:1:1 ratios of pyridoxal/ $\alpha$ -amino acid/Al(III) or pyridoxamine/ $\alpha$ -keto acid/Al(III) in  $\text{D}_2\text{O}$  at  $10^\circ\text{C}$ . A negatively charged deprotonated Schiff base coordinated to Al(III) was detected in all systems that undergo transamination.<sup>25</sup>

Hydrolysis of  $\alpha$ -amino acid esters in ternary complexes of copper(II) with glycyl-DL-valine has been studied in detail, and rate enhancements of  $\text{ca. } 10^3$  observed in the base hydrolysis reactions.<sup>26</sup> Ternary complexes of copper(II) with diethylenetriamine and  $\alpha$ -amino acid esters have also been investigated.<sup>27</sup> The formation constants of the ternary complexes were determined and the kinetics of base hydrolysis of the amino acid esters in the mixed ligand complexes studied at  $25^\circ\text{C}$ . Significant rate enhancements were observed when comparisons were made with the kinetic data for the uncomplexed esters.

Other investigations include studies on the complexation of Ni(II) and Cu(II) with L- $\alpha$ -amino- $\beta$ -indolepropionic acid,<sup>28</sup> and the kinetics of anation of  $[\text{Cr}(\text{H}_2\text{O})_6]^{3+}$  by L-hydroxyproline,<sup>29</sup> and L-phenylalanine.<sup>30</sup> The reaction of  $[\text{Cr}(\text{H}_2\text{O})_6]^{3+}$  follows an associative ( $\text{I}_\text{a}$ ) pathway.

A range of kinetic studies of redox reactions have been reported, including the reduction of cystine by V(II) in the pH range 7.5 to 12,<sup>31</sup> the anaerobic oxidation of cysteine to cystine by iron(II) in both acidic,<sup>32</sup> and basic,<sup>33</sup> solution, the oxidation of  $\beta$ -phenylalanine by  $\text{MnO}_4^-$  in aqueous perchloric acid,<sup>34</sup> and the oxidation of L-methionine by Cr(VI).<sup>35</sup> The photolytic release of copper from the complex  $[\text{Cu}(\text{II})_6\text{Cu}(\text{I})_8(\text{D-penicillamine})_{12}\text{Cl}]^{5-}$  has also been studied.<sup>36</sup>

**2.3 Solution Structures and Biological Activity.**— The reactions of  $\text{cis-[Pt(NH}_3)_2(\text{H}_2\text{O})_2]^{2+}$  with *S*-methyl-L-cysteine (MecysH) and L-methionine (MetH) have been followed by  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$  and  $^{195}\text{Pt}$  n.m.r.<sup>37</sup> With a small excess of platinum at pH 5, the chelates  $[\text{Pt}(\text{NH}_3)_2(\text{Mecys-}\underline{\text{S}},\underline{\text{N}})]^+$  and  $[\text{Pt}(\text{NH}_3)_2(\text{Met-}\underline{\text{S}},\underline{\text{N}})]^+$  are formed. In each case, the different configurations about sulphur give two slowly interconverting diastereoisomers.

The  $^{13}\text{C}$  n.m.r. chemical shifts of DL-selenomethionine have been measured and compared with L-methionine and 2-aminovaleric acid in neutral and basic aqueous solution.<sup>38</sup> The interaction of L-methionine and DL-selenomethionine with aurothiomalate was also studied. The results show that while L-methionine does not bind to gold(I) at any pH weak binding occurs with DL-selenomethionine in basic solution. The current interest in the biological chemistry of gold results from the clinical use of 1:1 gold(I)-thiolate compounds such as aurothiomalate ("Myocrisin") and aurothioglucose ("Solganol") as antiarthritic agents.

Copper(II) complexes of tridentate Schiff bases derived from pyruvic and amino acids have been shown to be catalytic intermediates in non-enzymatic transamination reactions. The *N*-pyruvideneglycinatocopper(II) complexes  $[\text{Cu}(\text{Pyrgly})(\text{L})(\text{H}_2\text{O})_x]$  ( $\text{L} = \text{H}_2\text{O}$ , pyridine, quinoline and aniline) have been prepared and their ligand field spectra studied.<sup>39</sup> The complexes show antimicrobial activity, being most active against fungi. Tetrakis- $\mu$ -acetatodirhodium(II),  $[\text{Rh}_2(\text{O}_2\text{CMe})_4]$  exhibits anticancer activity against many types of tumour. The dinuclear Rh(II)-Rh(II) complex  $[\text{Rh}_2(\text{Pro})_4][\text{NET}_4]_2$  has been synthesised<sup>40</sup> and its structure studied by a range of spectroscopic techniques. Two proline molecules serve as bridging ligands, while the other two are axially coordinated via their N atoms.

The interaction of Co(II), Ni(II), Cu(II) and Zn(II) with D-penicillamine disulphide, oxidised glutathione and L-cysteinylglycine has been studied by pH-metric, spectrophotometric and e.p.r. methods.<sup>41</sup> D-penicillamine disulphide forms binuclear complexes with all the metal ions studied. The formation of 1:1 complexes is characteristic of oxidised glutathione. L-Cysteinylglycine disulphide behaves like dipeptides, but the presence of two separate peptide moieties also

results in the formation of various binuclear complexes. Metal ion binding to the disulphide linkage was not observed in any case.

Bis-(N-hydroxyiminodiacetate)vanadate (IV), a synthetic model of "amavadin" has been prepared.<sup>42</sup>

**2.4 Equilibrium Studies.**— A variety of reports on the formation constants of metal complexes of amino acids and their derivatives, have been published and these are summarised in the Table.<sup>43-58</sup> Tabata and Tanaka<sup>59</sup> have shown that the formation of binary or ternary copper(II) complexes with amino acids is enhanced by noncovalent interactions between side chains of the amino acids. The enhanced stability was linearly correlated with the hydrophobicity of the side chains for both aromatic and aliphatic amino acids. Thallium interactions with biological fluids are attracting attention, and in a recent paper a potentiometric investigation of dimethylthallium complex equilibria with some typical amino acids is described.<sup>60</sup>

**2.5 Crystal Structures.**— Gold complexes are used in the treatment of rheumatoid arthritis and also exhibit some antitumour activity. The reaction between  $\text{Ph}_3\text{PAuCl}$  and silver salts of N-substituted glycines leads to the complexes  $\text{Ph}_3\text{PAuX}$  where X is the substituted glycinate; the X-ray structure of the hippurato-complex confirms the presence of an Au-O bond.<sup>61</sup> Cysteine and its derivatives forms stable purple complexes with the vanadyl ion,  $\text{VO}^{2+}$ . The crystal structure of the purple complex with L-cysteine methyl ester has been determined.<sup>62</sup> The coordination geometry around the central vanadium atom is square pyramidal with two fold symmetry and the nitrogen and sulphur atoms are in trans positions (5). Recently there has been considerable interest in the use of metal ions as protecting groups for both N- and C-terminal residues of a peptide chain. Complexes of Cu(II), Co(II) and Pt(II) have all been employed. The synthesis and crystal structure of trans-bis(L-alanine)dichloroplatinum(II) has been described in conjunction with preliminary solution studies using i.r. and <sup>1</sup>H n.m.r. spectroscopies.<sup>63</sup>

A number of DNA and RNA binding proteins have been shown to contain zinc and to be characterised by  $\text{Zn}(\text{S-Cys})_x$  coordination.



Table      Formation constant measurements for metal-amino acid complexes and their derivatives

| cation(s)                        | ligand, complexes                                       | method, conditions, comments                          | Ref. |
|----------------------------------|---------------------------------------------------------|-------------------------------------------------------|------|
| $H^+, Co^{2+}, Ni^{2+}, Cu^{2+}$ | <u>N</u> -hydroxy-D-asparagine                          | potentiometry at 25°C, I = 0.5M (KCl)                 | 43   |
| $H^+, Cu^{2+}$                   | (3-aminopropyl)malonate                                 | potentiometry at 25°C, I = 0.1M (NaCl)                | 44   |
| $Zn^{2+}, Cd^{2+}, Co^{2+}$      | Isoserine                                               | potentiometry at 25°C                                 | 45   |
| $H^+, Ca^{2+}, Mg^{2+}$          | $\gamma$ -carboxyglutamate                              | potentiometry at 25°C                                 | 46   |
| $Ti^{3+}$                        | Glycine-malonic acid                                    | potentiometry at 25°C, I = 1.0M (KCl)                 | 47   |
| $Cd^{2+}$                        | $\alpha$ -Alanine-oxalic acid                           | polarography                                          | 48   |
| $Ni^{2+}$                        | Bipyridine/Histidine-glycollic/lactic/malic/amino acids | potentiometry                                         | 49   |
| $Cu^{2+}$                        | Dipeptides(Ala-norVal, Ala-Met)-amino acids             | potentiometry at 25°C, I = 0.1M (NaClO <sub>4</sub> ) | 50   |
| $Cu^{2+}$                        | Glycine-tartaric acid                                   | spectrophotometry                                     | 51   |

| cations                             | ligand, complexes                                                                      | method,<br>conditions,<br>comments                                  | Ref. |
|-------------------------------------|----------------------------------------------------------------------------------------|---------------------------------------------------------------------|------|
| Pb <sup>2+</sup>                    | Glycine                                                                                | potentiometry at<br>25°C, I = 3M<br>(NaClO <sub>4</sub> )           | 52   |
| Cu <sup>2+</sup>                    | L-Histidinehydroxamic<br>acid                                                          | potentiometry at<br>25°C, I = 0.1M<br>(NaClO <sub>4</sub> )         | 53   |
| Cu <sup>2+</sup>                    | Diethylenetriamine-<br>α-amino acid esters                                             | potentiometry at<br>25°C                                            | 27   |
| Cu <sup>2+</sup>                    | Gly-Val-amino acid<br>esters                                                           | potentiometry at<br>25°C                                            | 26   |
| Cu <sup>2+</sup>                    | <u>N</u> -Salicyloyl derivatives<br>of amino acids                                     | potentiometry                                                       | 54   |
| Cu <sup>2+</sup>                    | <u>N</u> -Naphthylideneamino<br>acids                                                  | potentiometry                                                       | 55   |
| Cu <sup>2+</sup> , Ni <sup>2+</sup> | <u>N</u> , <u>N</u> , <u>N</u> ', <u>N</u> '-Tetrakis<br>(carboxymethyl)-L-<br>lysine  | potentiometry                                                       | 56   |
| Alkaline<br>earth ions              | <u>N</u> , <u>N</u> , <u>N</u> ', <u>N</u> '-Tetrakis<br>(carboxymethyl)-<br>cystamine | potentiometry                                                       | 57   |
| Cu <sup>2+</sup>                    | Sarcosinehydroxamic<br>acid                                                            | potentiometry at<br>25°C, I = 0.1M<br>(NaClO <sub>4</sub> ), e.p.r. | 58   |

For example, it has recently been established that the gene 32 protein (g32P) isolated from bacteriophage T4 contains a mole of tightly bound zinc. A series of Zn, Co and Cd thiolate complexes have now been characterised as models for the  $\text{Zn}(\underline{\text{S}}\text{-Cys})_3(\text{His})$  centre in the gene 32 protein. X-ray crystallographic studies establish that  $[(n\text{-Pr})_4\text{N}][\text{M}(\underline{\text{S}}\text{-2,3,5,6-Me}_4\text{C}_6\text{H}_3)(1\text{-Meim})]$  complexes of Co(II) and Cd(II) are isomorphous and the structure of the cobalt complex has been determined.<sup>64</sup> The Co(II) ion is coordinated by three thiolate ligands and the nitrogen of the 1-methylimidazole (1-Meim).

The complexes  $[\text{Cu}(\text{bipy})_2(\text{Ac-Asp})]\cdot\text{EtOH}\cdot 4\text{H}_2\text{O}$ ,  $[\text{Cu}(\text{bipy})_2(\text{Bz-Asp})]\cdot 2\text{H}_2\text{O}$  and  $[\text{Cu}(\text{bipy})_2(\text{Bzl-Asp})]\cdot\text{EtOH}\cdot\text{H}_2\text{O}$  (bipy = 2,2'-bipyridine) have been prepared. The crystal structure of the N-acetyl-L-aspartate complex establishes that in-plane coordination is from a nitrogen atom of each bipy ligand and a  $\beta$ -carboxylate oxygen of the aspartate anion.<sup>65</sup> Two nitrogen atoms, from each bipy molecule are the axial donors. The interaction between N-tosyl-L-leucine and copper(II) in water-methanol solution gives the complexes  $[\text{Cu}(\text{Tos-Leu})_2]\cdot 4\text{H}_2\text{O}$  and  $\text{Na}_2[\text{Cu}(\text{Tos-Leu})_2\cdot 2\text{H}_2\text{O}]\cdot 2\text{H}_2\text{O}\cdot\text{MeOH}$ .<sup>66</sup> In the second complex the coordination about the Cu atom is distorted square pyramidal arising from ligation of two trans amino acid dianions acting as N,O donors in the basal plane, and of one water oxygen in the axial site.

The dimeric complex  $\text{Fe}_2\text{L}(\text{OCH}_3)\text{L}'$ . (solvent) where L = N,N'-ethylenebis(salicylamine) and  $\text{L}' = \underline{\text{N,N'}}$ -ethylene(o-hydroxyphenylglycine)salicylamine has been characterised and the crystal structure determined.<sup>67</sup> The structure shows that the two iron atoms are coordinated to two different ligands and are bridged by a methoxide ion and a  $\mu$ -monodentate acetate oxygen from the pendant arm of  $\text{L}'$ . The crystal structures of a number of polymeric amino acid complexes have been determined. These include catena-poly $[\mu\text{-(}\underline{\text{N}}$ -acetyl-DL-valinato)- $\text{O}^1\text{:O}^3]$ copper(II)<sup>68</sup> and catena-diaqua-tris- $\mu\text{-(L-proline)}$ neodymium(III) perchlorate,  $[\text{Nd}(\text{Pro})_3(\text{H}_2\text{O})_2](\text{ClO}_4)_3$ .<sup>69</sup> Other X-ray crystal structures reported include trisglycinatocobalt(III) dihydrate<sup>70</sup> and chloro $[\underline{\text{N,N}}$ -bis(2-hydroxyethyl)glycinato]copper(II).<sup>71</sup>

**2.6 Miscellaneous.-** The use of N,N-diocetyl-glycine for the solvent extraction of palladium(II) from chloride media has been described.<sup>72</sup> Equilibrium constants for replacement in aqueous solution of the axial ligand in tetrakis(4-N-methylpyridyl)-porphine-zinc(II) by a variety of amino acids at pH 9 have been determined spectrophotometrically.<sup>73</sup> The equilibrium constant for the glycine derivative was also determined as a function of pH. From the pH dependence it appears that the substitution of glycine for OH<sup>-</sup> at the zinc centre is thermodynamically less favourable than for replacement of water.

Complexes of the type  $ML_2 \cdot nH_2O$  ( $M = Co^{II}, Ni^{II}, Zn^{II}, Cd^{II}$ ) have been prepared where HL is racemic 4-thiazolidine-carboxylic acid (6).<sup>74</sup> A variety of physical techniques establish that the ligand coordinates to a metal via the nitrogen and oxygen atoms. Interest in (S)-4-thiazolidinecarboxylic acid arises as it has been reported to be a new type of anticancer agent which can induce reverse transformation. Other synthetic studies have dealt with the characterisation of copper(II) complexes of the biologically active Schiff bases of 7-hydroxy-5-methoxy-2-methyl-chromonellidene amino acids,<sup>75</sup> and copper, nickel, palladium and platinum(II) complexes of C-allylglycine and N-(diphenylmethylene)-C-allylglycine ester.<sup>76</sup>

Preparation, characterisation, and intramolecular electron-transfer studies on pentammineruthenium histidine-modified high potential iron-sulphur protein from Chromatium Vinosum has been described.<sup>77</sup> The interaction of Fe(III)-pyridoxal with glycinehydroxamic in aqueous solution has been investigated in detail<sup>78</sup> and iridium(III) complexes of Schiff bases derived from amino carboxylic acids investigated.<sup>79</sup> The synthesis and characterisation of new hydroxobridged chromium(III) complexes with L-aspartic acid and L-serine has been described.<sup>80</sup>

The preparation and complexation properties of a chelating resin containing L-lysine-N,N,N'-triacetic acid groups have been described,<sup>81</sup> as has the separation of optical isomers of amino acids by reversed phase liquid chromatography in the presence of benzyl-L-proline copper(II).<sup>82</sup> Zinc(II)-bis-L-pyroglutamate has been prepared by cyclisation of L-glutamic acid in the presence of zinc oxide<sup>83</sup> and platinum(II) complexes with precursors of unsaturated  $\alpha$ -amino acids have also been prepared.<sup>84</sup> Dioxygen

complexes of cobalt(II) containing histidine and nucleosidemonophosphates have been characterised,<sup>85</sup> as has the mercury(II) chloride complex with N-(2,6-dimethylphenyl)-N-(2-methoxyacetyl)alanine methyl ester.<sup>86</sup>

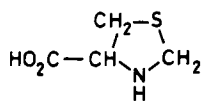
Renal handling of amino acid <sup>99m</sup>Tc chelates has been discussed,<sup>87</sup> and the synthesis of rare earth complexes of salicylidene-β-alanine described.<sup>88</sup> Other papers of interest describe the determination of the molar volume and electrostriction behaviour of [Co(en)<sub>2</sub>(GlyO)]<sup>2+</sup>,<sup>89</sup> the Raman spectra of L-phenylalanine, L-tyrosine and their peptides absorbed on silver surfaces,<sup>90</sup> the reactivity of some transition metal complexes of antracitic acid with leucine and monochloroacetic acid,<sup>91</sup> and the effect of coordinated ligands on the stability of hydrido-cobalt(II) complexes of diamino amino acids.<sup>92</sup>

### 3 Peptides

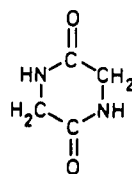
As in previous years the majority of work reported on metal peptide complexes deals with solution equilibria and reactions including formation, ionization and rate constant measurements and species distribution curves. However a number of interesting papers which describe the synthesis and study of complexes in the solid state have also appeared. In most cases the structures and behaviour of the metal peptide complexes serve as useful models for the physical and chemical properties of a number of metalloproteins and metalloenzymes.

Complexes in the Solid State.- The crystallisation of K[Cr(Gly-Gly)<sub>2</sub>] from aqueous basic solution produced crystals of K[Cr(Gly-Gly)<sub>2</sub>].L.2H<sub>2</sub>O (L=piperazine-2,4-dione), the diketone (7) resulting from the metal ion catalysed self condensation of the ligands.<sup>93</sup> The crystal structure of this complex by X-ray diffraction shows that incorporation of the dione in the structure results in some changes in bond lengths and angles and a multifold increase in the luminescence lifetime of the complex.

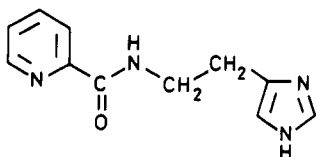
The antitumour drug bleomycin has metal chelating groups which may play a role in its biological activity. Recently the task of producing synthetic analogues of these chelating residues has been undertaken using the ligands PypepH (8) and PmpepH (9)



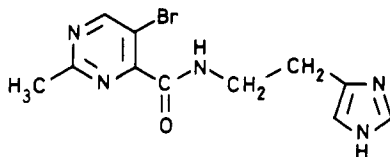
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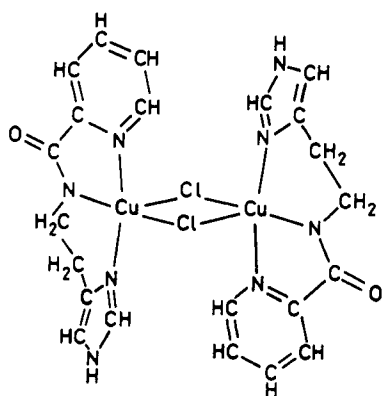
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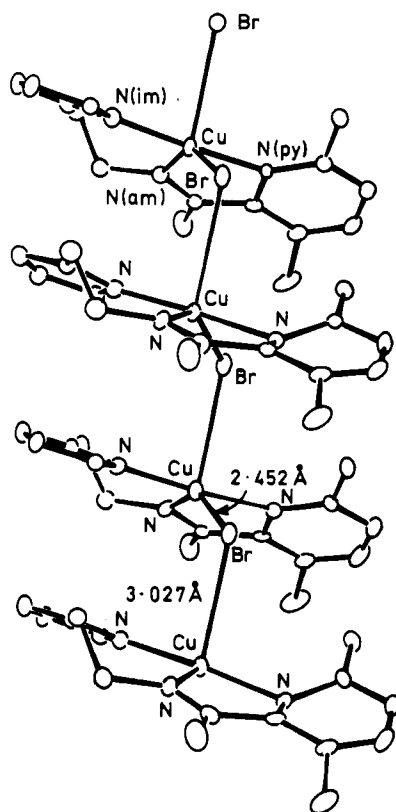
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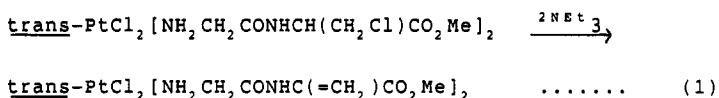
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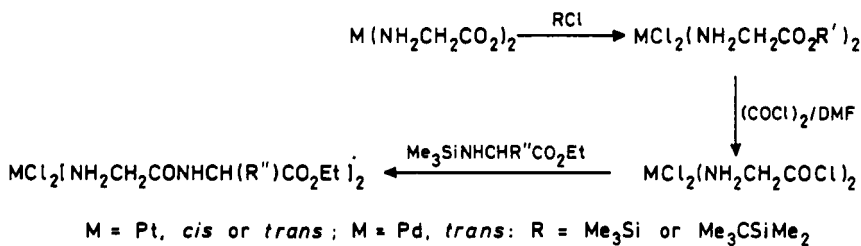
(11)

which mimic three of the five binding sites of various bleomycins. Reaction of  $\text{CuX}_2$  ( $\text{X}=\text{Cl}, \text{Br}$ ) with these ligands in methanol gave four halobridged peptide complexes, the structures of two of which were determined by X-ray crystallography.<sup>94</sup> In  $[\text{Cu}(\text{Pyep})\text{Cl}]_2 \cdot 2\text{H}_2\text{O}$  (10) there is a centrosymmetric  $\text{CuCl}_2$  core and each copper lies in a distorted square-pyramidal ligand environment in which the three N atoms and the Cl trans to the deprotonated amido group occupy the basal plane and the second bridging Cl occupies the apical position. The Cu-Cl apical bond is 0.51 Å longer than the Cu-Cl basal bond. A similar structure is proposed for  $[\text{Cu}(\text{Pyep})\text{Br}]_2$ . The structure of  $[\text{Cu}(\text{Pmep})\text{Br}]_\infty$  (11) contains units bridged by  $\text{Br}^-$  ions to give a novel zig-zag chain structure. Each copper(II) lies in a distorted square pyramidal ligand field containing three N atoms and a  $\text{Br}^-$  in the basal plane with the bridging  $\text{Br}^-$  of the previous unit in the apical position. A similar structure is proposed for  $[\text{Cu}(\text{Pmep})\text{Cl}]_\infty$ . In solution ( $\text{H}_2\text{O}$ ,  $\text{CH}_3\text{OH}$  or DMF) the bridged complexes dissociate into monomeric tetragonal copper(II) species the e.s.r. spectra of which are characteristic of the non-halide ligands. Variable temperature magnetic susceptibility measurements demonstrate that the metal centres in (10) are coupled antiferromagnetically while those in (11) are coupled ferromagnetically.

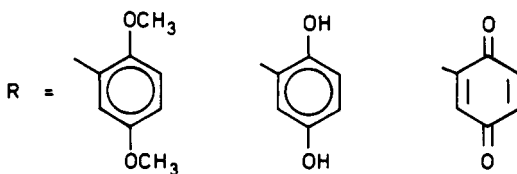
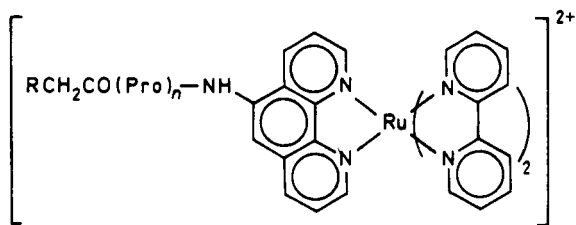
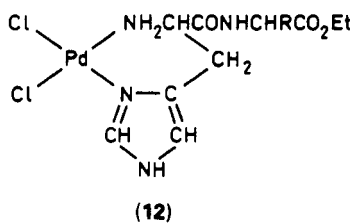
A number of Pd(II) and Pt(II) complexes of biologically important  $\alpha, \beta$ -dehydro and  $\beta$ -chloro- $\alpha$ -amino acids and peptides have been synthesised.<sup>95</sup> These include cis- and trans- $\text{MCl}_2[\text{NH}_2\text{C}(\text{CO}_2\text{Et})=\text{CHMe}]_2$  and  $\text{MCl}_2[\text{NH}_2\text{C}(\text{CO}_2\text{Et})=\text{CMe}_2]_2$  ( $\text{M}=\text{Pd}, \text{Pt}$ ), trans- $\text{PdCl}_2[\text{NH}_2\text{CH}(\text{CH}_2\text{Cl})\text{CO}_2\text{Me}]_2$  and  $\text{PdCl}_2[\text{NH}_2\text{CH}(\text{CH}_2\text{OAc})\text{CO}_2]_2$  and trans- $\text{PtCl}_2(\text{Gly}-\beta\text{-Cl-AlaOMe})_2$ . The structures of two of the palladium-amino acid complexes have been determined by X-ray diffraction. Elimination of HCl from the peptide complex gave a dehydrodipeptide complex, equation (1)



Amino acid complexes of Pd(II) and Pt(II) have been used for the synthesis of peptide complexes by the route shown in Scheme 1.<sup>96</sup> The following peptide complexes were isolated:  
trans- $\text{PtCl}_2(\text{AA-AA'OR})_2$  (AA = Gly AA' = PhGly, L-Ala, L-Val, Ser, Tyr R = Me, SiMe<sub>3</sub>, H : AA = L-Ala AA' = L-Ph-Gly, L-Ser R = Me,

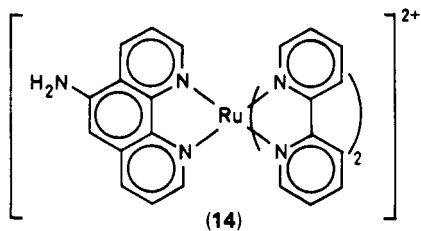


Scheme 1



$n = 1 - 4$

(13)





H), cis-PtCl<sub>2</sub>(AA-AA'OR)<sub>2</sub> (AA = L-Leu AA' = Gly, L-PhGly R = Me, Et), trans-PdCl<sub>2</sub>(AA-AA'OR)<sub>2</sub> (AA = Gly AA' = L-PhGly, L-Tyr, L-Phe R = Me, Et : AA = L-Ala AA' = Gly R = Et : AA = L-Leu AA' = Gly, L-Leu R = Et). Coupling of cis-PtCl<sub>2</sub>(His) with NH<sub>2</sub>CHR'CO<sub>2</sub>Et (R' = H, -CH<sub>2</sub>CHMe<sub>2</sub>, -CH<sub>2</sub>Ph) in the presence of Et<sub>3</sub>N=C=N(CH<sub>2</sub>)<sub>3</sub>NMe<sub>2</sub> gave peptide complexes of the type cis-PdCl<sub>2</sub>(His-AA) (AA = Gly, L-Leu, L-Phe) (12).

Platinum(II) complexes with the dipeptide ligands Gly-L-Tyr, Gly-L-His and Gly-L-Val have been synthesised from cis-Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub> and characterised by i.r. and n.m.r. spectroscopy.<sup>97</sup> The structure proposed for complexes of the first two peptides is of the form Pt(HL)(L)Cl which contains monodentate (HL) and bidentate (L) peptide ligands. The peptide complexes showed no significant antitumour activity in contrast to some cis-Pt(NH<sub>3</sub>)<sub>2</sub>(RCO<sub>2</sub>)<sub>2</sub> complexes (R = Me, ClCH<sub>2</sub>, Et, Me<sub>2</sub>CHCH<sub>2</sub>) described in the same paper.

The ruthenium(II) complexes (13) have been synthesised from complex (14) and characterised by microanalysis and by electronic and <sup>1</sup>H n.m.r. spectroscopic data.<sup>98</sup> While the *p*-dimethoxy substituents only slightly modify the metal to ligand charge transfer (MLCT) excited state the *p*-benzoquinone groups quench the yield and lifetime of the Ru MLCT emission, the quenching efficiency diminishing with increasing number of peptide spacers.

Other publications describe an i.r. study of the interactions of Ca(II), Cu(II), Ni(II), Fe(III) and Pr(III) with Ac-Glu-Val-Asp-Ala, a model for the metal sites of calcium binding proteins,<sup>99</sup> and the synthesis of cobalt carbonyl complexes of biologically active peptides (tachykinin hormone analogues) as i.r. spectroscopic markers.<sup>100</sup>

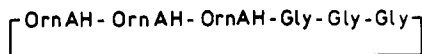
**Complex Formation in Solution.**— A number of papers reporting on aspects of complex formation in solution have been published and the majority of these deal with complexes of copper(II). The cyclic peptide cyclo(L-Leu-L-Phe-L-Pro)<sub>4</sub> has been synthesised and its conformation and complexation with metal ions in acetonitrile solution were investigated by c.d. and n.m.r. spectroscopy.<sup>101</sup> Selectivity towards alkaline earth cations was observed and the binding constant for Ba<sup>2+</sup> was found to be greater

than that of the hexapeptide  $\text{cyclo(L-Leu-L-Phe-L-Pro)}_2$ . When complexed with  $\text{Ba}^{2+}$  the conformation of the ligand changes to one in which all the peptide bonds are trans and which also contains four  $\beta$  turns with transannular hydrogen bonds. Complex formation between cobalt(II) and the cyclopeptides  $\text{cyclo(Pro}^{17}\text{O-Gly)}$  and  $\text{cyclo(Gly}^{17}\text{O-Pro)}$  has been studied by  $^{17}\text{O}$  and  $^{14}\text{N}$  n.m.r. spectroscopy.<sup>102</sup>

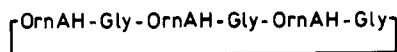
The recent discovery of two vanadium containing enzymes has contributed to the view of it being a widespread biometal. These enzymes are a vanadate(V)-dependent haloperoxidase, present in marine algae and in a lichen, and nitrogenases which contain vanadium instead of molybdenum. In order to model potential binding sites in proteins the complexation of vanadate by amino acids (Gly, Asp, Ser) and dipeptides (Gly-Asp, Gly-Gly, Gly-Tyr, Gly-Ser, Gly-Glu, Z-Gly-Glu, Z-Glu-Tyr, Val-Asp, Pro-Gly, Gly-Pro, Gly-Sar) has been investigated by  $^{51}\text{V}$  n.m.r. spectroscopy.<sup>103</sup> The peptides coordinate to vanadium via the amino groups, the peptide oxygens and either the carboxylate groups or side chain functional groups such as  $\text{OH}^-$ .

There is continuing interest in Ferrichrome, a representative iron(III)-siderophore which contains a cyclic hexapeptide ligand (desferriferrichrome, 15) composed of three consecutive glycyl and  $\text{N}^5$ -acetyl- $\text{N}^5$ -hydroxy-L-ornithyl residues.<sup>104</sup> A number of modified desferriferrichromes(16)-(19) have been synthesised. These form 1:1 tris(hydroxamate) complexes with iron(III) the stabilities of which to  $\text{H}^+$  attack follow the order  $15 > 18 > 16 > 17 > 19$  and to  $\text{OH}^-$  attack  $15 > 18 > 17 > 16 > 19$ . The stability against iron transfer to excess EDTA follows the order  $16 > 15 > 18 > 17 > 19$ . The c.d. spectra of the complexes are similar to that of ferrichrome indicative of similar octahedral, cis, configurations around the metal ion. Complexes of iron(III) with carboxylic acids, amino acids and peptides have also been investigated over a broad pH range.<sup>105</sup> While Gly forms mononuclear complexes,  $\beta$ -Ala,  $\gamma$ -Aba and Gly-Gly form binuclear ones and 6-Aha and Gly-Gly-Gly form trinuclear species.

Complexes of nickel(II) and nickel(III) with  $\alpha$ -aminoisobutyric acid ( $\text{Aib}_2$ ) have been investigated.<sup>106</sup> On raising the pH the high spin complex  $[\text{Ni}(\text{H}_1\text{Aib}_2)\text{Aib}_2]^-$  changes to low spin  $[\text{Ni}(\text{H}_1\text{Aib}_2)_2]^{2-}$  with loss of  $\text{H}^+$  ( $\text{pK}_a = 10.0$  at  $25^\circ\text{C}$ ) and



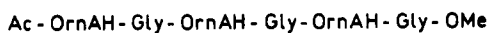
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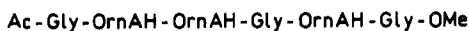
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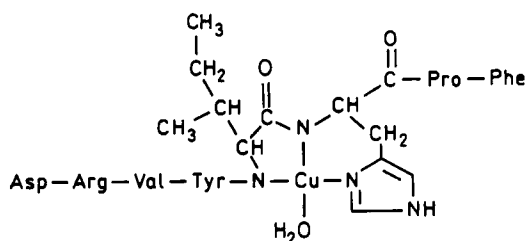
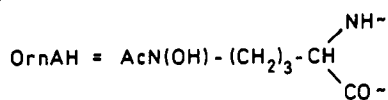
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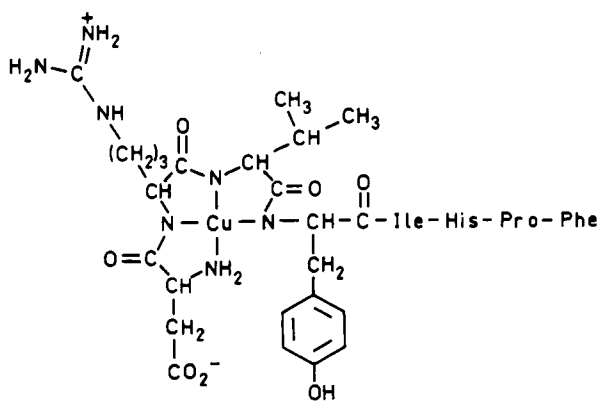
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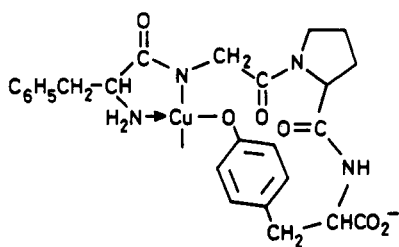
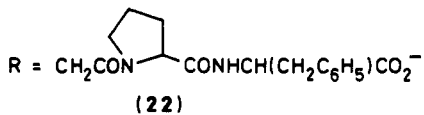
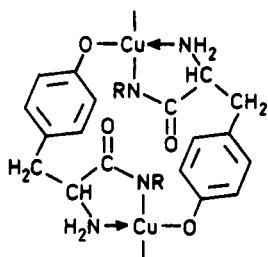
(21)

formation of a second Ni-N(peptido) bond. Generally in Ni(II) dipeptide complexes this spin change is observed only if Aib is the second residue and is attributed to the electron releasing effect of the  $\alpha$ -C methyl substituent on the ligand field strength. Oxidation of the orange, low spin Ni(II) complex gives the dark green tetragonally compressed nickel(III) complex  $[\text{Ni}(\text{H}_{-1}\text{Aib}_2)_2]^-$  which is stable in neutral and basic media. In acid solution it decomposes in a three step process to give two other nickel(III) species before final decomposition to nickel(II). The standard reduction potential of the Ni(III, II) couple is only 0.34 V vs (NHE) and a self exchange rate constant of  $48 \text{ M}^{-1} \text{ s}^{-1}$  was determined for  $[\text{Ni}(\text{H}_{-1}\text{Aib}_2)_2]^{-/2-}$ .

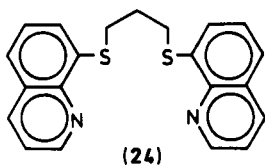
Angiotensin II is an octapeptide hormone which influences a range of physiological activities including the regulation of blood pressure as well as salt and water excretion by the kidneys. The conformation and effects of cations on the conformation of this peptide in solution are areas of current controversy. Protonation and copper(II) complexation of angiotensin(II) and two of its constituent peptide fragments (the N-terminal tetrapeptide residue Asp-Arg-Val-Tyr and the N-protected intermediate tripeptide residue  $\text{CH}_3\text{CO-Tyr-Ile-His}$ ) were studied by potentiometric and spectrophotometric (electronic absorption, c.d. and e.s.r.) methods.<sup>107</sup> At pH 6-8 Cu(II) is coordinated to the imidazole of His as well as the neighbouring deprotonated peptide nitrogens (20). Above pH 8 however a coordination switch to the terminal amino group and its neighbouring deprotonated peptide nitrogens occurs (21).

Investigations of break points in coordination of prolyl-containing peptides to metal ions such as copper(II) continue.<sup>108</sup> These break points arise because of the inability of Pro within a peptide chain to undergo peptide group deprotonation. The tetrapeptides Phe-Gly-Pro-Phe, Phe-Gly-Pro-Tyr and Tyr-Gly-Pro-Phe have been synthesised and investigated by potentiometry and spectroscopy (electronic absorption, c.d. and e.s.r.) at  $25^\circ\text{C}$ .<sup>109</sup> In the Tyr-containing peptides Cu(II)-phenolate bonding occurs at pH>8 either through the formation of a dimer (22) or a large chelate ring (23).

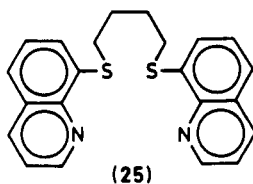
A number of studies on stereoselectivity in complex formation have been reported. Stability constants of Cu(II)-dipeptide-ATP



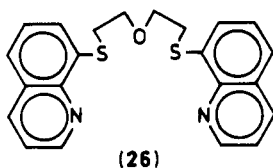
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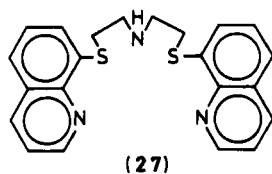
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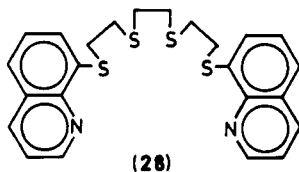
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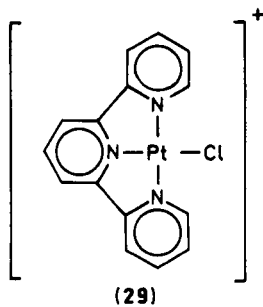
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(28)



(29)

complexes studied by potentiometry and by electronic spectroscopy for Gly-Gly, Gly-Ala, Ala-Ala, Gly-Val, Gly-Leu, Gly-Ahx, Gly-Avl, Val-Gly and Leu-Gly complexes increase with the hydrophobicity of the peptide side chains.<sup>110</sup> It has also been shown that copper(II) complexes of optically homogeneous peptides such as L-Leu-L-Leu-Gly are more stable than those of L-Leu-D-Leu-Gly but that the stereoselectivity, which resides in the amide deprotonation step, is lost if the leucyl residues are separated by Gly.<sup>111</sup> Similar optical selectivity for complexes of copper(II) with dipeptide ligands (Ala-Ala, Met-Met, Met-Val, Leu-Leu, Met-Phe and Leu-Ile) has been shown to correlate linearly with the hydrophobicity of the peptide chains.<sup>112</sup>

Complex formation between Cu(II) and the dipeptides Gly-L-Glu and L- $\alpha$ -Glu-Gly has been studied by potentiometry, calorimetry and e.s.r. spectroscopy.<sup>113</sup> The following species were detected in the pH range 1-9 : MHA, MA,  $MH_{-1}A$ ,  $MH_{-1}(OH)A$  and  $MH_{-1}A_2$ , all of which with the exception of MHA contain deprotonated peptide linkages. Potentiometric methods were also used to determine formation constants for the ternary complexes  $Cu(AA)L$  where AA = Gly, Ala,  $\beta$ -Ala, Phe, Val, Avl, Ser, Thr, Tyr, Asn, Trp, Asp and Glu ; L = Ala-Met or Ala-Avl.<sup>114</sup> The stabilities of the ternary complexes which are formed at pH 6.5-9.0 are compared with those of the binary peptide complexes  $CuL_2$  which exist in the pH range 6.5-9.0.

Homo- and heteronuclear complexes of Cu(II) and Cd(II) with L-His-Gly (HL) have been investigated by potentiometry, visible spectrophotometry and calorimetry in aqueous solution.<sup>115</sup> Formation constants are reported for the species HL,  $H_2L^+$ ,  $H_3L^{2+}$ ,  $CuL^+$ ,  $CuL_2$ ,  $[CuLH]^+$ ,  $[CuL_2H]^+$ ,  $[Cu_2LH_{-1}]^{2+}$ ,  $Cu_2L_2H_{-2}$ , CdL,  $CdL_2$ ,  $[CdLH]^{2+}$ ,  $[CuCdLH_{-1}]^{2+}$ ,  $[CuCdLH_{-2}]^+$  and structures for the complexes have been predicted on the basis of the thermodynamic and spectrophotometric results.

Two papers reporting formation constants of thiol and thiocarbonyl containing peptides have been published. The dipeptide Cys-Gly forms stable bis complexes with both Co(II) and Zn(II) in which it is S,N coordinated to the metal ions.<sup>116</sup> In contrast complexes of Gly-Cys are S,O coordinated to these ions. Peptide group deprotonation is not observed below pH 10 in any of these systems. Complex formation between Cu(II) and Ni(II) and the thioamide dipeptides  $^+NH_3CH(CH_3)CSNHCH(CH_3)CO_2CH_3$

(Alat-AlaOMe) and  $^+NH_3CH(CH_2CO_2H)CONHCH(CH_2Ph)CSNH_2$  (Asp-PhetNH<sub>2</sub>) have been investigated.<sup>117</sup> At pH<3 spectroscopic evidence suggests NH<sub>2</sub>, S coordination of Alat-AlaOMe(L) to Cu(II) and the stability constants of the CuL and CuL<sub>2</sub> complexes are two-fold and five-fold respectively greater than for complexes with the non-sulfur analogue ligands. In basic solution deprotonation of the thiopeptide group with concomitant coordination of N<sup>-</sup> to the metal occurs leading to the formation of CuAH<sub>-1</sub> and CuAH<sub>-2</sub>. Similar coordination and deprotonation is observed with Ni(II) but a change in geometry from octahedral NiA<sub>2</sub> to square planar NiA<sub>2</sub>H<sub>-2</sub> accompanies the deprotonation. Stability constants and possible structures are also reported for the complexes of Asp-PhetNH<sub>2</sub>.

Equilibria in solutions containing Ln<sup>3+</sup> ions (Dy, Ho, Yb) and the peptides Gly-Phe, β-Ala-L-His (carnosine) and β-Ala-L-Me-L-His (anserine) were studied by <sup>1</sup>H n.m.r. spectroscopy.<sup>118</sup> Lanthanide ion complexes of pyridoxal-dipeptide azomethines as models for the active centres of pyridoxal containing metalloenzymes have been studied by <sup>1</sup>H n.m.r. spectroscopy.<sup>119</sup> Eight 13-residue synthetic analogues of the metal coordinating sites of troponin C, a calcium binding protein involved in muscle contraction, have been synthesised and the binding of La<sup>3+</sup> to each studied by high field <sup>1</sup>H n.m.r. spectroscopy.<sup>120</sup> The effects of Asp residues at various positions on the association constants have been investigated.

**Reactions of Complexes in Solution.**— In view of the toxicity and carcinogenicity of Cr(VI) and the fact that it is reduced to Cr(III) by cellular reductants such as glutathione there have been many recent studies on the reduction mechanism and some of these have established the existence of stable Cr(V) intermediates. As part of these investigations reduction of Cr(VI) by glutathione was investigated by e.s.r. spectroscopy.<sup>121</sup> Above pH 5 two Cr(V) intermediates were observed and these were most stable at pH 7. However at pH values of 3 and 4 only one Cr(V) species was evident. The reduction of copper(II) complexes of the thiaza ligands L,(24)–(28), by glutathione (GSH) has also been investigated as models for the non-enzymatic oxidation of glutathione *in vivo* by blue copper proteins.<sup>122</sup> Evidence has been obtained for the formation of intermediates of the type [CuL(SG)]<sup>+</sup> and it is proposed that intramolecular transfer from glutathione

sulfur to copper(II) is the rate determining step of substrate oxidation.

The formation of hydroxy radicals by the reaction of a number of copper(II) complexes with  $H_2O_2$  has been investigated. Of these, the bis(1,2-diaminoethane) complex is more active than peptide complexes while copper(II)-polyaminopolycarboxylate complexes are inactive in the absence of reducing agents such as  $NaDH$ .<sup>123</sup>

It is known that the iron(II) complex of bleomycin, the antitumour antibiotic, (see above under 'Complexes in the Solid State') is readily air oxidised to an inactive iron(III) complex via an intermediate, another iron(III) complex, which possesses DNA cleaving activity. The redox potentials of the active iron(III) complex of bleomycin and two of its derivatives (iso-bleomycin and deglyco-bleomycin) have been measured by cyclic voltammetry at 25°C under an atmosphere of hydrogen.<sup>124</sup> A reversible one electron  $Fe(III)-Fe(II)$  coupled redox reaction at -0.225 V vs SCE was observed for the active complex. The magnetic susceptibilities (6-300K), electronic and e.s.r. spectra of complexes of haemoctapeptide from cytochrome c with azide, pyridine, imidazole and cyanide have been measured and compared with those of cytochrome c and haemoglobin.<sup>125</sup>

The selectivity of  $[Pt(terpy)Cl]^+$ , (29), towards amino acids and related ligands (Cys, His, imidazole and  $SHCH_2CH_2OH$ ) peptides ( $\gamma$ -Glu-Cys-Gly, Gly-His-Gly) and cytochrome c (from two sources) has been investigated and its potential as a tagging agent for proteins assessed.<sup>126</sup> Although it shows selectivity for thiol over imidazole ligands nevertheless both His 33 and His 39 in the proteins are labelled in preference to Cys 102. This implies that Cys 102 is not exposed at the protein surface. The  $[Pt(terpy)His]^{2+}$  and  $[Pt(terpy)Cys]^+$  chromophores were detected by their characteristic u.v. bands. Labelling with the complex does not alter the structural or redox properties of the proteins which augurs well for its potential as a non-invasive tagging agent. The reaction of the complex  $[PtCl(dien)]Cl$  with glutathione (GSH), oxidised glutathione (GSSG) and  $\underline{S}$ -methylglutathione (GS-Me) has been investigated by  $^1H$ ,  $^{13}C$  and  $^{195}Pt$  n.m.r. spectroscopy and by potentiometry.<sup>127</sup> Two steps were observed for this reaction the first (pH-dependent) leading to the formation of  $[Pt(dien)GS]^+$



which then reacts in a pH-independent process to give the S-bridged dimer,  $\{[Pt(dien)]_2GS\}^{3+}$ . At pH<7 the rate of the first step is slow compared to the second but at pH>10 this is reversed. The dimer may also be obtained from GSSG and  $[Pt(dien)Cl]^+$  while GS-Me with this complex gives  $[Pt(dien)GS-Me]^{2+}$ .

The hydrolysis of Leu-Gly-NHNH-Dns (Dns = dansyl) and Leu-Gly-NH(CH<sub>2</sub>)<sub>2</sub>NH-Dns by porcine kidney cytosol leucine aminopeptidase (LAP) in the absence of and in the presence of Mg(II), Mn(II), Ni(II), Cu(II) and Zn(II) has been investigated by stopped-flow spectrofluorescence.<sup>128,129</sup> The hydrolysis of enkephalin and related peptides by dipeptidyl aminopeptidase III has also been studied. The activity of the enzyme was markedly stimulated by Co<sup>2+</sup>.<sup>130</sup>

**Miscellaneous.**— The use of high performance immobilised metal ion affinity chromatography to separate in excess of 30 biologically active synthetic peptides has been reported.<sup>131</sup> The metal chelating gel contains iminodiacetic acid (IDA) coupled to a hydrophilic resin based matrix. The retention of the peptides on copper(II), nickel(II) and zinc(II) ions immobilised on the chelating gel showed that while some of them could be separated by isocratic elution the majority of them could be distinctly separated by elution with a linear imidazole or a continuously decreasing pH gradient. The Cu(II)-IDA chelate column gave the best resolution as demonstrated in the separation of angiotensin I and II and four of their synthetic analogues. The use of specific metal chelating peptides attached to the amino terminus of a protein to purify that protein by immobilised metal ion affinity chromatography is also described.<sup>132</sup> The technique is particularly useful for recombinant proteins.

The use of metal salts as additives in peptide synthesis reactions is described. In carbodiimide-mediated peptide couplings the use of 1-hydroxybenzotriazole and CuCl<sub>2</sub> gave peptides in high yields without racemization.<sup>133</sup> The use of these additives improved the yield of PhCH<sub>2</sub>OCO-Gly-Val-Val-OMe for example from 22% to 88% and reduced racemization from 38% to zero.

The tripeptide Gly-L-His-L-Lys has been isolated from human plasma. The copper(II) complex of this peptide was found to

stimulate collagen synthesis by human fibroblasts.<sup>134</sup> Evidence for the liberation of the tripeptide by proteases at the site of the wound to exert in situ healing effects is reported. The yeast C. glabrata expresses both metallothionein and  $\gamma$ -glutamyl peptides for metal detoxification. Hence exposure of the cells to copper(II) salts stimulates the formation of 2 metallothioneins with a Cys content of 30%, while exposure to cadmium salts leads to the formation of  $\gamma$ -glutamyl peptides.<sup>135</sup> A rapid and sensitive peptide mapping technique based on h.p.l.c. analysis of fragments obtained by tryptic cleavage of S-carboxamidomethylated thioneins is reported.<sup>136</sup>

The interaction of  $\text{Na}^+$  with basic amino acid residues e.g. His, Arg, Lys in peptides has been studied by examining the collision induced dissociation spectra of the  $[\text{M}+\text{Na}]^+$  ions of the peptides Gly-His, Gly-Lys, His-Gly-Gly, Gly-His-Gly, Gly-Gly-His, Gly-Ala-Tyr, Gly-Tyr-Ala and Tyr-Ala-Gly.<sup>137</sup>

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# Appendix

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## IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN)

## Nomenclature of glycoproteins, glycopeptides and peptidoglycans

### Recommendations 1985

Drafted by Nathan SHARON, The Weizmann Institute of Science, Rehovot, Israel

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#### 1. INTRODUCTION

Various types of compound consisting of carbohydrates covalently linked with other types of chemical constituent are classified under the general name of *glycoconjugates*. The major groups of glycoconjugates are the glycoproteins, glycopeptides, peptidoglycans, glycolipids and lipopolysac-

charides. The first three of these are considered in the present document. The nomenclature of glycolipids has been the subject of an earlier document [1], which is now under revision.

As early as 1907, when very little was known about glycoproteins, they were defined by the Committees on Protein Nomenclature of the American Society of Biological Chemists and the American Physiological Society as 'compounds of the protein molecule with a substance or substances containing a carbohydrate group other than a nucleic acid' [2]. In spite of the enormous progress in our knowledge of the occurrence, biosynthesis, properties and functions of the glycoproteins, this definition remains appropriate.

Glycoproteins are widely distributed in all forms of life, with the possible exception of the eubacteria. They occur in cells, both in soluble and membrane-bound forms, as well as in the extracellular matrix and in extracellular fluids. The commonest glycoproteins are those in which the carbohydrate is linked to the protein by glycosyl linkages. Glycosylation represents one of the important co-translational and post-translational modifications of proteins.

The term 'glycoprotein' should include proteoglycans, which in the past were considered as a separate class of compound. Proteoglycans are those glycoproteins whose carbohydrate moieties consist of long, unbranched chains of alternating residues of hexosamine and uronic acid or galactose, often sulfated. Such polysaccharides belong to the class of glycosaminoglycans. Proteoglycans were not considered as glycoproteins in the past because their carbohydrate seemed to differ so greatly from the comparatively small, branched, usually unsulfated carbohydrate units, devoid of repeating units, found in other glycoproteins. Another reason was their unique distribution: the proteoglycans are found mainly in connective tissues, where they contribute to the organization and physical properties of the extracellular matrix. It is known, however, that proteoglycans too are glycosylated proteins, synthesized by the action of glycosyltransferases, and that repeating disaccharide units are present in typical glycoproteins as well. Proteoglycans are therefore considered to be a subclass of glycoproteins.

The terms 'protein glucosylation (or glycosylation)' and 'glucosylated (or glycosylated) hemoglobin' have been used improperly to refer to the products of non-enzymic reactions between glucose or other sugars and free amino groups of proteins. Compounds formed in this manner are not glycosides, however, as they result from the formation of a Schiff base followed by Amadori rearrangement to 1-deoxyketosyl derivatives of the proteins. For example, the product of the reaction between glucose and hemoglobin is not glucosylated hemoglobin but *N*-(1-deoxyfructosyl)hemoglobin. The term 'glycation' is suggested for all reactions that

These are recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). They have been approved for publication by the Executive Committee of IUB, and for publication as provisional recommendations by the Interdivisional Committee on Nomenclature and Symbols of IUPAC. The members of JCBN are H.B.F. Dixon (Chairman), A. Cornish-Bowden (Secretary), C. Liébecq (representing the IUB Committee of Editors of Biochemical Journals), K.L. Loening, G. P. Moss, J. Reedijk, S.F. Velick, and J.F.G. Vliegthart. Comments may be sent to any member of the commission, or to its secretary: A. Cornish-Bowden, Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham, England, B15 2TT. JCBN thanks the following for comments on these recommendations: C.E. Ballou, E. Buddecke, E.A. Davidson, R.J. Doyle, D. Duksin, J.-M. Ghysen, T. Hardingham, V.C. Hascall, D. Heinegård, F.W. Hemming, D. Horton, R.C. Hughes, W.B. Jakoby, R.W. Jeanloz, O. Kandler, T. Laurent, Y.C. Lee, U. Lindahl, B. Lindberg, H. Lis, F.G. Lontiens, J. Montreuil, H. Muir, A. Neuberger, E.F. Neufeld, T. Osawa, L. Rodén, H. Schachter, R. Schauer, K. Schmid, G.D. Shockman, J.E. Silbert, C.C. Sweeley and I. Yamashina, as well as members, associate members and former members of the Nomenclature Committee of IUB (NC-IUB), namely H. Bielka, C.R. Cantor, P. Karlson, N. Sharon, E.J. Van Lenten and E.C. Webb.

Table 1. Structure of glycosaminoglycans

Based mainly on Lindahl, U. & Höök, M. (1978), *Annu. Rev. Biochem.* 47, 385–417; and Rodén, L. (1980) in *The biochemistry of glycoproteins and proteoglycans* (Lennarz, W. J., ed.), pp. 267–371, Plenum Press, New York

| Name                         | Repeating disaccharide                                                                                                                       | Sulfation                                                                                                                                                                                                                                                                                   | Linkage region                                                                                                                   |
|------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Hyaluronic acid              | GlcNAc( $\beta$ 1 $\rightarrow$ 4)GlcA( $\beta$ 1 $\rightarrow$ 3)                                                                           | None                                                                                                                                                                                                                                                                                        | not proved to be linked to protein                                                                                               |
| Chondroitin 4- and 6-sulfate | GalNAc( $\beta$ 1 $\rightarrow$ 4)GlcA( $\beta$ 1 $\rightarrow$ 3)                                                                           | GalNAc-4- <i>O</i> -sulfate, 6- <i>O</i> -sulfate or hybrids                                                                                                                                                                                                                                | GlcA( $\beta$ 1 $\rightarrow$ 3)Gal( $\beta$ 1 $\rightarrow$ 3)Gal( $\beta$ 1 $\rightarrow$ 4)Xyl( $\beta$ 1 $\rightarrow$ O)Ser |
| Dermatan sulfate             | GalNAc( $\beta$ 1 $\rightarrow$ 4)GlcA( $\beta$ 1 $\rightarrow$ 3) and GalNAc( $\beta$ 1 $\rightarrow$ 4)I-IdoA( $\alpha$ 1 $\rightarrow$ 3) | GalNAc-4- <i>O</i> -sulfate, or 6- <i>O</i> -sulfate; L-iduronic acid may be sulfated at position 2                                                                                                                                                                                         | GlcA( $\beta$ 1 $\rightarrow$ 3)Gal( $\beta$ 1 $\rightarrow$ 3)Gal( $\beta$ 1 $\rightarrow$ 4)Xyl( $\beta$ 1 $\rightarrow$ O)Ser |
| Heparin and heparan sulfate  | GlcNR( $\alpha$ 1 $\rightarrow$ 4)GlcA( $\beta$ 1 $\rightarrow$ 4) and GlcNR( $\alpha$ 1 $\rightarrow$ 4)I-IdoA( $\alpha$ 1 $\rightarrow$ 4) | In heparin (highly sulfated), R mostly SO <sub>3</sub> H, little acetyl; in heparan sulfate generally less SO <sub>3</sub> H and more acetyl than in heparin. Some of the <i>N</i> -acetyl-D-glucosamine is 6- <i>O</i> -sulfated, and some of the L-iduronic acid is 2- <i>O</i> -sulfated | GlcA( $\beta$ 1 $\rightarrow$ 3)Gal( $\beta$ 1 $\rightarrow$ 3)Gal( $\beta$ 1 $\rightarrow$ 4)Xyl( $\beta$ 1 $\rightarrow$ O)Ser |
| Keratan sulfate              | GlcNAc( $\beta$ 1 $\rightarrow$ 3)Gal( $\beta$ 1 $\rightarrow$ 4)                                                                            | GlcNAc-6- <i>O</i> -sulfate; Gal-6- <i>O</i> -sulfate                                                                                                                                                                                                                                       | see Fig. 1                                                                                                                       |

link a sugar to a protein or peptide, whether or not they form a glycosyl bond. The product of glycation is a glycoprotein, or, in the special case of the reaction with hemoglobin, glycohemoglobin. When appropriate, a more precise name such as deoxyfructosylhemoglobin may be used.

Peptidoglycans, sometimes referred to as mureins, are glycoconjugates found only in bacterial cell walls. Although they are also composed of carbohydrates and amino acids covalently linked, they are a distinct class of compound as (a) they do not contain a protein portion; (b) they contain sugars not found elsewhere; and (c) they consist of linear polysaccharides (of the class of glycosaminoglycan) crosslinked by oligopeptides, thus forming a huge and rigid network.

No official nomenclature of glycoproteins, glycopeptides and peptidoglycans has been available hitherto. In drawing up the present document, we follow the general rules of biochemical nomenclature [3], especially the more recent ones on carbohydrates [4–7] and amino acids and peptides [8].

## 2. NOMENCLATURE

### 2.1. Glycoproteins, proteoglycans and glycosaminoglycans

A *glycoprotein* is a compound containing carbohydrate (or glycan) covalently linked to protein. The carbohydrate may be in the form of a monosaccharide, disaccharide(s), oligosaccharide(s), polysaccharide(s), or their derivatives (e.g. sulfo- or phospho-substituted). One, a few, or many carbohydrate units may be present. *Proteoglycans* are a subclass of glycoproteins in which the carbohydrate units are polysaccharides that contain amino sugars. Such polysaccharides are also known as *glycosaminoglycans*.

### 2.2. Glycopeptides, glyco-amino-acids and glycosyl-amino-acids

A *glycopeptide* is a compound consisting of carbohydrate linked to an oligopeptide composed of L- and/or D-amino

acids. A *glyco-amino-acid* is a saccharide attached to a single amino acid by any kind of covalent bond. A *glycosyl-amino-acid* is a compound consisting of saccharide linked through a glycosyl linkage (*O*-, *N*- or *S*-) to an amino acid. (The hyphens are needed to avoid implying that the carbohydrate is necessarily linked to the amino group.)

### 2.3. Peptidoglycans

A *peptidoglycan* consists of a glycosaminoglycan formed by alternating residues of D-glucosamine and either muramic acid {2-amino-3-*O*-[(*S*)-1-carboxyethyl]-2-deoxy-D-glucose} or L-talosaminuronic acid (2-amino-2-deoxy-L-taluronic acid), which are usually *N*-acetylated or *N*-glycoloylated. The carboxyl group of the muramic acid is commonly substituted by a peptide containing residues of both L- and D-amino acids, whereas that of L-talosaminuronic acid is substituted by a peptide consisting of L-amino acids only.

## 3. COMMENTS

### 3.1. Form of carbohydrate in glycoproteins

In many glycoproteins (e.g. plasma glycoproteins such as human  $\alpha_1$ -acid glycoprotein or fetuin) the carbohydrate is in the form of oligosaccharides, linear or branched, the latter containing up to about 20 monosaccharide residues; glycoproteins containing mono- or disaccharide units are also known [e.g. collagens, fish antifreeze glycoproteins, sheep submaxillary (or submandibular) glycoproteins], as well as those that contain oligosaccharides that consist of repeating units of *N*-acetylglucosamine (e.g. band 3 of the human erythrocyte membrane).

### 3.2. Proteoglycans

A *proteoglycan* is a protein glycosylated by one or more (up to about 100) glycosaminoglycans (Table 1). The glycosaminoglycans of the proteoglycans are linear polymers



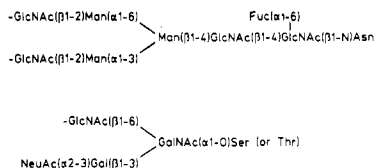


Fig. 1. Linkage region of corneal and skeletal keratan sulfate. The upper structure shows the linkage region in keratan sulfate type I (corneal); the lower shows that in keratan sulfate type II (skeletal).

of up to about 200 repeating disaccharide units that consist of a hexosamine (D-glucosamine or D-galactosamine) alternating with a uronic acid (D-glucuronic or L-iduronic) or a neutral sugar (D-galactose). The hexosamines are usually *N*-acetylated, and in some of the glycosaminoglycans the D-glucosamine is *N*-sulfated. Varying degrees of sulfation occur in other positions of the hexosamines as well as on the L-iduronic acid. The chain of repeating units is linked to the protein by an oligosaccharide of a structure different from the repeating units. This linkage region (Table 1) is identical in most of the proteoglycans (chondroitin sulfates, dermatan sulfate, heparin, and heparan sulfate), but is different in keratan sulfate. This last glycosaminoglycan contains repeating units of *N*-acetylglucosamine that are *O*-sulfated. Because of their content of uronic acid and/or ester sulfate, the glycosaminoglycans of the proteoglycans are anionic polyelectrolytes and have been referred to as 'acidic glycosaminoglycans' (equivalent to the older term 'acid mucopolysaccharides'). Proteoglycans may also contain one or more oligosaccharides of structures similar to those found in other glycoproteins.

Several diseases of proteoglycan metabolism are recognized, including the Hunter and Hurler syndromes; they are referred to as mucopolysaccharidoses, but strictly speaking should go by the term 'glycoproteinoses', which encompasses other diseases of glycoprotein metabolism such as mannosidosis and fucosidosis.

### 3.3. Microheterogeneity

A particular glycoprotein may occur in forms that differ in the structure of one or more of its carbohydrate units, a phenomenon known as *microheterogeneity*. Such differences may affect both the size and charge of individual glycoproteins; occasionally, the differences may be solely due to changes in linkage position in a carbohydrate unit. For example, chicken ovalbumin contains a single glycosylated amino acid residue (Asn-293), but more than a dozen different oligosaccharides have been identified at that site. In proteoglycans, individual glycosaminoglycan chains may differ in structure, e.g. the degree of sulfation, the ratio of glucuronic acid to L-iduronic acid, and chain length. Proteoglycans are therefore highly polydisperse, in contrast to typical glycoproteins, which, in spite of their microheterogeneity, are not markedly polydisperse in their molecular size.

### 3.4. Glycopeptides, etc.

Glycopeptides, glyco-amino-acids, glycosyl-amino-acids and glycosylpeptides are obtained by enzymic or chemical

cleavage of glycoproteins, or by chemical or enzymic synthesis.

Examples of glycosyl-amino-acids that constitute the common linking compounds between the carbohydrate and protein in glycoproteins are as follows (Table 2): 2-acetamido-*N*-(L-aspart-4-yl)-2-deoxy- $\beta$ -D-glucopyranosylamine, i.e. *N*<sup>4</sup>-(*N*-acetyl- $\beta$ -D-glucosaminyl)asparagine, which is abbreviated to (GlcNAc)-Asn (parentheses here around the carbohydrates placed next to the symbol for an amino-acid residue indicate substitution on its side chain; see section 3AA-17.2 in ref. 8); *O*<sup>3</sup>-(*N*-acetyl- $\alpha$ -D-galactosaminyl)serine and threonine, (GalNAc)-Ser and (GalNAc)-Thr; *O*- $\beta$ -D-xylosylserine, (Xyl)-Ser; *O*<sup>3</sup>- $\alpha$ -D-galactosylhydroxyllysine<sup>1</sup>, (Gal)-Hyl; and  $\beta$ -L-arabinosylhydroxyproline<sup>2</sup>, (L-Ara)-Hyp. Another example of a glyco-amino-acid is [Man<sub>2</sub>-GlcNAc(31-4)-GlcNAc]-Asn, isolated from a proteolytic digest of soybean agglutinin. Such a compound may be referred to as *oligosaccharylasparagine*.

### 3.5. *N*,*O*-Glycoproteins

If desirable, the linkage between carbohydrate and protein may be indicated by the locants *N*- or *O*-. The locant *N*- is used for the *N*-glycosyl linkage to asparagine. *N*-Linked oligosaccharides are divided into two major classes: the *N*-acetylglucosamine type containing *N*-acetyl-D-glucosamine, D-mannose, D-galactose, L-fucose and sialic acid; and the *oligomannose* type containing *N*-acetyl-D-glucosamine and a variable number of D-mannose residues. Structures containing both oligomannose- and *N*-acetylglucosamine-type oligosaccharides are designated as *hybrid* type. Examples of *N*-glycoproteins (or *N*-glycosylproteins) are chicken ovalbumin, pig ribonuclease, human  $\alpha_1$ -acid glycoprotein and soybean agglutinin.

The locant *O*- is used for *O*-glycosyl linkage to serine, threonine, hydroxyllysine or hydroxyproline. Sheep submaxillary glycoprotein, collagen, fish antifreeze glycoproteins and potato lectin are *O*-glycoproteins (or *O*-glycosylproteins).

Two types of carbohydrate-peptide linkage in the same protein or peptide chain may be indicated by a combination of the locants. Thus, calf fetuin, procollagen, human erythrocyte membrane glyophorin and human chorionic gonadotropin are *N*,*O*-glycoproteins (or *N*,*O*-glycosylproteins).

### 3.6. Asialoglycoproteins and asialo-agalactoglycoproteins

Glycoproteins from which the sialic acid has been removed (by treatment with enzyme or mild acid) are designated by the prefix *asialo*-, e.g. *asialo*- $\alpha_1$ -acid glycoprotein, and *asialofetuin*. Removal of both sialic acid and galactose results in *asialo*-agalactoglycoproteins.

### 3.7. Condensed representation of sugar chains

For writing the structure of sugar chains, the non-reducing terminus of the carbohydrate chain should always be on the left-hand end (see ref. 6). Current practice allows the use of either an *extended* form (a) or a *condensed* form (b), which

<sup>1</sup> This compound could more strictly be described as 5( $\alpha$ -D-galactopyranosyloxy)-L-lysine, but hydroxyllysine is regarded as a trivial name [8], so names are based on it.

<sup>2</sup> Hydroxyproline is the trivial name for *trans*-4-hydroxy-L-proline.

Table 2. Commonly occurring glycosyl-amino-acids

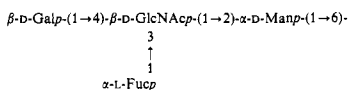
Based on Sharot, N. & Lis, H. (1982) in *The proteins*, 3rd edn (Neurath, H. & Hill, R. L., eds) vol. 5, pp. 1–144, Academic Press, New York

| Example                                                                                                      | Structure | Occurrence                                               |
|--------------------------------------------------------------------------------------------------------------|-----------|----------------------------------------------------------|
| N-Glycoside*<br>$\beta$ -N-Acetylglucosaminyl-<br>-asparagine, (GlcNAc-)Asn                                  |           | widely distributed in animals, plants and microorganisms |
| O-Glycosides<br>$\alpha$ -N-Acetylgalactosaminyl-<br>-serine or -threonine,<br>(GalNAc-)Ser,<br>(GalNAc-)Thr |           | glycoproteins of animal sources                          |
| $\beta$ -Xylosyl-serine<br>(Xyl-)Ser                                                                         |           | proteoglycans, human thyroglobulin                       |
| $\beta$ -Galactosyl-hydroxylysine<br>(Gal-)Hyl                                                               |           | collagens                                                |
| $\beta$ -L-Arabinosyl-<br>-hydroxyproline, (L-Ara-)Hyp                                                       |           | plant and algal glycoproteins                            |

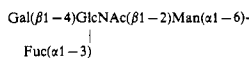
\* Traditionally the term 'glycoside' referred explicitly to O-linked compounds; we use the term 'N-glycoside' rather than 'nitrogen analogue of glycoside', which is unnecessarily cumbersome.

allows structures to be shown in one line as well as in two or more, and in which the longest chain should always be the main chain:

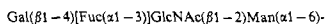
## a) Extended form



## b) Condensed form in two lines



or condensed form in one line



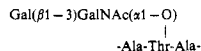
The condensed form is still unnecessarily long, however, and there should be no serious loss if it is shortened further

by (i) omitting locants of anomeric carbon atoms, (ii) omitting the parentheses around the specification of linkage, and (iii) omitting hyphens if desired. We therefore suggest a more condensed or *short form* of writing (c):

## c) Short form



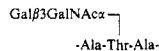
Similarly a glycopeptide sequence, represented in the condensed form in two lines as



or in the condensed form in one line as



may be written in the short form in two lines as

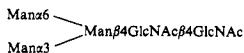


or in the short form in one line as

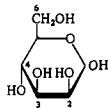


### 3.8. Representation of N-linked oligosaccharides

As a rule, N-linked oligosaccharides contain a common pentasaccharide core as follows:



For the sake of uniformity, the location of substitution should be written as above in accordance with Haworth's representation of the pyranose structure of monosaccharides, in analogy with the glycogen molecule.



### 3.9. Peptidoglycans

In peptidoglycans, peptide units of adjacent polysaccharides (glycosaminoglycans) may be cross-linked by a peptide bond between the C-terminal alanine residue of one peptide subunit and the  $\omega$ -amino group of the diamino acid residue of the other (e.g. L-lysine or *meso*-diaminopimelic acid), thereby giving rise to a giant macromolecule that forms the rigid cell wall ('sacculus'). This macromolecule is known to occur as a monomolecular layer between the inner and outer membrane in Gram-negative bacteria and as a multimolecular layer, often associated covalently or non-covalently with various additional compounds (teichoic acids, neutral polysaccharides, etc.) in Gram-positive bacteria.

Extensive investigations of peptidoglycans in thousands of bacterial strains demonstrated the existence of more than 100 chemotypes in the eubacteria [9]. The peptidoglycans of eubacteria have been classified into two major groups (A and B) and several subgroups according to the mode of cross-linkage. Within group A two main subgroups are recognized: one in which the C-terminal alanine residue is directly bound to the  $\omega$ -amino group of the diamino acid at position 3 of the peptide subunit of an adjacent polysaccharide (glycosaminoglycan) and one in which the cross-linkage is mediated by an interpeptide bridge consisting of either one or up to 5 amino acid residues (e.g. Lys-[Gly]<sub>n</sub>-type, Fig. 2). Depending on the kind of diamino acid at position 3 and the amino acids serving as interpeptide bridges, many variations of these subgroups of murein are known. In the peptidoglycan types of group B, the cross-linkage does not occur at position 3 of the peptide subunit but at position 2, utilizing the  $\alpha$ -carboxyl group of the D-glutamic residue. The interpeptide bridge must contain a diamino acid, which may be lysine, ornithine or diaminobutyric acid, in either L- or D-configuration. As a second special characteristic of the group B peptidoglycan types, the L-alanine residue at position 1 of the peptide subunit is replaced by either glycine or serine.

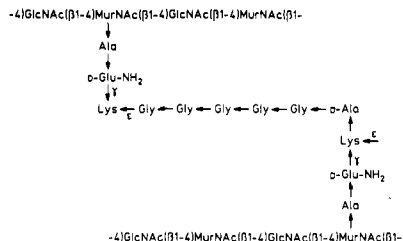


Fig. 2. Primary structure of a peptidoglycan, in which the cross-linkage between adjacent peptide-substituted polysaccharides is mediated by an interpeptide bridge consisting of Lys-[Gly]<sub>n</sub>.

In the archaeobacteria, several organisms contain a peptidoglycan that differs in certain respects from those described above, typical for the eubacteria [10, 11]. It consists of a polysaccharide formed by alternating ( $\beta$ 1-3)-linked N-acetylated residues of D-glucosamine or D-galactosamine and ( $\beta$ 1-3)-linked N-acetylated residues of L-talosaminuronic acid, and a peptide containing exclusively L-amino acids attached to the carboxyl group of L-talosaminuronic acid. The peptide units of adjacent polysaccharides may be cross-linked by a peptide bond between the  $\gamma$ -carboxyl group of the glutamic acid of one peptide subunit and the  $\alpha$ -amino group of the lysine residue of the other, thus giving rise to the rigid cell wall of the methanogenic bacteria. This multimolecular layer is often associated covalently, but also non-covalently, with neutral polysaccharides. Only a few chemotypes of such peptidoglycans have so far been described.

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## 5. APPENDIX: IMPLIED CONFIGURATIONS AND RING SIZES

In the condensed system of symbols for sugar residues the common configuration and ring size (usually pyranose) are implied in the symbol. Thus, Gal denotes D-galactopyranose; Man, D-mannopyranose; Fuc, L-fucopyranose; GlcNAc, 2-acetamido-2-deoxy-D-glucopyranose or N-acetyl-D-glucosamine; Neu5Ac (which may be abbreviated to NeuAc), N-acetylneuraminic acid. The symbol Sia stands for sialic acid, a general term that can also be used when the exact structure is unknown.

Whenever the configuration or ring size is found to differ from the common one it must be indicated by using the appropriate symbols for the extended system. The configuration of amino acids is L unless otherwise noted. Although symbols such as Gal and Man are useful in representing oligosaccharide structures they should not be used in the text to represent monosaccharides.