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Amino Acids and Peptides

Volume 18

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Amino Acids and Peptides

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A Review of the Literature Published during 1985

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Preface

Our syllabus and approach are as in the previous volume, but we have been obliged to submit to the discipline of producing camera-ready copy for the first time. The formulae have been drawn for us by the R.S.C., but the layout of the text has been our own responsibility. Some of us are novices at this art form, and crave indulgence accordingly.

The most notable relevant book[1] published during 1985 is edited by our colleague John Davies. It is largely a derivative work, drawn from the 1982 edition of the 'Dictionary of Organic Compounds', but the entries have all been reviewed and updated where necessary, and some three hundred completely new ones have been added. There are getting on for two thousand all told, and perhaps ten thousand literature citations. The compilation, which is arranged alphabetically, gives physical, chemical, and bibliographic data on all the important amino acids and peptides. The amino acids of proteins have very full entries, and their principal protected derivatives also appear individually. The rarer amino acids are covered as well, together with nearly all known dipeptides; higher peptides are listed if they are of biological or pharmaceutical importance. Coverage extends not only to peptide antibiotics, including β-lactams, but also to natural products such as the peptide alkaloids.

A number of valuable monographs have appeared on biological aspects of amino acid and peptide chemistry. Although aimed at advanced biomedical students, readers of this Report will also find them useful as introductory background reading. Bender's well-known 'Amino Acid Metabolism'[2] is now out in a second edition. Wallis, Howell, and Taylor's 'The Biochemistry of the Polypeptide Hormones'[3] has a chapter on each main class of peptide

hormone and will be very helpful to any chemist entering the field. So will 'Immunology'[4] by Roitt, Brostoff, and Male, which is remarkable for its profuse, clear, and elegant diagrams.

Balliol College, Oxford *John Jones*
July 1986

References

1. 'Amino Acids and Peptides', ed. J.S. Davies, Chapman and Hall Ltd., 1985.
2. D.A. Bender, 'Amino Acid Metabolism', 2nd Edn., John Wiley and Sons Ltd., 1985.
3. M. Wallis, S.L. Howell, and K.W. Taylor, 'The Biochemistry of the Polypeptide Hormones', John Wiley and Sons Ltd., 1985.
4. I. Roitt, J. Brostoff, and D. Male, 'Immunology', Gower Medical Publishing Ltd., 1985.

Contents

Abbreviations

Abbreviations for amino acids and their use in the formulation of derivatives follow, with rare exceptions, the 1983 Recommendations of the I.U.P.A.C.-I.U.B. 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

All major sub-divisions of amino acid science are represented in this Chapter as in previous Volumes of this Specialist Periodical Report (formerly named 'Amino acids, Peptides, and Proteins'), though with some waxing and waning as topic areas develop or become exhausted. The emphasis continues to reside in chemical studies but covers the biological literature to the extent that chemical and analytical studies are included there.

2 Textbooks and Reviews

Reference texts[1] and compendia of data[2,3] include the second supplementary list of amino acid derivatives that are useful in peptide synthesis (taking in the literature to the end of 1982).[3] Other topics reviewed include 1-aminocyclopropanecarboxylic acid,[4] synthesis of N-methylamino acids,[5] applications of uncommon amino acids in natural-products synthesis,[6] the role of S-adenosylhomocysteine[7] and of L-ergothioneine,[8] and boron analogues of amino acids[9] including p-borono-L-phenylalanine.[10]

3 Naturally Occurring Amino Acids

3.1 Occurrence of Known Amino Acids.- Close relatives of the common amino acids are covered here, and no attempt is made to review the routine literature of the distribution of well-known amino acids.

The first natural appearance of methionine sulphoximine is reported;[11] it is the toxic principle of Chestis glabra. L-DOPA-3-Sulphate has been located in the brown alga Ascophyllum nodosum,[12] and α-hydroxymethylserine, not previously reported to be a natural product, has been found in Vicia pseudo-orobus.[13]

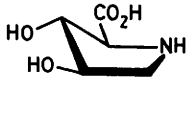
N-Substituted amino acids continue to arise in a variety of systems: N-trimethylalanine at the N-terminus of myosin light chains,[14] L-pyrrolidone-2,4-dicarboxylic acid in the muscle of the mollusc abalone Haliotis discus hannai,[15] and 3R,4R-dihydroxy-L-proline (1), present in virotoxins.[16] (1), like (+)-3,4,5-trihydroxypipecolic acid (2) from Baphia seeds,[17] competitively inhibits cattle β-D-glucuronidase.[16] Leucinopine, one of a group of N-(1-carboxyalkyl)amino acids often categorized as 'opines', has been shown[18] to possess the L-threo stereochemistry; in other words, this amino acid, N-(1,3-dicarboxy-

propyl)leucine, has the 'L-glu-,L-leu-' configuration and in this respect is unique amongst the other opines octopine ('D-ala-,L-arg-'), nopaline ('D-glu-,L-arg-'), and succinamopine ('D-glu-, L-asn-').[18]

Plant, fungal, and microbial sources of less common amino acids: Asplenium unilaterale (4-hydroxy-L-2-aminopimelic acid as well as D-2-aminopimelic acid and trans-3,4-dehydro-D-2-aminopimelic acid),[19] Dactylosporangium aurantiacum (L-threo-β-hydroxyaspartic acid, previously only found in Arthrinium and in various Streptomycetes),[20] and Amanita pseudoporphyria (L-2-aminopent-4-ynoic acid and L-2-aminopent-4-enoic acid, L-2-aminohex--4-ynoic acid and L-2-aminohept-4-en-6-ynoic acid, as well as L-2-amino-4-chloropent-4--4-enoic acid and L-2-aminohexa-4,5-dienoic acid as previously reported).[21] Another cyclic tetrapeptide from Helminthosporium carbonum has been described,[22] containing a 2-amino-8-oxo-9,10-epoxydecanoic acid residue. L-Phenylalanine and its 3S-methyl homologue (3) occur as their N-acetyl derivatives esterified with the unusual 8R-hydroxy-9S-methyl oxiranyl-2E,4Z,6E-decatrienoic acid as AK-toxins I and II from Alternaria alternata pear fungus (black spot disease).[23]

Cross-linking amino acid residues in mammalian proteins continue to attract interest, a recent citation referring to the identification of pyridinoline in Type 1 collagen.[24]

3.2 New Natural Amino acids.- New aliphatic α-amino acids include 2S-aminohex-5-ynoic acid (from Cortinarius claricolor var. tennipes),[25] D$_s$-erythro-2-amino-4-ethoxybutanoic acid (from the edible mushroom Lyophyllum ulmarium),[26] erythro-γ-hydroxyhomo-L-arginine (from the seed of Lonchocarpus costaricaensis; the threo diastereoisomer is already known to be a natural product),[27] and the sulphate ester of trans-4-hydroxypipecolic acid (seeds of Peltophorum africanum).[28] This is the first naturally occurring sulphate ester of a non-protein amino acid to be reported. The bulgecins contain O-glycosylated 5-hydroxymethyl-4-hydroxyproline amides (4; R = glycosyl residue).[29,86] 'Dealanylalahopcin' (5), found with alahopcin in Streptomyces albulus cultures, is (2S,3R)-2-amino-4-formyl-3-(hydroxyaminocarbonyl)butyric acid[30] (wrongly named as the 4-(hydroxyaminocarbonyl)acid in the original paper). A high level of interest in N-(1-carboxyalkyl)amino acids (the 'opines'; see listing in preceding Section) is reflected in three new examples from the 1985 literature: crown-gall tumours incited by Agrobacterium tumefaciens produce agropine and related mannityl opines and leucinopine and in addition large amounts of a new member of the family N-{(1S)-1-carboxy-2-carbamoylethyl}-(S)-glutamic acid ('LL-succinamopine').[31a] The D,L-diastereoisomer having been isolated previously from the same source, this is the first example of the natural occurrence of epimeric opines. The other two new opines are N-(1-carboxyethyl)-L-methionine[31b] and a phosphorylated example (agrocinopine A) secreted by healthy crown-gall cells induced by the same bacterium.[32]



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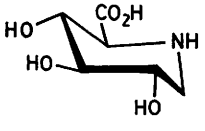

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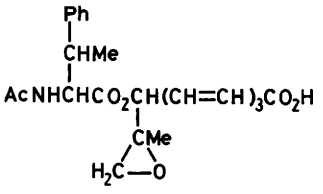

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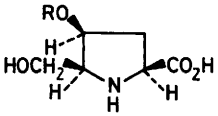

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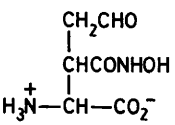

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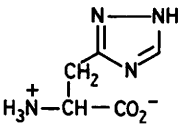

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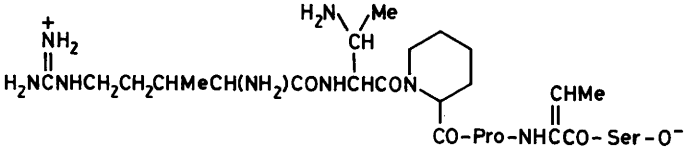

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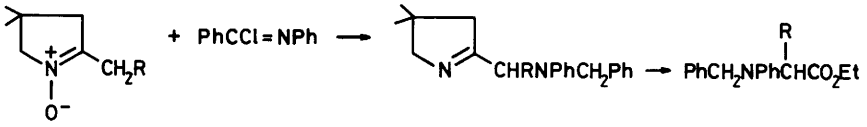

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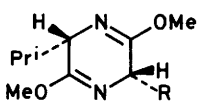

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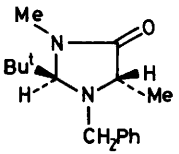

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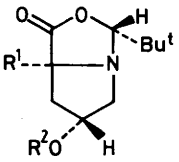

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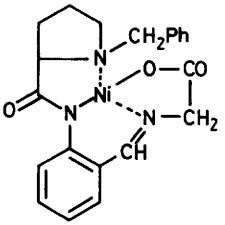

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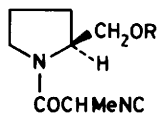

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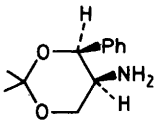

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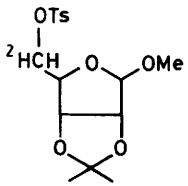

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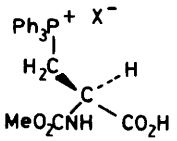

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Streptomyces KM-10329 produces β-(1,2,4-triazol-3-yl)-L-alanine (6).[33]

3.3 New Amino acids from Hydrolysates.- This section specifically refers to natural products that in principle or in practice can release new amino acids on hydrolysis.

Lavendomycin from Streptomyces lavendulae is an unusual peptide (7) containing some close analogues of common amino acids.[34]

Carzinophilin contains (2S,3S)-4-amino-2,3-dihydroxy-3-methylbutanoic acid.[35]

4 Chemical Synthesis and Resolution of Amino Acids

4.1 General Methods of Synthesis.- The major standard methods, mostly of many years' standing, continue to be fully used. It is not necessary to do more than cite most of these with literature references (recent review coverage is available[36]), and some further details of the synthetic objectives are given in later sections of this Chapter. The alkylation methods in which the side chain of the α-amino acid is put in place include alkylation of acetylaminomalonates[37] and other glycine derivatives $(MeS)_2C{=}NCH_2CO_2Et$,[38] $Ph_2C{=}NCH_2CO_2Et$,[39,40] $PhCH{=}NCH_2CO_2Et$,[41] $CNCH_2CO_2Et$,[42] and azlactones.[43]

Alkylation of methyl 2-acetamidoacrylate with a Grignard reagent in the presence of copper(I) iodide gives moderate yields of 3-substituted alanines,[44] and N-alkylamino acid esters, benzaldehyde, and alkenes react in refluxing toluene to give prolines.[45] The latter process involves cycloaddition to intermediate azomethine ylides.

Strecker synthesis of α-amino nitriles[46,47] involving reaction of an aldehyde, a secondary amine, and Me_3SiCN in MeOH can be accomplished within less than 5 minutes, thus providing some assistance in the synthesis of α-amino acids labelled with short-lived radioactive isotopes.[47] Dehydrogenation of aliphatic secondary amines by phenylseleninic acid (or its anhydride), under mild conditions in the presence of NaCN or Me_3SiCN, is a new route to α-amino nitriles.[48]

A full paper has been published on the synthesis of N-acyl α-amino acids through the isomerization - amidocarbonylation of allylic alcohols by primary amides and H_2 with carbon monoxide, using a homogeneous binary catalyst system $HRh(CO)(PPh_3)_3$ with $Co(CO)_8$ or $Fe_2(CO)_9$: $R^1R^2C{=}CR^3CH_2OH + RCONH_2 \longrightarrow R^1R^2CHR^3CH(NHCOR)CO_2H$.[49]

A new amino acid synthesis adding to the group of methods in which the amino function is introduced into an alkanoic acid (or a precursor of it) has been reported.[50] Ethanolysis of the pyrroline formed after reaction of the corresponding N-oxide (8) with N-phenylbenzimidoyl chloride yields an N-phenyl-N-benzylamino acid ethyl ester, from which the various N and C substituents can be removed by standard methods.

4.2 Asymmetric Synthesis.- Further examples have accumulated in the literature during 1985 to extend established methods in the amino acid area. The already voluminous output of Schöllkopf and co-workers, based on the alkylation of bis-lactim ethers (9) derived from piperazine-2,5-diones, has been augmented to include syntheses of D-tryptophan methyl ester and (R)-α-methyltryptophan methyl ester,[51] and other alkylation processes in which very high diastereoselectivity is achieved.[52-56] One of these[54] deals with asymmetric synthesis of D-threonine through reaction of acetaldehyde with the $Ti(NMe_2)_3$ complex of the bis-lactim ether. Another is concerned with the synthesis of chiral deuteriated α-aminoisobutyric acid through reaction of the bis-lactim ether (10) with C^2H_3I.[56]

Further results from Seebach's group[57-59] on the alkylation of chiral enolates with what has been called 'self-reproduction of the centre of chirality' – i.e. the incoming group takes the place of the proton that is substituted — confirm the high (>90%) diastereoselectivity that accompanies this approach. Enantiomerically pure pivalaldehyde aminals (11) derived from N-benzyl-L-alanine can be alkylated and elaborated into (R)- or (S)-α-methylDOPA, depending on the cis or trans orientation, respectively, of the aminal.[57] Other α-methyl analogues prepared in this study in high optical purity include α-methyl-L-methionine and α-methyl-L-valine. Pivalaldehyde N,O-acetals (12) from O-acyl-4-hydroxy-L-proline[58] and the corresponding compound from L-thiazolidine-4-carboxylic acid[59] have also been studied in what is clearly the start of a programme seeking to understand the relationship of structure to carbanion stereochemical integrity, in which the alkylating agent no doubt plays a role.[279]

Highest optical purity was observed in the stereoselective synthesis of L-aspartic acid through alkylation of a di-alkyl malonate with N-benzyloxycarbonyl-L-alanyl-2-chloroglycine methyl ester, followed by hydrolysis, when the malonate carried bulky alkyl groups.[60]

Other studies based on recent pioneering work include alkylation of chiral nickel(II) complexes, to yield either D-serine in better than 80% enantiomeric excess, using 0.2M NaOMe as base, but L-serine in 80-98% enantiomeric excess, using NEt_3,[61] when the complex (13) formed between N-benzyl-L-proline N-arylamide and the contiguous glycine Schiff base is alkylated with formaldehyde. A less puzzling result is seen for the corresponding alanine complex, used[62] for the preparation of α-methyl amino acids in optically pure form after separation of diastereoisomers over silica gel.

Alternative asymmetric synthesis of α-methyl amino acids has been established[63] but in much lower enantiomeric excess (31.7% for the R-enantiomer) when the alanine-based isonitrile (14) participates in Michael addition to acrylonitrile. Variable results (10-45% enantiomeric excess of the R-enantiomer)were obtained in the corresponding reaction with methyl acrylate, in relation to the asymmetric synthesis of α-methyl-D-glutamic acid and α-methyl-D-ornithine.[63]

The chiral-template approach employed in these examples underpins other examples, based on initial explorations already familiar to readers of earlier volumes of this Specialist Periodical Report. Weinges and co-workers have provided further examples of the use of the chiral 5-amino-1,3-dioxan (15) as a component for asymmetric Strecker synthesis of α-amino nitriles. (2S,4S)-(-)- and (2S,4R)-(+)-5,5,5-trifluoroleucine have been prepared in this way,[64] the latter from (R)-$CF_3CHMeCH_2CHO$ prepared from (E)-$CF_3CMe{=}CHCO_2Et$ through successive Pd-catalyzed hydrogenation, $LiAlH_4$ reduction, and resolution. D- and L-2-(2-thienyl)- and -2-(3-thienyl)glycines were prepared in an analogous fashion;[65] in both cases the α-amino nitriles were converted into the α-amino acids and the chiral dioxan moiety removed by HIO_4 cleavage. 'Chiral glycine', $H_3\overset{+}{N}{-}CH^2H{-}CO_2^-$, has been prepared from the(R)-toluene-p-sulphonyl ribofuranoside (16) through aminolysis with N_3^- followed by reduction; aminolysis with potassium phthalimide gave the N-phthaloyl derivative in better than 93% enantiomeric purity, after oxidative cleavage ($KMnO_4$).[66]

Fewer research groups are studying asymmetric hydrogenation methodology for the introduction of a chiral centre into an αβ-unsaturated α-amino acid. Stille has described the use of a catalyst system with a chiral phosphine attached to polystyrene in combination with a Rh(I) salt.[67]

4.3 Prebiotic Synthesis Models.- Interest in this topic covers broader areas than synthesis alone, and later sections cover models for chiral discrimination.

Synthesis of α-amino acids from simple compounds and elements, with energy input of the sort that might be available in Nature, has been a long-running topic. ^{60}Co-γ-Irradiation of oxygen-free aqueous HCN and NH_4CN gives mixtures of amino acids,[68] and some of these arise by further reactions involving glycine, which is a major initial product.[69] Aqueous KCN irradiated with u.v. light yields amino acids (but mainly oligoglycines), through a $HO^{\cdot}$-initiated chain reaction.[70] U.v. irradiation of solutions of bisglycinatonickel(II) dihydrate andan aldehyde yields mixtures of amino acids.[71] Formaldehyde gives mainly glycine, alanine, aspartic acid, and serine, acetaldehyde gives glycine, aspartic acid, threonine, and allothreonine, while benzaldehyde somewhat surprisingly also gives glycine, serine, and aspartic acid (with two unidentified products).[71] Glyceraldehyde reacts with ammonia in phosphate buffer at pH 7 to give alanine.[72] Seventeen amino acids, with glycine, alanine, serine, aspartic acid,and glutamic acid predominating, are formed in a radiofrequency plasma of H_2 and N_2 with cellulose as carbon source placed between the electrodes of the reaction cell.[73]

4.4 Synthesis of Protein Amino Acids and Other Naturally Occurring α-Amino Acids.- The literature supporting this Section can only be described as expansive and expanding, judging

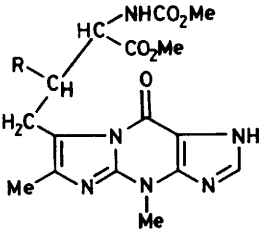
by the literature of the 1980's and particularly 1985. Frequently, the authors' primary interest is in an application of a novel synthetic procedure rather than in the establishment of a route to an amino acid for which efficient methods of synthesis already exist.

Reviews of large-scale production of protein amino acids[74,75] and peptides[75] have appeared. This topic and its fine details as represented in the biosynthesis literature can only be hinted at here, with representative citations (production of L-tryptophan and 5-hydroxy-L-tryptophan by Escherischia coli,[76] pilot-scale production of L-phenylalanine from D-glucose,[77] and L-aspartic acid production by Brevibacterium flavum[78]), with special reference to the conversion of one amino acid into another in this way (L-tyrosine and its N-formyl derivative into L-DOPA and its N-formyl derivative by Mucuna pruriens,[79] hydroxylation of phenylalanine by hypoxanthine and xanthine oxidase via H_2O_2 and the superoxide anion to give o-, m-, and p-tyrosines,[80] and DL-methionine into D-α-aminobutyric acid through the action of methionine γ-lyase and D-amino acid aminotransferase[81]).

Most of the syntheses to be described in this Section are side-chain hydroxylated amino acids; some are similarly close analogues of familiar amino acids. Few of these studies have contributed to generally applicable methodology, except the introduction of the phosphonium salt (17) as a generally useful chiral building block for β-substituted alanines. It is easily prepared from L-serine methyl ester and has been used[82] in a synthesis of S-(-)-wybutine (18), a fluorescent minor base from yeast phenylalanine tRNA through Wittig condensation with the corresponding formylpurine.

Relatively simple operations are involved in the non-enzymatic and glutamate dehydrogenase-catalyzed reduction of Δ^1-pyrroline-2-carboxylic acid, as part of a mechanistic study involving NAD(P)H and ^2H isotope effects,[83] and diborane reduction of N-benzyloxycarbonyl-α-methyl-L-glutamate to give N-benzyloxycarbonyl-L-proline methyl ester (40% yield after a 6 hour reaction in THF).[84] A much more extensive procedure is involved in the synthesis of bulgecinine (4),[29] a constituent of the bulgecins (from Pseudomonas acidophila),[85] that employs D-glucose as chiral synthon.[86] The other hydroxylated amino acids that have received attention are: (2S,3R,4R)-3,4-dihydroxyproline (stereoselective synthesis from the erythro-β-hydroxy-α-amino acid, 19) and its 2S,3S,4S-diastereoisomer[87] (the 2S,3R,4R-diastereoisomer has been prepared[88] through the separation of the mixture of stereoisomers formed through a long-established route[89]); erythro-β-hydroxy-L-aspartic acid and erythro-β-hydroxymethyl-L-serine through the common intermediate (20), formed from L-tartaric acid by oxirane ring opening and selective reduction of the resulting azido-ester;[90] and 2-amino-4-hydroxy-4-(p-hydroxyphenyl)-3-methylbutanoic acid as a mixture of stereoisomers formed through 1,3-dipolar cycloaddition of ethoxycarbonylmethane nitrile oxide to (E)-(4-methoxyphenyl)propene.[91]

(2S,4R)-Erythro- and (2S,4S)-threo-4-methylglutamic acids have been prepared[92] from



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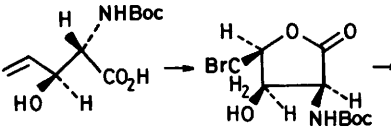

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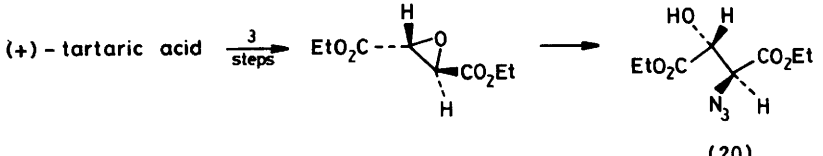

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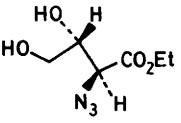

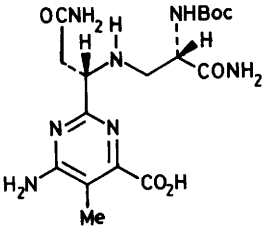

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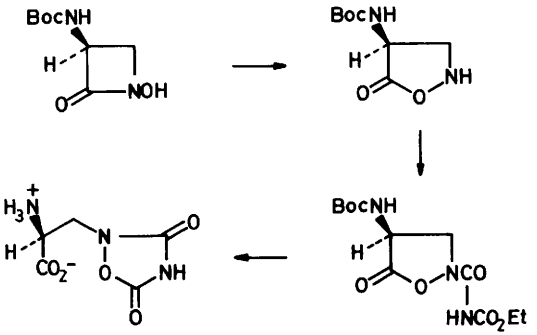
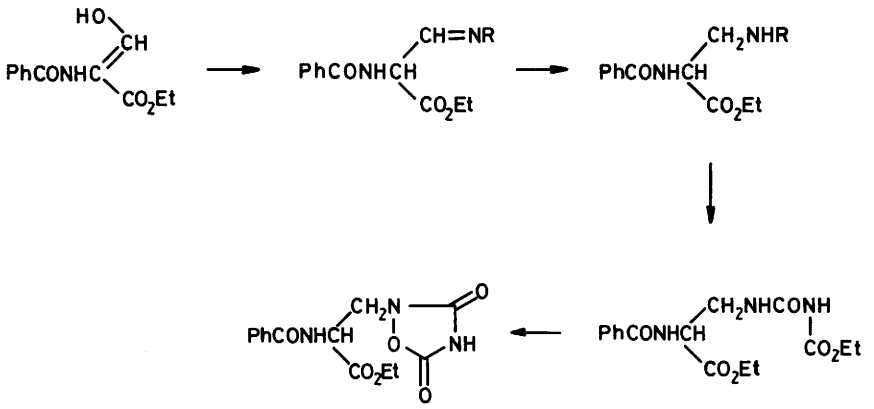
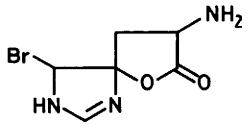


Scheme 1



Scheme 2



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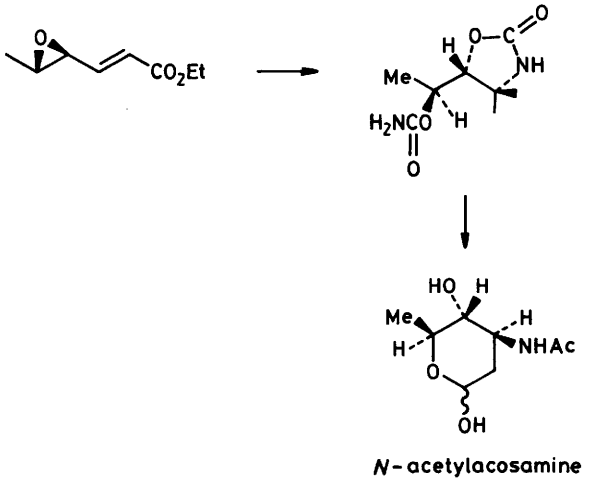


N-acetylacosamine

Scheme 3

(RS)-4-methyl-2-oxoglutaric acid by glutamate dehydrogenase-catalyzed reductive amination.

α-Amino acids with side-chain nitrogen functions that have been synthesised include N^{α}-Boc-L-2,3-diaminopropionic acid amide for use in a total synthesis of bleomycin.[93] The application of the Hofmann rearrangement to Boc-L-asparagine constituted the essential step in this synthesis. The side-chain Schiff base of this product was used in the synthesis of the bleomycin constituent pyrimidoblamic acid (21).[94] Quisqualic acid has been synthesised by routes that permit approaches to analogues at some future time: a mild new general synthesis of the isoxazolin-5-on-2-yl ring system is a notable feature of Baldwin's work (Scheme 1).[95,96] It involves a favoured 5-endo-dig cyclization and the overall route constitutes the first enantio-efficient chemical synthesis of the natural product, L-β-(isoxazolin-5-on-2-yl)-alanine, and is adaptable for the synthesis of other β-amino-alanines.[96] Bycroft's group uses a dehydroserine, PhCONHC(=CHOH)CO_2Et, as a framework on which the isoxazoline side chain is built (Scheme 2).[97]

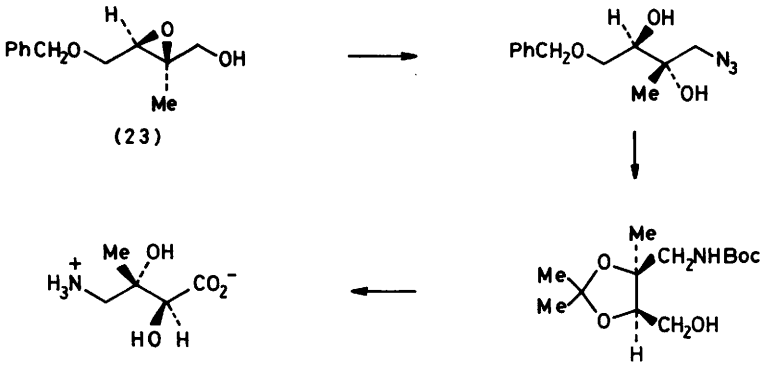
Cysteine derivatives that have been synthesized include the four stereoisomers of β-methyllanthionine, through a conventional route in which (2S,3S)- or (2R,3R)-β-methyl-cysteine is reacted with D- or L-β-chloro-alanine.[98] 2'-(S-Cysteinyl)-L-histidine, an unusual amino acid found in a tyrosinase from Neurospora crassa, has been synthesized via (22), formed from L-histidine methyl ester through reaction with Br_2.[99]

4.5 Synthesis of β- and Higher Homologous Amino Acids.- Novel oxirane ring-opening reactions are in vogue for syntheses of unusual amino acids that carry side-chain hydroxy groups (see preceding section). This is a key feature of a synthesis of N-acetyl derivatives of acosamine and ristosamine (Scheme 3).[100] Azide-ion ring opening of the oxirane (23) is also a crucial step in an efficient enantioselective synthesis of (2S,3S)-4-amino-2,3-dihydroxy-3-methylbutanoic acid, a constituent of carzinophilin (Scheme 4).[35]

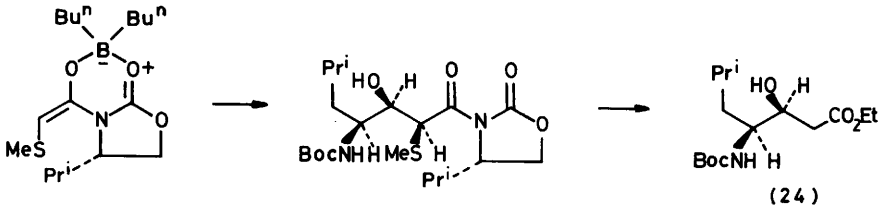
Previous syntheses of statine (24) were not diastereoselective, unlike the route shown in Scheme 5 that starts with Boc-L-leucinal and involves enantio- and erythro-selective aldol condensation with (S)-4-(1-methylethyl)-3-[(methylthio)acetyl]-2-oxazolidinone.[101] More conventional chemistry is all that is called for in syntheses of (S)-4-amino-3-hydroxybutanoic acid from (S)-malic acid via its cyclic anhydride[102] and its N-trimethyl analogue DL-carnitine, from $Me_2NCH_2COCH_3$ and diethyl carbonate in the presence of NaH, followed by reduction and N-methylation of the resulting γ-dimethylamino-β-keto-ester.[103]

Cystathionine, methionine, and lysine are utilized by Streptomyces cattleya in the formation of thienamycin (25).[104]

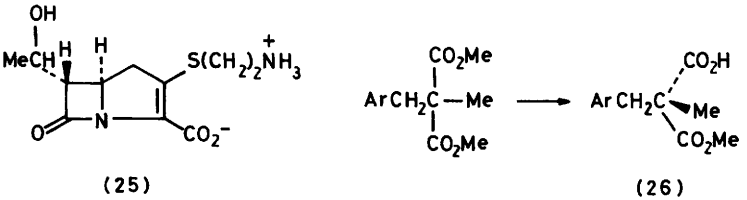
4.6 Synthesis of α-Alkyl Analogues of Protein Amino Acids.- A number of examples answering to this title have been mentioned in earlier Sections. The synthesis of α-methyl



Scheme 4



Scheme 5



amino acids through Strecker synthesis of alkylation of 1-substituted 4-methyl-2-imidazolin-5-ones has been reviewed.[105] Optically pure (S)-α-methyl analogues of phenylalanine, tyrosine, and DOPA are accessible through pig liver esterase and α-chymotrypsin-catalyzed hydrolysis of corresponding dimethyl 2-benzyl-2-methylmalonates, giving the (R)-ester (26) that is open to conversion into the acyl azide for use in a Curtius rearrangement reaction.[106]

α-Phenyl serine has been prepared by Strecker synthesis starting with $PhCOCH_2OAc$, and a similar procedure has given α-hydroxy-α-phenyl-β-alanine.[107]

4.7 Synthesis of Other Aliphatic and Alicyclic Analogues of Protein Amino Acids.- To the extent that amino acids under this heading may in due course be found in Nature, and that synthetic methods described here are equally applicable to examples found elsewhere in this Chapter, the reader seeking to know the currently used range of synthetic methods will need to read the whole of Section 4. The overlap is apparent in a use of the N-benzyloxycarbonyl Schiff base $ZN{=}CHCO_2Me$ (see also Section 4.1) for cycloaddition to cyclopentadiene to give 2-azabicyclo[2.2.2]heptane-3-carboxylic acid,[108] and a similar new proline synthesis using the azomethine ylide formed from $Me_3SiCH_2N{=}CHCO_2Me$.[109] Another route to alkylprolines[110] is based on copper(II)-catalyzed Michael addition of nitroacetic esters to αβ-unsaturated ketones, followed by reductive cyclization (H_2/Pd-C) of the resulting 2-nitro 5-oxo-esters.

Specific functional-group chemistry is involved in an improved preparation of L-homoglutamic acid from N-acetyl-L-lysine ethyl ester, using ^tBuOCl for N-chlorination, followed by dehydrochlorination and hydrolysis.[111] More general methodology is used for the conversion of αε-di-aminopimelic acid into α-amino-ε-ketopimelic acid by transamination with pyridoxal.[112]

4.8 Synthesis of α-Alkoxy-α-Amino Acids and Related α-Hetero-atom Substituted α-Amino Acids.- α-Substitution of Schiff bases $Ph_2C{=}NCH_2CO_2Et$ through 'allylic' bromination with N-bromosuccinimide in the presence of simple heteroatom nucleophiles in DMF gives the title compounds (e.g. NaOAc yields the α-acetoxy Schiff base).[40] These are valuable as electrophilic glycine synthons, $RC(NHR)CO_2R$; they react readily with organocopper reagents to give the corresponding 1-naphthyl, 2-thienyl, and t-butyl amino acids, for example.[40] A similar outcome but involving the nucleophilic glycine synthon $(MeS)_2C{=}N{-}\bar{C}H{-}CO_2Et$,[38] has been reported, reaction with aromatic aldehydes giving oxazolines.

4.9 Synthesis of Halogeno-alkyl Amino Acids.- Interest is particularly high in fluorine analogues of the protein amino acids, associated with their potential as enzyme inhibitors. β-Fluoro-α-amino acids have been reviewed.[113]

Synthesis of trifluoroalanine[114] proceeds through an unusual oxazole synthesis (Scheme 6). Other syntheses either employ variants of standard amino acid syntheses [erythro- and threo-β-fluorophenylalanines from a glycine Schiff-base benzyl ester with α-bromo-α-fluorotoluene;[115] amination of (E)-CHF=CMeCHBrCO$_2$Et, made from Me$_2$C=CHCO$_2$Et, to (E)-β-fluoromethyleneglutamic acid[116] in a route which can be used[117] to give (E)-$H_3\overset{+}{N}CH_2C(=CHF)CO_2^-$, capable of inhibition of GABA transaminase[117]] or involve manipulation of side-chain functional groups (erythro- and threo-β-fluoroglutamic acids from N-acetyl-β-hydroxyglutamic acid through fluorodehydroxylation[118]).

4.10 Synthesis of Aliphatic Amino Acids Carrying Side-chain Hydroxy Groups.- Frequent mention has been made in the preceding Sections of amino acids of this type, whether as new natural products or as compounds useful in synthesis.

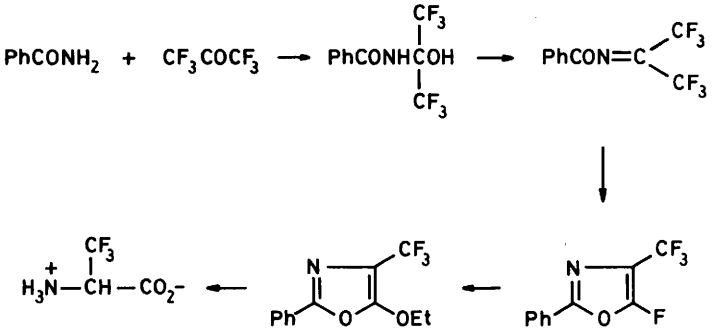
α-Aminosilylketene acetals provide β-hydroxy amino acids through Lewis acid-catalyzed addition to aldehydes (Scheme 7).[119] The 'bislactim ether' route (see Section 4.2) has been used for the preparation of β-hydroxy-γ-azido-L-valine (reaction of (27) with N_3^-).[120]

4.11 Synthesis of Aliphatic Amino Acids with Unsaturated Side chains.- While interest continues in routes to 'dehydro amino acids' (i.e. αβ-unsaturated α-amino acids), there is also increasing attention being given to homologues where the unsaturation is either further away from, or placed between, the amino and carboxy groups.

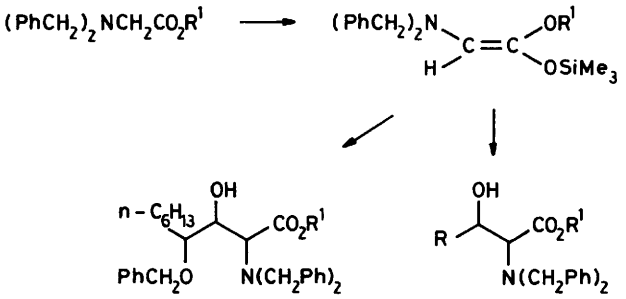
Protected dehydroglutamic acids have been prepared from corresponding α-ketoglutaric acids by condensation with benzyl carbamate.[121] An alternative approach[122] is based on the addition of malonic esters to nitriles catalyzed by tin(II) chloride, (28)→(29).

Protected βγ-unsaturated α-amino acid esters can be formed by oxidative rearrangement of γ-phenylseleno-αβ-unsaturated esters in the presence of an alkyl carbamate (Scheme 8). The route[123] has been used for the preparation of (±)-vinylglycine in 48% yield after removal of protecting groups by acid hydrolysis. An intermolecular ene reaction (Scheme 9) between allylglycine and ethyl glyoxylate shifts the unsaturation to the βγ-position and has been used[124] to prepare representative 2,6-disubstituted aminopimelic acids. A similar outcome is achieved[125] in the rearrangement of β-hydroxyallylglycine (30), obtained through SeO_2-^{t}BuOOH oxidation of protected allylglycine.[126] The acetate of (30) undergoes palladium(II)-catalyzed [3,3]-sigmatropic rearrangement to give the corresponding (E)-3-acetoxy-1-propenyl glycine.[125] Addition of diazomethane gives a 1:1 mixture of stereoisomers from which the natural α-(methylenecyclopropyl)glycine (31) was obtained through standard procedures.

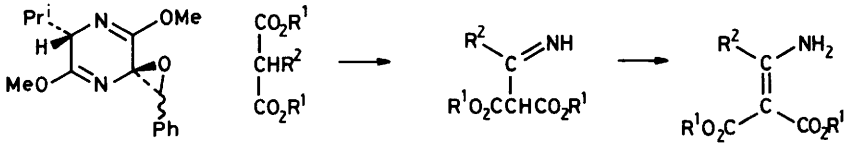
Reaction of 9-allyl- and 9-but-2-enyl-9-borabicyclo[3.3.1]nonane with Schiff bases (S)-(-)-PhCHMeN=CHCO$_2$Bu gives high yields of allylglycines (e.g. 92% yield of the (S)-enantiomer in 92% enantiomeric excess, for allylglycine itself).[127]



Scheme 6



Scheme 7



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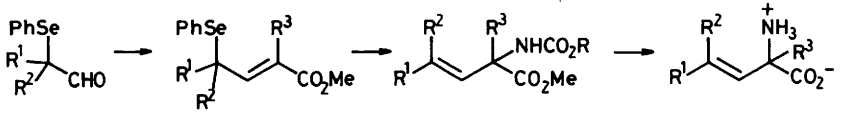
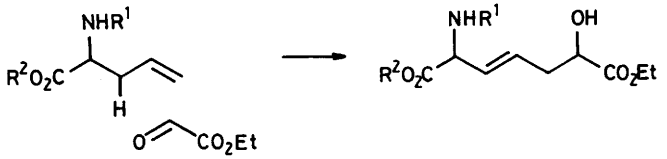
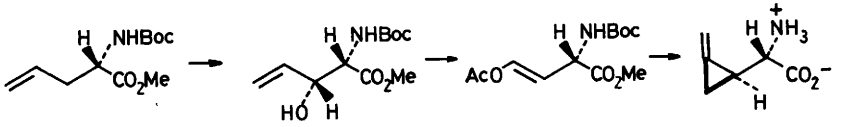


Scheme 8



Scheme 9



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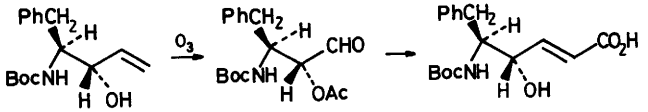
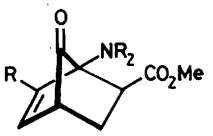


Scheme 10



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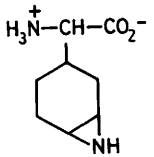

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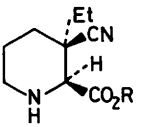

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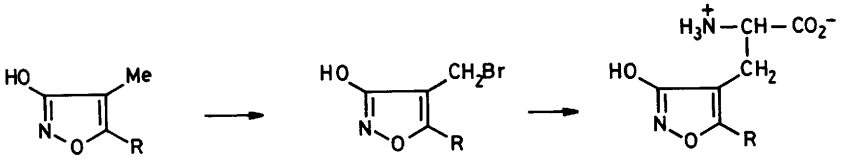

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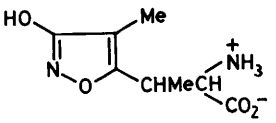


(36)

2-Aminobutadienecarboxylates can be obtained from β-allenyl-α-amino acid esters by a prototropic shift (reaction with ^{t}BuOK during 10 minutes).[128] The products can undergo cycloaddition to give more complex β-amino acids [e.g. (32) with methyl acrylate].

Acetylenic amino acids, an unconventional but common usage for amino acids with carbon-carbon triple bonds in the side chain, are represented by (2R,3R)- or (2S,3S)-2-acylamino-3-phenylhex-5-ynoic acids in the current literature. They were prepared from $HC{\equiv}CCH_2CHPhCH(CO_2Et)_2$ by conventional amination-decarboxylation or modified Curtius rearrangement procedures.[129]

Where the amino and carboxy functional groups are separated by a four-carbon moiety containing unsaturated groupings, then the compound can be variously described (e.g. as a 'keto-vinyl' or 'hydroxyethylidene' isostere of a dipeptide, if such groupings are present in the requisite locations). Synthesis of these novel dipeptide analogues employs stereocontrolled reactions (Scheme 10), starting with Boc-L-phenylalaninal.[130]

4.12 Synthesis of Amino Acids with Aromatic or Heterocyclic Side-chain Groupings.- Modifications of the protein amino acids (phenylalanine is converted into o- and m-hydroxy-L-tyrosines through the involvement of phenylalanine hydroxylase and tyrosine hydroxylase,[131] and 3-cyano-L-tyrosine is obtained from tyrosine via the 3-formyl derivative[132]) are frequently reported in this Section in preceding volumes. Synthesis is often not the main objective in such studies, which are supported by biosynthetic considerations.

Saturated heterocyclic side chains are rarely encountered in natural amino acids but feature occasionally in pharmaceutical studies. (3,4-Iminocyclohexyl)glycine (33) has been prepared from the corresponding cyclohexenylglycine by reaction with INCO.[133] Compounds more closely related to natural amino acids, e.g. the pipecolic acid derivative (34) prepared from L-asparagine, are also represented in the recent literature.[134]

2-Amino-4-(3-pyridyl)butyric acid has been prepared through a Strecker synthesis that starts with 3-(3-pyridyl)propionaldehyde,[135] including α-chymotrypsin resolution (Section 4.15).

A considerable number of ibotenic acid analogues (35) has been prepared from the isoxazoles through side-chain bromination with N-bromosuccinimide, the resulting bromomethyl compound being used to alkylate acetamidomalonate esters in a conventional application of this general method. Where the substituent R of the isoxazole is an ethyl group, bromination places the halogen on the carbon atom adjacent to the isoxazole ring, and use of acetamidomalonate leads to the analogues (36).[136]

4.13 Synthesis of Amino Acids with Side chains Containing Sulphur or Selenium.- This Section regularly depends on cysteine derivatives and near relatives for its existence, and the same applies this year. S-Adenosyl-L-homocysteine can be prepared from L-homocysteine and

5'-deoxyadenosine, employing sodium in liquid ammonia to generate the thiolate anion that adds to the purine.[137] S-(5'-Deoxy-5'-adenosyl)-(±)-2-methylhomocysteine has been prepared similarly;[138] with α-methylmethionine as starting material, sodium in liquid ammonia is used to cleave the methionine into the corresponding thiolate, exploiting the attack at the S - CH_3 bond that is somewhat surprisingly predominant, almost exclusive.

O-Acetylhomoserine sulphydrylase- catalyzed synthesis of L-selenocystine and L-selenohomocysteine from Na_2Se_2 and O-acetylserine and O-acetylhomoserine has been described for potential large-scale implementation.[139] The new selenium-containing amino acid, L-selenodjenkolate, has been prepared from selenocysteine, which in this case was obtained by conventional reductive selenation of β-chloro-L-alanine using Se and $NaBH_4$.[140]

4.14 Amino Acids Synthesised for the First Time.- This 'catch-all' Section again serves to record amino acids newly synthesized by methods that need no special discussion. The reader seeking comprehensive coverage of the title of this Section will need to browse in other parts of Section 4 of this Chapter where new amino acids are occasionally described, often arising incidentally as the outcome of studies of new or modified synthetic methodology.

Amino acid	Reference
3-Aminopyrrolidine-3-carboxylates (from cucurbitin)	141
O-Dipropionylglyceryl NNN-trimethylserine	142
β-(3,4-Diaminophenyl)alanine	43
3-[p-3-(Trifluoromethyl)-3H-diazirin-3-yl] phenylalanine	39
Astatinotyrosine	143
β-(5-Nitro-2-furyl)serine	144

4.15 Synthesis of Labelled Amino Acids.- Examples covered in this Section are grouped on the basis of the label - ^2H, ^3H, ^{11}C, and ^{13}N - and in the order of increasing atomic mass number.

Methods range from conventional general processes to individually designed routes to suit the particular objective. Examples of the former category include a synthesis of L-tryptophan-3,3-$^2H_2$ using the acetylaminomalonate synthesis followed by resolution employing L-acylase,[37] and ^1H - ^2H exchange of the α-proton in DL-α-amino acids in ^2H_2O catalyzed by a salicylaldehyde - formaldehyde copolymer.[145] (4R)- and (4S)-[4-^2H]Homoserine has been prepared, as a mixture of diastereoisomers since the chiral centre at C-2 remained in its initial (RS) state, through straightforward elaboration of (±)-p-$MeOC_6H_4CH(\overset{+}{N}H_3)CH_2CO_2^-$ via (±)-p-$MeOC_6H_4CH(NHBoc)CH_2C^2HO$.[146] 4-[$^2H_2$]-L-Glutamic acid has been obtained in excellent yield by enzymic reductive amination of 4-[$^2H_2$]-2-ketoglutaric acid.[147] The

formation of stereospecifically deuteriated (4R)- and (4S)-[4-$^2H_2$]-L-glutamic acids through separation of the products of $Na(CN)B^2H_3$ reduction of (2RS,4S)- and (2RS,4R)-4-hydroxyglutamic acid derivatives has been shown to involve 75% inversion of configuration in the labelling step.[147]

The well established alkylation of methyl 2-acetamidoacrylate with a Grignard reagent has been used to give fair to good yields, quenching with ^2H_2O giving the α-deuteriated amino acid derivative.[44] The use of CuI in the alkylation step with RMgI as alkylating agent is advocated.[44]

An extensive study in which regiospecifically 2-alkylated 3-deuteriated-1-aminocyclopropane-1-carboxylic acids are obtained starts with 1-deuterio-1,2-dibromoalkanes.[148] Conversion into the cyclopropane-1,1-dicarboylic esters by treatment with di-t-butyl malonate was followed by ester exchange and selective hydrolysis (KOH/MeOH) of the resulting dimethyl ester, leaving the more hindered ester unchanged, and allowing it to be manipulated into an NH_2 group through standard procedures.

3-[^{3}H]-5-(4'-Azobenzenearsonic acid)-L-tyrosine has been prepared from N-Boc-di-iodo-L-tyrosine through reaction with $^3H_2$, substitution with diazotized arsanilic acid, and removal of the Boc group.[149] Meso-2,6-diamino-[3,4,5-$^3H_3$]-1,7-heptanedioic acid is obtained through $^3H_2$/Pd-C-catalyzed dehydrochlorination of 3- or 4-chloro diaminopimelates formed by chlorination of meso-diaminopimelic acid by Cl_2 in conc. HCl.[150]

Several papers have appeared, extending the already substantial literature on synthesis of ^{11}C-labelled amino acids.[151-156] The short half-life of the light carbon isotope calls for rapid procedures covering synthesis, purification, and resolution, let alone rapid use in medical applications where the movement and accumulation of particular amino acids is of interest. Phenylmagnesium bromide treated with $^{11}CO_2$ and reduction gives $Ph^{11}CHO$, employed in the azlactone route with hydrogenation over a chiral Rh(I) catalyst, and resolution, to give 3-^{11}C-L-phenylalanine.[151] The labelled benzaldehyde has also been used in an accelerated Bucherer-Strecker synthesis of 2-^{11}C-DL-phenylglycine (cf. Volume 16 of these Specialist Periodical Reports, p.1).[152] The same group of workers have described a synthesis of labelled methionine, in which S-benzyl-L-cysteine is converted into the $^{11}CH_3$-amino acid through standard sulphur functional-group chemistry after methylation with $^{11}CH_3I$.[153] From what has been mentioned above, ever more speedy chemical operations offer the clinical research worker the best opportunity for time-consuming metabolic studies of these labelled amino acids, as well as merely their accumulation in particular body sites. A preparation time of 50 minutes has been reported[154] for [1-^{11}C]-DL-ornithine and the correspondingly labelled lysine, prepared by the carboxylation of corresponding α-lithioisocyanides by $^{11}CO_2$. Although shorter times have been reported (see earlier Volumes of these Specialist Periodical Reports)

for other syntheses (Strecker methods in particular), problems of side-chain functional groups have to be taken into account in methods based on the construction of parts of the 'glycine' moiety of the target amino acid as the final stage. The same carboxylation route has been used in a synthesis of [1-^{11}C]-DL-proline from α-lithiopyrrolidinyl N-t-butyl formamidine.[155]

[ω-^{13}N]-L-Citrulline, L-[ureido-^{11}C]-L-citrulline, and [carbamyl-^{11}C,^{13}N]carbamyl-L-aspartic acid have been synthesised, employing either ornithine transcarbamylase or aspartic acid transcarbamylase.[156]

4.16 Resolution of DL-Amino Acids.- The crop of recent papers, taken as a whole, amounts to consolidation of established methods. There is some overlap between this and a later section in which analysis of enantiomer mixtures employs separation methods that are essentially the same as those used, or usable, on a preparative scale.

(S)-(-)-Carbamalactic acid, PhNHCOOCHMeCO$_2$H, has been proposed[157] for use in classical diastereoisomeric salt formation resolution of amines and amino acid esters. The same principle is used for the resolution of numerous aryl-substituted phenylglycines by the use of $(+)_{589}$-tartaric acid,[158] and this research group has also reported[159] asymmetric transformation data for the same system to which a carbonyl compound has been added. The principle here is based on reversible Schiff-base formation and the reversible release of the proton at the chiral centre, an equilibrium that is shifted in favour of one enantiomer in the presence of an enantiomer of a chiral catalyst. Another research group has used the same principle combined with the preferential crystallization technique, for asymmetric transformation of DL-p-hydroxyphenylglycine in 95% AcOH at 100°C in the presence of small amounts of salicylaldehyde.[160] Here, preferential crystallization of the toluene-p-sulphonate of one enantiomer by seeding the reaction mixture with the desired enantiomer provides the enantiospecific driving force that is essential to the asymmetric transformation principle.

The preferential crystallization principle continues to accumulate its own literature, partly composed of problem cases as well as a substantial list of examples for which the method is successful. Preferential crystallization of one enantiomer is often inefficient due to the co-crystallization of often substantial amounts of the other isomer, and improvements of a practical nature have been proposed for large-scale resolution of amino acid salts.[161]

Enzymatic methods have been particularly well represented in the recent literature, but no new principles have emerged. Uses of aminoacylases[37,162,163] that leave the D-enantiomer of an N-acylamino acid unchanged by mild hydrolysis include an example of the longest extant laboratory method under this heading, N-chloroacetyl DL-1-aminocyclopropane-1-carboxylic acids[148] were subjected to porcine kidney acylase I-catalyzed hydrolysis and reaction mixtures worked up by standard methods.[162] The same underlying principle is exploited in the catalyzed hydrolysis of DL-phenylthiohydantoins illustrated in the preparation of D-phenyl-

glycine (catalyzed by hydroxypyrimidine hydrase and N-carbamyl-D-amino acid hydrolase),[164] in the use of benzylpenicillinacylase with N-phenylacetyl-DL-α-methyl-α-amino acids,[165] in papain-catalyzed condensation of N-benzyloxycarbonyl-γ-carboxy-DL-glutamic acid with phenylhydrazine (followed by removal of the phenylhydrazide grouping from the L-enantiomer with $FeCl_3$)[166] and extension of this method to all 20 'protein amino acids',[167] and in the production of D-arylglycines in a two-phase liquid system employing immobilized subtilisin for catalyzed esterification of the L-enantiomer.[168] At the other end of the scale, separation of picomole levels of ^{11}C-labelled L-amino acids as their L-aminoacyl-tRNA complexes from the free D-amino acid has been employed[169] in an assay for enantiomeric purity of products from a necessarily rapid synthesis, purification, and resolution procedure (see also refs.151-153). Refs. 135 and 324 describe the use of α-chymotrypsin in resolution of DL-amino acids.

Methods based on physical separations of diastereoisomer mixtures are represented by the flash-chromatographic separation of N-acetyl DL-amino acid L-phenylalanine amides.[170] An aqueous two-phase polymer system based on Dextran 40 - PEG 6000 with serum albumin restricted to the lower phase has been used to resolve DL-tryptophan by counter-current distribution (L-tryptophan is retained in the lower phase).[171] Somewhat similar principles underlie the 'host-guest' approach, in which selective incorporation of one enantiomer into a chiral host molecule is involved. Initially promising results[172] with a chiral Schiff-base polymer that is capable, after complexation to cobalt(II) ions, of hosting D-phenylalanine preferentially from the racemate, have been developed further to give almost 100% stereospecificity. The latter result was obtained[173] for a copolymer prepared from the N-benzyl-D-valine-Co(III) complex of (37) with styrene and divinylbenzene from which the valine was removed to give the 'host'.

Kinetic resolution accompanies the stereoselective reduction of N-hydroxy-DL-amino acids by a chiral thiol in the presence of Fe^{2+} salts.[174] In this fascinating study it is shown that L-amino acids accumulate when (-)-dihydrolipoic acid is the reducing agent, while the D-enantiomers are more rapidly formed when L-cysteine is involved.

A topic of continuing interest, related to the prebiotic predominance of L-amino acids with respect to their enantiomers, concerns a role for chiral radiation in preferential destruction of (or other chemical change to) the D-isomer. The Vester-Ulbricht theory (that natural β-radiation that is inherently chiral behaves in such a way) has been discarded in recent years following the reasoning of Keszthelyi and co-workers.[175] However, recent theoretical work shows that it seems wrong to reason that there is no discrimination between amino acid enantiomers.[176] Clearly, further exploration of these ideas would be useful, and the debate will no doubt continue. There is no better next step than to obtain experimental evidence, and some further papers are cited in the later section of this Chapter (6.1 Racemization) on the unequal outcome, as far as the two enantiomers are concerned, of irradiating amino acids.

5 Physical and Stereochemical Studies of Amino Acids

5.1 Crystal Structures of Amino Acids and Their Derivatives.- The papers under this heading are mainly factual reports, though often there are points of interest that emerge from the specific content of the subject of the study, or more general principles when the results are correlated with other literature reports.

The crystal structures of L-glutamic acid hydriodide,[177] N-acetyl-L-histidinamide,[178] N-benzoyl-DL-alanine ethyl ester,[179] Z-N-acetyl-α,β-dehydrophenylalanine methylamide,[180] N-tritylaziridinecarboxylic acid and N-tritylproline,[181] 2-alkyl-1-aminocyclopropane-1-carboxylic acids,[148] N-acetyl-DL-methionine,[182] L-asparagine monohydrate and L-asparagine + L-aspartic acid monhydrate,[183] and β-amino-γ-hydroxybutyric acid.[184]

A review of a large number of X-ray crystal structures of amino acids[185] from the point of view of minimum contact distances between the non-polar side chains (leucine, isoleucine, valine, and phenylalanine) shows that the preferred interatomic distances in the crystalline state are 0.3-0.5 Å greater than the minimum contact distances.

5.2 Nuclear Magnetic Resonance Spectrometry.- Many physical and stereochemical studies are based on more than one technique, particularly in the spectroscopic area as far as amino acids are concerned. Some cross-referencing between this and succeeding sections is therefore necessary.

Simultaneous appearance of thorough reviews of the n.m.r. of amino acids and peptides is to be noted; one of them[186] covers the literature that appeared mostly within the period 1983-4, while the other[187] is more narrow in its timescale.

^{1}H-N.m.r. studies, excluding the routine uses in support of synthetic work, cover linear relationships that appear to exist between the chemical shift of the amide proton of N-acetyl aspartic acid and temperature.[188] This relationship permits a novel proposal to be made: that this resonance is a useful index for the local temperature within a tissue sample.[188] Other straightforward applications of ^{1}H-n.m.r. form part of an X-ray/n.m.r. study of inter- and intramolecular interactions in N-benzoyl-DL-alanine dithioester[179] and part of a c.d./n.m.r. assignment of absolute configuration to galantinic acid, established[189] to be (2S,4S,5S)-5-amino-2-carboxymethyl-4-hydroxytetrahydropyran (38).

Enantiomeric purity measurements are described[190] for N-acyl-, N-aroyl-, or N-heteroaroyl amino acid methyl esters, based on the separate signals seen for the methyl groups in the presence of the chiral shift reagent Eu(tfc)$_2$. Similar non-equivalence is induced into a range of solutes by methyl esters of N-(3,5-dinitrobenzoyl)-L-amino acids.[191]

The new generation of ^{1}H-n.m.r. techniques is increasingly entering the amino acid field, with consequent benefits in the peptide and protein areas. 2D Double-quantum

coherence values allow the identification of ^{1}H-n.m.r. resonances arising from methyl groups in proteins, for example, and the spectral characteristics associated with methyl groups in alanine, valine, isoleucine, leucine, and threonine have been illustrated.[192] Similar studies (CIDNP - COSY and CIDNP - NOESY) have been reported for photochemically induced dynamic nuclear polarization 2D ^{1}H-n.m.r. spectra of N-acetyl-L-tyrosine and N-acetyl-L-tryptophan.[193]

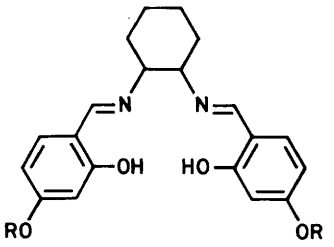
A solution to a simple but frustrating problem, the differentiation of side-chain N-substituted histidines, has been found in n.m.r. spectrometry. Unambiguous differentiation between π- and τ-substitution by a substituent of the general structure RCH_2- is obtained by methylation, followed by nuclear Overhauser effect n.m.r. study. There is an enhanced CH_2 signal for a π-substituent when the adjacent proton between the ring nitrogen atoms is irradiated.[194,195]

As well as accumulating results from several physical methods in tackling problems of structure and dynamics in solution, it is often beneficial to use n.m.r. measurements based on two or more nuclei. ^{1}H- and ^{13}C-N.m.r. spectra of aspartic acid as a function of pH indicate the intramolecular non-bonded interaction of carboxy groups that sets in at higher pH, involving a six-membered ring structure.[196] Vicinal ^{13}C - ^{1}H, ^{15}N - ^{1}H, and ^{13}C - ^{15}N spin coupling constants derived from ^{1}H and ^{13}C n.m.r. spectra for various ionic forms of amino acids, and their ^{15}N isotopomers, have been determined. When reviewed in the light of the potential information in terms of conformational assignments that these can offer, it seems that the ^{13}C - ^{15}N data have relatively little value.[197]

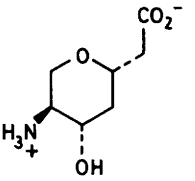
^{2}H-N.m.r. studies of solid amino acids have been interpreted to reveal the re-orientation of deuterons in the $N^{2}H_3$ group of the α-glycine crystal.[198] Similar solid-state dynamics studies of phenylalanine, but including ^{13}C-n.m.r. data as well, reveal that the amino acid is in a state that allows about half the molecules in the crystal to undergo rapid two-fold flips when crystallized from water.[199] The C^{β} - C^{α} bond is the site at which the rotation occurs; the situation is quite different for other methods of crystal formation, and this is clearly opening up a valuable role for n.m.r. studies in solid-state assessment.

^{13}C-N.m.r. chemical-shift values for the α-carbon of an amino acid mainly reflect the electronic shielding of that atom by nearby groupings and has been proposed[200] as a useful parameter for QSAR studies. A straightforward extension of ^{1}H-n.m.r. methodology is seen in the splitting of the resonances of the enantiomeric methylene carbon atoms in 1-aminocyclopropane-1-carboxylic acid, when this amino acid is condensed into a dioxopiperazine (39) with an L-amino acid.[201]

^{17}O-N.m.r. studies have seemed to offer little of distinctive diagnostic value in the past, whether to amino acid studies in particular or relatively complex organic structures in



(37) R = *p* - vinylbenzyl



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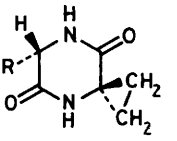

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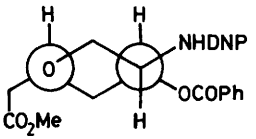

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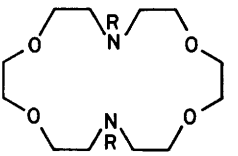
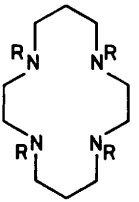
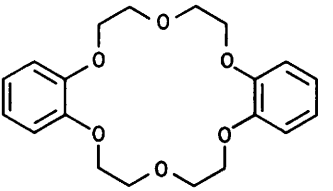


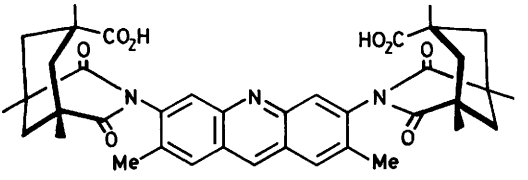
(41) R = $PhCH_2$ or PhCO, also RN in place of O



(42)



(43)



(44)

general. ^{17}O-N.m.r. chemical shifts of the carboxylate-group oxygen atoms of 20 'protein amino acids', together with hydroxyproline, sarcosine, and N,N-dimethylglycine, fall in the range 250 - 257 ppm at low pH but somewhat more downfield (266 - 273 ppm) at neutral pH, with reference to water as an external standard.[202] This study seems a little at odds with a parallel investigation[203] in which the range of chemical shifts in water is found to be identical (249 - 257 ppm) with that reported by Lauterwein et al. for solutions of low pH.[202] Common α-amino acids show ^{17}O-n.m.r. resonances at 234 - 243 ppm in FSO_3H.[203] Comparisons of factors controlling the ^{17}O-n.m.r. chemical shift have been made[204] for cis- and trans-N-acetyl-L-proline and the corresponding N-acetylsarcosine geometrical isomers, taking account of the values for the amino acids themselves. There now seems to be scope for extending the technique to N-acetylamino acid amides and peptides since the reproducibility and resolution of the instrument have been established.

^{19}F-N.m.r. of 3-fluorophenylalanine and 3-fluorotyrosine in bicarbonate buffer reveal a pH-dependent upfield resonance accompanying reversible carbamate formation (NH_2- ⇌ $^-$OCONH-), as well as the resonance for the free amino acid seen in aqueous solutions.[205] This offers a simple method for measuring the equilibrium constant for the condensation.

5.3 Optical Rotatory Dispersion and Circular Dichroism.- A quiet year for these techniques; a representative citation[189] describes the assignment of absolute configuration to (+)-galantinic acid (38; Volume 17 of this Specialist Periodical Report, p.8) based on Kawai's 'DNP - aromatic rule': the positive Cotton effect at ca. 400 nm is consistent with configuration (40).

5.4 Mass Spectrometry.- Discussion of the analytical use of mass spectrometry in conjunction with another technique can be found in the later Section 7: Analytical Methods.

Routine measurements are not covered for this Section, but even the more sophisticated ionization methods are entering into a consolidation-of-existing-knowledge phase. There is continuing interest, of course, in obtaining spectra for the amino acids themselves, avoiding the need to derivatize. Chemical ionization mass spectrometry of amino acids with aromatic side chains, including compounds related to DOPA and tryptophan, gives good spectra with minimum fragmentation when methylamine is used as reactant gas, and analyzing for positive ions.[206] Negative-ion spectra using CCl_4 as reactant gas are also successful.[206] Amino acid methyl esters, converted into oxazolidinones by reaction with 1,3-dichloro-1,1,3,3-tetrafluoroacetone, have provided negative-ion mass spectra by the same method, leading to $M+Cl^-$ amongst other ion - molecule species.[207]

Fast atom bombardment mass spectrometry[208,209] have much to offer for involatile samples, no more so than in the amino acid and peptide field. A distinction between leucine and isoleucine by f.a.b. - tandem mass spectrometry[209] is possible, based on m/z 86 ions;

other examples include the identification of the amino acids in trofopar after derivatization as N-trifluoroacetyl methyl esters [210] and structure assignment to pipecolic acid derivative (2).[17]

Straightforward applications have been described for trimethylsilyl derivatives of N-(1-deoxyhexitol-1-yl)amino acids,[211] $\alpha\beta$- or $\beta\gamma$-unsaturated α-amino acid esters,[212] imino acids (proline and pipecolic acid and their derivatives) in biological fluids at nanomole levels,[213] and assessment of ^{15}N-enrichment in the D-alanine content of bacterial cell walls,[214] for cells grown in $^{15}NH_3$-containing media. In the last-mentioned study,[214] the spectra were measured after conversion of the amino acid into its N-heptafluorobutyroyl D-2-butyl ester.

5.5 Other Physical Studies.- Whereas the preceding Sections would be of most interest to the practising scientist employing amino acids for synthetic and structural studies, there is clearly a wide range of other physical methods that can be applied. The scope of this Section indicates that all available methods are being applied, and in increasing detail. Perhaps this is hardly surprising for a group of organic compounds of such supreme importance in biological contexts in particular, but also in chemical studies.

Spectroscopic and related physical techniques not covered in the preceding sections include ultraviolet Raman spectroscopy of aromatic amino acids (the spectra show considerable dependence on the excitation wavelength),[215] fluorescence spectroscopic study of the binding of tryptophan derivatives to lipid bilayers,[216] infrared spectrometric study of 1-deoxy-1-glycino-D-fructose (the Amadori condensation product of D-glucose with glycine),[217] spectroscopic study of the barrier to rotation about the thioamide bond in N-thionaphthoylsarcosine,[218] dielectric spectra of amino acids in aqueous solutions over the frequency range 1 MHz to 40 GHz,[219] and electron spin resonance spectroscopy of the tyrosyl radical in aqueous solutions.[220] In the last-mentioned study, spectra measured at elevated temperatures (above 60°C) refer to all the possible rotational processes that are conceivable and provide the first complete characterisation of the tyrosyl radical.[220]

Measurements determined with relatively simple apparatus usually based in the teaching laboratory include dissociation constants of amino acids using the ionophoretic technique,[221] volumetric virial coefficients of N-substituted amino acids,[222] enthalpy of interaction measurements for β-alanine with urea in aqueous solution (essentially the same as that for alanine itself in the same system),[223] enthalpies of dilution of N-acetyl-L-prolinamide with equimolal solutions of other N-acetylamino acid amides,[224] and of solutions of these solutes in NN-dimethylformamide compared with data for aqueous solutions.[225] These properties reflect all facets of solvent - solute and solute - solute interactions of particular interest. The heats of dilution data for aqueous solutions of D-alanine amides containing various amounts of the L-enantiomer reveal chiral recognition arising through diastereoisomeric pairwise

interactions.[226]

Some simple physical properties that include important biological roles, at least in principle, have been reported. Proline is suggested to be a natural cryoprotectant,[227] preserving membrane structure and function as far as Ca^{2+} transport is concerned, under freezing conditions. Seleno-DL-methionine offers some protection for human platelets against freezing injury.[228] The sites at which these amino acids exert their effect may not be identifiable without knowledge of the transport properties of the amino acids themselves, as well as of other solutes. Transport properties of amino acids is a long-standing research topic, and recent studies cover the transport of β-alanine and non-protein α-amino acids across brush-border membranes of rabbit ileum.[229] These studies frequently employ labelled amino acids, and the uptake of [^{3}H]-L-tryptophan by rabbit forebrain synaptosomes[230] is an example of this. In this study, the kinetics of the process were of particular interest, with the demonstration that extracellular sodium ions reduce the rate of uptake.[230] In model systems of potential biological relevance, polyamine and polyamide macrocycles (41) and (42) and polyethers (43) are excellent cation carriers for the transport of amino acid ester salts.[231] In the same category, the compound (44) shows a remarkable ability for the extraction of phenylalanine, tryptophan, and o-methyltyrosine into chloroform from water.[232] There is thought to be three-point binding involving particularly the acridine moiety in a stacking-type interaction with the aromatic groupings of these amino acids.

Interactions between amino acid amides and polyribonucleotides are also a long-standing subject of research, and can be detected by measuring melting temperatures of such systems as a function of concentration.[233]

A series of N-acyl-DL-amino acids has been studied by differential scanning calorimetry to add to knowledge of structures known to form solid racemic compounds.[234] Other points of interest concerning amino acids and their derivatives in the condensed state arise for N-(n-dodecanoyl)-L-alanine (adopts the cholesteric mesophase liquid crystalline state)[235] and a fascinating observation[236] that a structural correlation exists between an etched crystal surface (either enantiopolar glycine or enantiomorphic L-asparagine hydrate) and the etchant. This provides a new means of manual sorting of enantiomorphous crystals and also a new method for assigning absolute configuration to a crystalline enantiomer.

5.6 Molecular Orbital Calculations.- Apart from calculations for atomic charges at all locations in amino acids and peptides,[237] most current papers address conformational problems. Representative citations from major research groups deal with N-acetyl dialkylglycine N-methylamides[238] and energy differences between low-energy conformers of N-acetyl glycine N-methylamide.[239]

The very different aspect of energy involved in calculations of parity-violating weak

neutral current perturbation of amino acid electronic energies has an important implication: the energy shifts that are caused are consistently favouring the L-enantiomer of a racemic pair.[240]

6 Chemical Studies of Amino Acids

6.1 Racemization of Amino Acids.- Aspects of resolution discussed in Section 4.15 are based on the separation of one enantiomer from a solution of a racemate in which the other enantiomer is being caused to racemize. The underlying chemistry has also been used in a study optimizing the racemization of amino acid esters in organic solvents by aromatic aldehydes as catalysts. The aldehyde may be a solute or bonded to a solid phase.[241]

A continuing research topic has been christened 'radioracemization' - the search for experimental proof that destruction of one enantiomer of a DL-amino acid by γ-radiolysis is more rapid than for the other. γ-Radiolysis of L-leucine and its hydrochloride, in aqueous solution or adsorbed on clays (kaolin or bentonite), has been continued so that between 2% and 89% of the amino acid is 'destroyed', and it is accompanied by small levels of racemization. L-Leucine in aqueous solution is least stable to radiolysis and radioracemization and the amino acid in the solid state is most stable to the conditions, with the amino acid adsorbed on clays occupying an intermediate position.[242; see also 348]

6.2 General Reactions of Amino Acids.- This Section is followed by one entitled 'Specific Reactions of Amino Acids', in which reactions of the amino acid side chain, in isolation or in concert with the amino and carboxy groups, are covered. Therefore, this Section is essentially concerned with reactions of amino acids irrespective of the side chain. This coverage falls broadly into three headings: reactions at the amino group, reactions at the carboxy group, reactions involving both carboxy and amino groups.

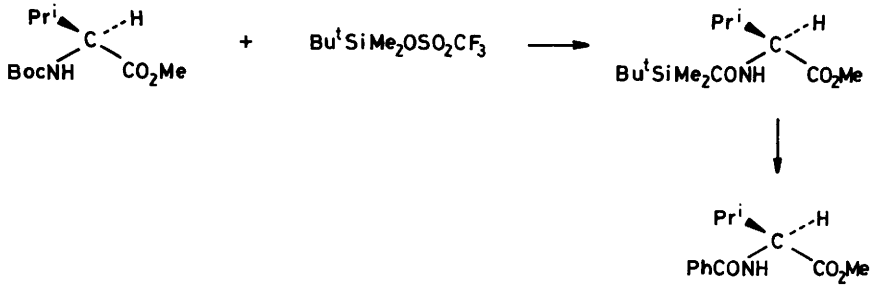
'Reactions at the amino group' and the following section largely exclude the synthesis of protected derivatives for use in peptide synthesis, unless there is any unusual interest. N-Boc-amino acids, for which a representative Organic Syntheses recipe now exists[243] (for Boc-L-phenylalanine) employing Boc-anhydride, can be converted into their N-ethyl analogues through treatment with ^{t}BuLi and then $Et_3O^{+}BF_4^{-}$.[244] A second Boc group can be introduced at the nitrogen atom of a Boc amino acid, if the carboxy group is esterified, using Boc anhydride in MeCN containing the non-nucleophilic base 4-dimethylaminopyridine.[245] The esterifying grouping used in this work was the benzyl group, permitting its selective removal from the product. A convenient preparation of carbamates of α-amino acids[246] involves reaction with a chloroformate in refluxing ethyl acetate. An interesting preparation of a trimethylsilyl carbamate, an intermediate that can be converted into any other alkyl

carbamate through reaction with an alkyl halide, has been described.[247] The general scheme (Scheme 11) allows the conversion of an N-Boc-L-amino acid ester into its N-benzyloxycarbonyl analogue without racemization, in 85% yield in the case of L-valine methyl ester. It is also compatible with other protection regimes, for example side-chain unsaturation and the protection of hydroxy groups as acetonides in multifunctional amino acids (45); see also ref 125.

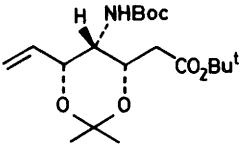
A group of papers that cover problems associated with standard analytical procedures contributes to ever more reliable assays. N-Methylation and NN-dimethylation accompany methyl ester formation with amino acids, when diazomethane is used as part of a procedure for the conversion of amino acids into volatile derivatives for g.l.c. analysis.[248] Metal ions interfere in reactions of the amino group with the fluorogens dansyl chloride, o-phthaldialdehyde, fluorescamine, but especially 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole.[249] The net result, the suppression of the fluorescence to variable extents, can be overcome if EDTA is added to the reaction mixtures. Surfactants also act to inhibit various colour reactions that are commonly used in amino acid analysis, and the effect is particularly noticeable with non-ionic surfactants.[250] Further attention has been given to the mechanism of the o-phthaldialdehyde procedure that has become a popular choice for pre- and post-column h.p.l.c. analysis of amino acids. The procedure involves a thiol as an essential component and this is shown to be incorporated into the eventual product, a 2-*mercapto*-isoindole, at a final stage. The initially formed imine may not be the only intermediate, as shown by detailed kinetics measurements and their interpretation.[251] It is believed that addition of the thiol to the imine to give an α-alkylaminobenzyl sulphide is followed by isoindole formation.[251,252]

Fluorescent products formed between some of the protein amino acids and 12-oxo-cis-9-octadecenoic acid have been characterized.[253] The aliphatic acid is otherwise known as 12-keto-oleic acid, a by-product of lipid peroxidation; apart from glycine, only the basic amino acids yield strong fluorescence, which is diminished by N-acetylation and inhibited by the addition of thiols. Methyl 12-keto-oleate reacts with amino acids to give 8-(N-substituted 4,5-dihydro-4-oxo-5-hexyl-5-hydroxy-2-pyrrolyl)octanoic acid methyl esters (46).[253] Other results of interest concerning reactions between amino acids and aliphatic compounds that accompany them in vivo have been reported: for melanoidins formed in the Maillard reaction between glucose and glycine, and their separation over a strong anion-exchange resin,[254] and for the 'eneaminol' (47), the initially formed adduct between D-xylose and glycine when reacted in equimolar amounts in water at 68°C.[255] The eneaminol is clearly formed between the reactants in a 2:1 ratio, and the use of equimolar amounts may simply have been the way that the experiment was actually carried out, rather than the outcome of a series of experiments designed to consign an optimized procedure to the literature.

Other reactions at the amino group include a careful study of the well-known reaction



Scheme 11



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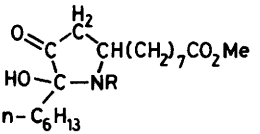

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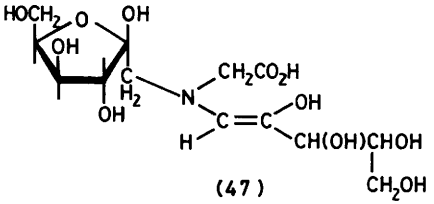

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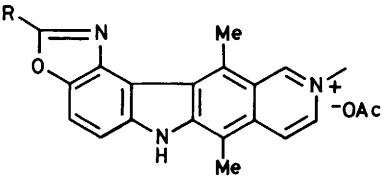

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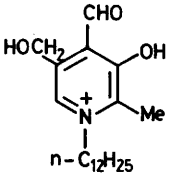

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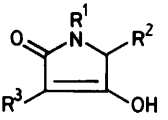

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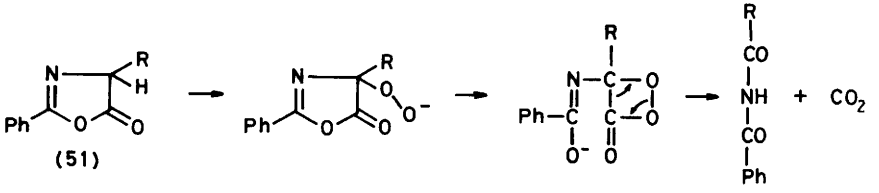

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Scheme 12

with nitrous acid in the presence of halide ions, leading to L-α-halogenoalkanoic acid esters from the corresponding α-amino acid esters,[256] peroxidase-catalyzed reaction with N-2-methyl-9-hydroxyellipticinium acetate leading to condensed oxazoles (48),[257] and correcting earlier structures assigned to the product, and Schiff-base formation with the pyridoxal 5'-phosphate model (49) and transamination reactions involving the product.[258]

Cleavage of promising N-protecting groups has been the subject of mechanistic study for diphenylphosphorylamino acids ($MeOH/H_3O^+$)[259] and N-(1-benzotriazolylcarbonyl)amino acid esters (Et_3N-catalyzed alkanolysis, but only in moderate yield).[260]

The already voluminous literature on oxidative degradation of amino acids is further augmented by kinetic studies using Chloramine-T,[261] phenyliodosyl acetate,[262] ClO_2,[263] bromate,[264] N-bromosuccinimide,[265] and N-bromoacetamide.[266] Catalysis of the oxidative decarboxylation by carboxylate anions is a curious observation arising from the last-mentioned study.[266]

N-Protected amino acids can be converted into their diphenylmethyl esters using benzophenone hydrazone, iodine, and phenyliodosyl acetate.[267] Rapid esterification of amino acids through temporary N-protection by condensation with ethyl acetoacetate, treatment with an alkyl chloride, and N-deprotection with toluene-p-sulphonic acid gives amino acid ester toluene-p-sulphonates in 75-89% yields.[268] Long-chain alkyl esters of amino acids have been obtained by reaction with the alkanol and $MeSO_3H$, using the alkanol ($C_{18}H_{37}OH$ was used in this study)as solvent as well as reactant.[269] A reaction mixture containing an amino acid, an alkyl toluene-p-sulphonate, and the corresponding alkanol proceeds to the amino acid ester utilizing the alkanol, not by transesterification of the sulphonate ester.[270]

Isopropenyl chloroformate has been proposed[271] for the preparation of carbonates of N-protected amino acids for use as 'active esters' in peptide synthesis. An interesting, though negative, result has been reported for cyanoacetylene, thought to be a peptide-forming agent relevant to ideas of prebiotic protein synthesis: it does not react with N-protected amino acids.[272] Conversion of N-trifluoroacetylamino acids into primary amides has been accomplished by condensation with hydroxylamine using dicyclohexylcarbodi-imide, followed by reaction with NH_3.[273]

Disproportionation of phosphinic - carboxylic anhydrides of N-protected amino acids has been subjected to kinetic study.[274] Other studies dealing with reactions of carboxy-group derivatives of amino acids deal with uses of N-trifluoroacetylamino acid chlorides in Friedel-Crafts acylation reactions leading to aryl N-trifluoroacetylaminoalkyl ketones,[275] formation of α'-amino-αβ-ynones via α-amino acid isoxazolidides,[276] and continuing studies of enantioselective hydrolysis of DL-amino acid p-nitrophenyl esters by micelles containing L-histidine.[277] A closely related study[278] with the same objective employs N-dodecanoyl-

DL-phenylalanine p-nitrophenyl ester as substrate and micelles containing N-benzyloxycarbonyl-L-phenylalanyl-L-histidyl -L-leucine as chiral catalyst.

Schiff bases formed between pivalaldehyde and an α-amino acid N-methylamide give trans-imidazolidin-4-ones (11) when cyclized with benzoyl chloride but cis isomers when benzoic anhydride is used.[279] Other studies in which both amino and carboxy functions of amino acids are involved include the formation of pyrrolidine-2,5-diones (50) by cyclization of N-acylalanine and glycine esters with NaH in DMF, and their alkylation and acylation behaviour.[280] Whereas acylation occurs exclusively on oxygen, alkylation leads to a mixture of O- and C-alkylated products. Oxazolin-5-ones (51) formed from N-acylamino acids undergo base-catalyzed decarboxylation after adding O_2, leading to diacylamines (Scheme 12).[281] A new synthesis of oxazolidin-2,5-diones ('amino acid N-carboxy anhydrides' has been reported[282] in which N-Boc-amino acids are reacted with $^tBuMe_2SiCl$ and the resulting silylester is reacted with oxalyl chloride.

Reactions of potential relevance to prebiotic condensation reactions of amino acids are discussed in a substantial set of Symposium papers, two of which are typical: thermal copolymerization of a mixture of 18 amino acids gives readily reproducible mixtures of peptides,[283] and the reactivity of amino and carboxy functional groups of amino acids can be assessed to account for preferred combinations between constituents in amino acid mixtures.[284] Acid catalysis of gas-phase cyclization of α-amino acids has been assessed,[285] and a co-operation of amino and carboxy groups is seen in the hydroxyl radical-induced decarboxylation of amino acids in alkaline solutions.[286] The radical is believed to add to the unprotonated NH_2 group in this system and thereby trigger off the loss of CO_2 and simultaneously involve water in the process.

1:1 Complexes formed between 18-crown-6 or dibenzo-18-crown-6 ethers with amino acids or their K^+, Ca^{2+}, or Na^+ salts have been described.[287]

6.3 Specific Reactions of Amino Acids.- Amino acids with side-chain hydroxy groups are again the subject of several recent papers covering reactions involving one or more functional groups. Sulphate ester formation is quantitative using H_2SO_4 in DMF with dicyclohexylcarbodi-imide as condensing agent.[288] A re-investigation of the oxidative degradation of amino acids by Chloramine-T concentrates on nitrile formation from threonine.[289]

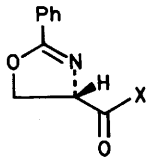
Base-induced $C^\alpha - H$ and $C^\alpha - C^\beta$ bond cleavages in Λ-and Δ-bis{N-salicylidene-(S)-O-acylthreoninato}cobaltate(III) have been compared, revealing the former to be 3 times faster,[290] and competing β-elimination has been assessed.[291,292] 2-Iminopropionate esters formed in this way from O-acetyl and O-alkanesulphonyl serine esters can undergo base-catalyzed addition to aldehydes to reform the γ-hydroxyamino acid ester.[292] During attempted conversion of N-trifluoroacetyl serine and threonine into their trimethylsilyl esters

by reaction with N,O-bis(trimethylsilyl)trifluoroacetamide, a straightforward process for other common amino acids, condensation to form the corresponding 2-trifluoromethyloxazolines, was observed.[293] The 2-phenyloxazoline derived from L-serine methyl ester reacts with 2-alkyl-2-lithio-1,3-dithians to give (52), from which corresponding chiral aminoketones may be obtained without racemization by reductive desulphurization.[294]

Enantiomerically pure N-Boc- or -benzyloxycarbonyl β-lactams (53) formed by the use of Mitsunobu reagents with the corresponding L-serine derivatives are susceptible to ring opening by powerful nucleophiles (e.g. methoxide ion) to give the β-substituted alanines.[295] β-Lactams formed by annelation of Schiff bases can involve D-threonine as starting material; the threonine-derived Schiff base (Scheme 13) gives almost 100% optically pure β-lactams.[296]

Some of the studies of serine derivatives described in the preceding paragraphs have also included cysteine analogues with similar results - the conversion of cysteine into its S-sulphonate[288] and β-elimination of methanethiol from S-methylcysteine co-ordinated to cobalt(III)[292] are accomplished in the ways described for the oxygen analogues. Another β-elimination study[297] employing S-benzyl-DL-cysteine describes the use of N-dodecylpyridoxal micelles for the purpose. A variety of reactions of the thiol group of cysteine are represented in the recent literature and need only brief description: formation of the thiolsulphonate RSH → RS-N=O[298] → $RSSO_2Ph$;[299] reduction of cystine thiolsulphinates to give unsymmetrical cystines and lanthionines, using tris(dialkylamino)phosphines;[300] formation of the methoxycarbonylsulphenyl derivative RSH + $MeO_2CSCl \rightarrow MeO_2CSSR$ that yields thiolcysteine RSS^-K^+ with KSH;[301] formation of thiocarbamoyl and carbamoyl derivatives from N-benzoylcysteine ethyl ester RSH + R-N=C=S → RNHCSSR and RSH + PhN=C=O → PhNHCOSR, respectively (but benzoyl cysteine fails to react with PhNCO);[302] formation of N-Boc- or N-Bpoc-N-methyl-4-aminobutanoyl derivatives RSH → Boc- or Bpoc-$NMe(CH_2)_3COSR$, the group being removeable by trifluoroacetic acid;[303] and preparation of N-Boc-{S-(3-nitro-2-pyridinesulphenyl)}cysteine N-hydroxysuccinimide ester as a heterobifunctional crosslinking agent for linking cysteine peptides to bovine serum albumin.[304]

S-Alkylation of cysteine derivatives for different purposes has been reported in a synthesis of a compound related to leukotriene E_4: $Me(CH_2)_{11}C{\equiv}CCH\{O(CH_2)_3CO_2R\}_2$ is added to $CF_3CONHCH(CH_2SH)CO_2Me$;[305] in preparations of chiral phosphines starting with L-cysteine, L-methionine, or D-penicillamine and subjecting them to S-alkylation, N-methylation (formaldehyde/H_2/Pd-C), and carboxy-group reduction to the primary alkanol ($LiAlH_4$);[306] and a conversion of S-t-butylhomocysteine into methionine through S-methylation (MeI and $AgClO_4$) and storage of the sulphonium salt at 40°C during 2 hours.[307] In the last-mentioned study methylation by excess MeI in aqueous emulsion during 2 hours converted the S-t-butyl compound into the S-dimethylsulphonium salt.



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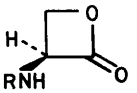

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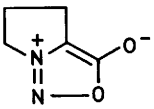

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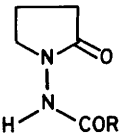

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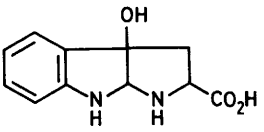

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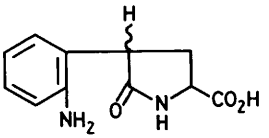

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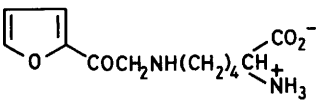

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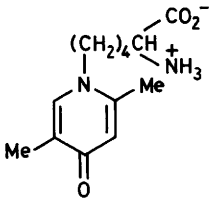

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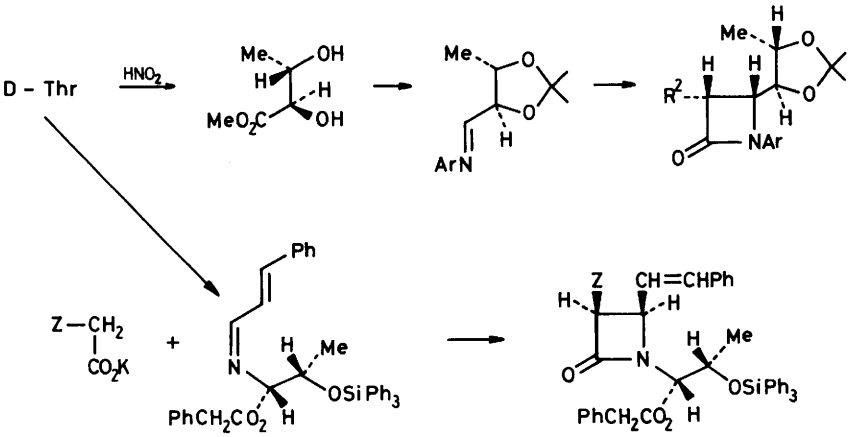

Scheme 13

Pyrolysis of solid L-cystine and its three stereoisomers either at 150°C or at 230°C gives a mixture of NH_3, H_2O, H_2S, and CO_2.[308]

Asymmetric oxidation of methionine derivatives to give the sulphoxide has been accomplished using t-butyl hydroperoxide in the presence of $(Me_2CHO)_4Ti$ - (+)-diethyl tartrate.[309]

Studies involving proline and other imino acids deal with the formation of N-nitroso-L-proline ethyl ester in millet inoculated with Fusarium moniliforme, a common fungus, when nitrite is present in the growth medium,[310] the formation of N-acylazaprolines from proline via a mesoionic derivative (54) → (55),[311] and a related cycloaddition of N-(2-pyridinecarbonyl) proline with 2-chloroacrylonitrile in acetic anhydride (which functions both as solvent and as reagent for forming a mesoionic intermediate from the proline derivative).[312] A surprising but useful oxidative cleavage of nopaline, a member of the 'opine' family discussed on p.2 of this Chapter (refs. 18,30-32), has been described that gives the two constituent amino acids L-arginine and D-glutamic acid without racemization and thereby allows absolute configuration to be assigned.[313] For no obvious reason, the normally straightforward method in this series for assigning absolute configuration - the reaction of L-arginine with enantiomers of 2-chloropentanedioic acid and comparison of products with the natural compound - failed.[313] Aziridine carboxylic acid has been converted, via its potassium salt, into series of esters and amides.[314]

1-Aminocyclopropanecarboxylic acid (whose synthesis in grapefruit tissue is inhibited by ethylene, which enhances the accumulation of its N-malonyl derivative and thereby reduces further the availability of 1-aminocyclopropanecarboxylic acid[315]) breaks down to give not only the well-known products ethylene and CO_2 but also CN^-.[316] Fortunately the cyanide does not accumulate but appears in biosynthesised asparagine. The sequential single-electron transfer pathway for ethylene biosynthesis is given further support through this novel finding.

By their nature, alkyl side chains are not susceptible to selective attack while the amino and carboxy groups are protected only by the relatively labile groups that are used in peptide synthesis. However, N-benzoylvaline methyl ester has been used in studies of free-radical halogenation,[317] with the finding that SO_2Cl_2 and N-bromosuccinimide show different regioselectivity. The stable reagent 4-formyl-2-methyl-1,3,4-thiadiazolin-5-thione converts N-hydroxyglycine into its N-formyl derivative (hadacin, an anti-tumour antibiotic) in 83% yield.[318]

A worrying finding, that 5-phenyl-2-pyridinamine is mutagenic,[319] has to be coupled with the fact that this pyrolysis product of phenylalanine is present in broiled sardines. Heteroaromatic amino acids are represented by tryptophan reactions: oxidative modifications to peptides carrying tryptophan at the N-terminus lead to the generation of its 3-anilinopyrrolidin-2-one derivative that is not susceptible to Edman degradation;[320] generation of light accompanies chlorination of the tryptophan indole moiety by the myeloperoxidase - H_2O_2 - Cl^-

or HOCl or taurine chloramine systems;[321] formation of strongly fluorescent derivatives ($\lambda_{exc.}$ 305 nm, $\lambda_{emiss.}$ 455 nm in dil. HCl) by reaction with chloroacetaldehyde;[322] β-cleavage by phenyliodosyl acetate in methanolic KOH to give 3-methoxymethyl-3H-indole through initial addition of C_6H_5IO to the indole nitrogen atom;[323] α-chymotrypsin-catalyzed esterification of N-acetyl-L-tryptophan and N-acetyl-L-tyrosine in ethanol containing less than 10% of water;[324] and photo-oxidative conversion into 3-(2-aminophenyl)-2-pyrrolidone-5-carboxylates and kynurenine (56) → (57).[325]

Strong acid treatment of α-amino-δ-hydroxyvaleric acid leads to the formation of proline and several unidentified products, presumed to be lactones.[326]

Reactions of the acidic amino acids reported in the recent literature are mostly relatively routine: formation of α-esters of γ-methyl N-benzyloxycarbonyl-L-glutamate;[327] N-trimethylsilylation of pyroglutamic acid with Me_3SiCl allows N-p-nitrobenzylation to be accomplished (but at 150°C);[328] decarboxylation of γ-carboxyglutamic acid by guanidine, a model for the possibility that arginine residues in γ-carboxyglutamic acid-containing proteins, may control the number and position of such residues.[329] A surprising result has emerged from attempted transamination of β-fluoroaspartic acid using pyridoxal 5'-phosphate, where dehydrofluorination occurred instead;[330] the possibility of carboxy-group assistance needs to be excluded by comparative studies with other β-fluoro-α-amino acids.

The basic amino acids are represented by lysine (further studies by Tyihak's group on the mechanism of N^{ϵ}-formylation by condensation with formaldehyde,[331] oxidative conversion into α-ketoglutaric acid catalyzed by saccharopine dehydrogenase and involving the NAD to NAD(H) reaction[332]), hydroxylysine [oxidation by HIO_4 to glutamic acid via the semi-aldehyde, but to Δ^1-pyrroline-5-carboxylic acid by $NaIO_4$ in alkaline conditions,[333] and N-protected α,ω-di-amino acids (RuO_4 oxidation to give the protected α-amino acid ω-amide[334])]. Controversy surrounding the identification of canavanine in alfalfa extracts was initiated some years ago[335] on the basis that the colour reaction on cellulose t.l.c. with pentacyanoammonioferrate was due to histidine instead; it has swung back to refutation in favour of the initial claim.[336] It seems that there is a substantial quantity (8 - 20 g Kg^{-1}) of the toxic amino acid in this plant.[336]

6.4 Non-enzymic Models of Biochemical Processes involving Amino Acids.- Some of the preceding Sections contain topics that could have been located here, too, but their major interest has appeared to lie in the techniques used. The binding of common amino acids to ^{14}C-thymine seems to be enhanced by γ-irradiation[337] and may involve a stacking interaction in the case of aromatic and heteroaromatic amino acids, as seen for tryptophan with uracil by hypochromic shifts and fluorescence characteristics.[338] Effects of glycine, β-alanine, and γ-aminobutyric acid[339] or glycine and glutamine[340] or amino acid amides[233] on the

stability of the DNA double helix have been determined by melting behaviour[233,340] or by c.d. measurements.[340]

6.5 Effects of Electromagnetic Radiation on Amino Acids.- Aromatic amino acids have been treated to vigorous irradiation: pulse radiolysis of 5- and 2,5-cysteinylDOPAs in aqueous solutions containing azide ions causing sequential semiquinone and quinone-imine formation,[341] and u.v. irradiation of aqueous solutions of phenylalanine, tyrosine, and tryptophan in the presence of nitrite or nitrate ions gives 'uncharacterized mutagens'[342] (a quite unacceptable piece of preliminary publication in its lack of information on its important claim).

Milder processes are involved in a study of fluorescence decay kinetics of L-tyrosine as a function of pH [343] and similar studies of homotryptophan.[344] In the latter study, the data were almost identical with those for tryptophan, bringing into question interpretations of the fluorescence behaviour of tryptophan based on assignment of lifetimes to structural states that involve interactions between the heteroaromatic moiety and ionic forms of the aliphatic moiety. A new method for fluorescence decay studies of tryptophan employs synchrotron radiation for pulsed excitation and photon-counting for decay kinetics.[345] More vigorous processes are described for photolysis of tryptophan under aerobic and anaerobic conditions at various pH values of solutions,[346] and an interesting finding that tryptophan radicals formed under irradiation can protect other species in the same environment - in other words, can act as endogenous antioxidants in vivo.[347]

An earlier Section, 6.1, refers to radioracemization of amino acids, a process that does not occur with amino acids crystallized from water by their γ-irradiation in the solid state but does occur for sublimed enantiomers of leucine.[348] These greatly differing quantum yields for decarboxylation through irradiation by non-polarized γ-radiation (D $\sim$ 2L $\approx$ DL) have been reported by a research group that has been active in the field for some time and adds another curious result that defeats explanation. It is significant that three papers reviewed in different parts of this Chapter[199,242,348] describe differing behaviour of solid amino acids as a function of their solid state; with the ever closer approach of chemistry and electrical properties of the solid state, the late 1980's and beyond are going to provide rich rewards to research workers who show the appropriate vigilance in these topic areas.

7 Analytical Methods

7.1 Gas-Liquid Chromatography.- The preparation of volatile derivatives introduces specific problems, and useful efforts continue to be made to eliminate sources of artefacts in these preparations. Heptafluorobutyroylation of arginine isobutyl ester, using a mixture of heptafluorobutyric acid and its anhydride, represents the standard technique of preparing the

most commonly used N and C derivatives. However, the area of the arginine peak in g.l.c. was less than expected, and two spurious peaks were seen.[349] The same workers[350] have described removal of glucose from plasma samples prior to g.l.c. analysis, by its enzyme-catalyzed conversion into glucose-6-phosphate. The same derivatives have been used in analysis of γ-carboxyglutamic acid,[351] and [1-^{13}C]leucine,[352] and the isopropyl ester analogue,[353] n-butyl ester analogue,[354] or ester analogue with (+)-2-butanol,[214] and N-trifluoroacetyl[354] and other perfluoroacyl[355] analogues. Pentafluorobenzoyl amino acid di-n-butylamides have been used for analysis of taurine in plasma at picomole levels [356] and similar applications.[357] Silylation procedures continue to be used occasionally, for analysis of asparagine and glutamine (dimethylsilylation after N- or O-t-butylation) [358] and analysis of selenomethionine.[359]

Mass spectrometric detection and identification of separated components has been used in a number of cases.[214,352,357,360]

Maillard reaction products formed between lysine and fructose, or lysine and lactulose, give hydrolysis products furosine (58) and pyridosine (59), according to g.l.c. analysis.[361]

Enantiomeric purity of amino acids can also be assessed by g.l.c., through methods that are now standard. Either the volatile derivative of the amino acid is separated into its enantiomers over a chiral stationary phase (N-pivaloylproline methyl ester over Chirasil-Val,[362] and a wide range of examples over chiral polysiloxanes of various types[363]) or the analysis sample is converted into a diastereoisomer mixture[364-366] (e.g. reaction of the D- and L-amino acid ester mixture into the N-trifluoroacetyl-L-prolyl derivative[366]). As was mentioned earlier in this Section, introduction of artefacts that cast doubt on the analytical accuracy can be anticipated in any derivatization procedure, and the inconsistent results reported in the last-mentioned study have been ascribed to partial racemization of the reagent, N-trifluoroacetyl-L-prolyl chloride, used in this case.[366] The conclusion is surprising, because the reagent would not be expected to undergo racemization even in the presence of triethylamine that is required for the process; however, the inconsistent results only arise at low D:L ratios (below 1:10).

7.2 Ion-Exchange Chromatography.- The small scope of this Section does not relate to the volume of data that is collected regularly under this heading, through a technique that is undergoing continuous instrumental development, merely that there is little of substantial chemical interest in the recent literature. Overlap with the later h.p.l.c. section should be borne in mind by readers seeking representative coverage.

General methodology is described using h.p.l.c. instrumentation and ninhydrin colorimetry;[367] other papers deal with specific analyses (di-aminopimelic acid reaching 2 nanomole levels [368] and glutamic acid - glutamine ratios with norleucine as internal standard [369]). A group of papers share a common interest in cysteine derivatives and near relatives: h.p.l.c.

ion exchange for homocysteine[370] and other routine studies (aminoethyl cysteine[371] and S-aminoethyl-3-mercaptolactic acid [372]) together with a study of the estimation of cysteine + cystine in proteins as 'cysteinoic acid' formed by hydrolysis of proteins in 2% dimethyl sulphoxide - 6M HCl.[373] The last-mentioned study follows earlier suggestions [374] and refers to the formation of cysteic acid; accompanying oxidation of tyrosine, serine, and methionine whether within the protein or after hydrolysis is avoided by adding phenol to the hydrolysis cocktail.

7.3 Thin-Layer Chromatography.- As for the preceding Section, small scope for this Section does not mean that the technique is in any way declining in its use.

Attention has been given to 2-dimensional t.l.c. identification of N^{π}-methylhistidine, since this runs close to abnormal metabolites present in physiological samples.[375] Estimation of free proline in mixtures has been based on isatin colour formation and estimation at 608 nm of the concentration from absorption data, for samples extracted from paper chromatograms.[376] T.l.c. of dansylamino acids on polyamide plates has been given thorough study,[377] and further data have also been secured for resolution of DL-amino acids by ligand-exchange chiral t.l.c. analysis.[378] The principle behind the latter study has a long history, since cellulose itself in the course of conventional paper chromatography and t.l.c. has the ability to separate enantiomeric pairs, but the newer ligand-exchange resolution procedure appears to offer the necessary flexibility to optimise particular analytical applications.

7.4 High-Performance Liquid Chromatography.- In contrast with the relatively static nature of the stage reached for g.l.c., ion exchange, and t.l.c. methods, there is still much development occurring in h.p.l.c. methodology and competition between the various alternatives in amino acid analysis. Six standard methods for h.p.l.c. analysis of amino acids have been compared,[379] and variables that influence the reliability of phenylalanine h.p.l.c. assays, in terms of the handling of samples prior to analysis, have been discussed.[380]

Procedures for amino acids in general (h.p.l.c. ion exchange,[381] isocratic elution employing aqueous copper(II) alkanesulphonates as solvent) and amino acids in particular are briefly cited here. NN-Dimethylglycine can be determined by h.p.l.c. ion exchange and specific detection using dimethylglycine dehydrogenase,[383] cysteine by amperometric detection at less than 4 picomole levels,[384] and S-adenosylmethionine and S-adenosylhomomethionine[385] tryptophan (either electrochemical[386] or fluorescence[387] detection), DOPA, and its m- and p-O-methyl derivatives (electrochemical detection[388]) and related catechol-based amino acids [389] have also been studied using standard methods. Aminohydroxyphenylalanine (a constituent of phaeomelanin),[390] [^{10}B]-p-boronophenylalanine,[391] O-phospho-L-serine, L-threonine, and L-tyrosine [392] and other phospho-amino acids[393] provide examples of specialized research interests.

Resolution based on the ligand-exchange principle employing an aqueous solution of copper(II) L-prolinate as stationary phase has been applied to aromatic amino acids,[394] [^{11}C]-DL-leucine and [^{11}C]-DL-tryptophan. The earlier Section 4.14 has included the point to be made concerning the very short half-life of the ^{11}C isotope, and preparation together with h.p.l.c. resolution took no more than 55 - 60 minutes for these labelled L-amino acids.[395] The classical chiral stationary-phase principle applied[396] to the separation of N-(3,5-dinitrobenzoyl)-DL-amino acids over (R)-N-(11-triethoxysilylundecanoyl)cyclohexyl-(6,7-dimethyl-1-naphthyl)methylamine; L-enantiomers are found to travel more slowly than their isomers in this h.p.l.c. system. The analogous process employing silicates carrying various L-valylamides has been developed further.[397] The remaining major h.p.l.c. resolution technique is represented by further studies of the use of Marfey's reagent,[398] prepared by reacting 1,3-difluoro-4,6-dinitrobenzene with one equivalent of L-alanine amide. DL-Amino acids treated with the reagent yield diastereoisomeric N-aryl-DL-amino acids that are found to be better for the purpose of resolving (RS)-β-leucine than long-standing alternatives such as diastereoisomer formation of the DL-amino acid benzyl ester with (-)-10-camphorsulphonic acid.[399]

As in the last few examples, h.p.l.c. methods have been applied most often to derivatized amino acids rather than to the amino acids themselves. This is related to raising a colour response of one sort or another so that high sensitivity can be achieved by the h.p.l.c. detector, or it may be because the amino acid derivatives have arrived in derivatized form in any case, as they would from a sequencing procedure, for example. Nevertheless, post-column derivatization continues to be favoured by some workers, and is mandatory for ion-exchange methods and the increasingly interesting ion-pair methods (for determination of hydroxyproline in tissue fluids, using ninhydrin as post-column reagent and monitoring at 440 nm,[400] for fully automatic routine amino acid analysis using dodecyl sulphate for pairing,[401] and for a broad range of amino acid analyses[402]).

Pre-column derivatization has been reported on, concerning dansyl-[403,404] and dabsyl-[404,405] amino acids, dinitrophenylamino acids,[406] phenylthiohydantoins,[407,408] and other derivatives arising from sequencing studies.[408] The o-phthaldialdehyde - thiol condensation products have become particularly widely used;[409] the method has been used to determine relative amounts of glutamic acid, pyroglutamic acid, and glutamine in samples [410] and in an interesting application in which N-acetylglutamic acid is separated from other materials by ion exchange and subjected to aminoacylase-catalyzed hydrolysis, then derivatized.[411] The h.p.l.c. of N-acyl-, N-alkoxycarbonyl amino and imino acids has been surveyed.[412] Other o-phthaldialdehyde - thiol derivatization studies include its coupling with electrochemical detection[413] rather than the usual fluorescence measurement, its use in an assay for N$^{\varepsilon}$-methyl

lysine,[414] and related studies,also including the use of 3-mercaptopropionic acid as the thiol component of the reagent,[415] and for the estimation of cysteinesulphinate, hypotaurine, and taurine.[416] Attempts are being made to improve this method and extend its scope, notably its sensitivity (use at 1.5 picomole level has been described[417]) and reproducibility. In the latter context, o-acetylbenzaldehyde has been found to give more stable isoindoles in the procedure as it is usually applied, in comparison with o-phthaldialdehyde. Further results will be welcome if they indicate that improved methodology can be established. A two-step procedure (iodoacetic acid followed by the o-phthaldialdehyde reaction) for the derivatization has been proposed,[418] though the benefits do not seem obvious. Bearing in mind that the o-phthaldialdehyde procedure is specific for amino acids, further studies have been described of a two-tier strategy that allows the unreacted imino acids to be determined after all the amino acids have been derivatized. This can be accomplished by the use of 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole in the case of proline and hydroxyproline, with 3,4-dehydroproline used as internal standard,[419] or by the use of 9-fluorenylmethoxycarbonyl chloride.[420]

Pre-column derivatization by phenyl isothiocyanate has been widely adopted and well supported. The essence of the method is the rapid mild conversion of amino acids into phenylthiocarbamoylamino acids and efficient h.p.l.c. analysis.[421]

7.5 Fluorescence Methods.- Introduction of specific fluorescence methods that have been tested through h.p.l.c. regimes has been announced for 5-pyrrolidone-2-carboxylic acid (derivatized with 4-bromomethyl-7-methoxycoumarin) [422] and for amino acids (derivatization with anthryldiazomethane).[423]

7.6 Other Methods.- Chromatographic methods that are not met in the amino acid context very often have been used for estimation of phenylthiohydantoins (electrokinetic chromatography, i.e. micellar solubilization and electrokinetic migration in a capillary)[424] and for determining binding constants for interaction of L-tryptophan with serum albumin (size exclusion chromatography).[425]

7.7 Determination of Specific Amino Acids.- Comprehensive coverage by the third edition of 'Methods of Enzymatic Analysis' continues to become available, the most recent Volumes including L-serine[426] and S-adenosylmethionine[427] (among many other amino acids). Other enzymatic assays appearing in the primary literature include homocysteine[428] and carnitine (in serum[429] as such, and in esterified form[430]). A rapid enzymatic method for the estimation of D-amino acids[431] employs D-amino acid oxidase and spectroscopic assay of the resulting keto-acids as their hydrazones.

Estimation of the relative amounts of lysine and lysinamide has been based on selective reaction of the former with furfural.[432] A radiometric assay has been reported for guanidinoxy-[^{14}C]-L-canavanine that allows the incorporation of [^{14}C]-canavanine into protein to be measured.[433]

Colorimetric methods are increasingly out of place for routine amino acid assay, beside the sensitive instrumental techniques described in the preceding pages. The red product formed between tyrosine and 4-aminophenazone and $NaIO_4$ in NH_4OH can be estimated spectrophotometrically (λ_{max} 470 nm), allowing the presence of this amino acid in hydrolysates to be determined within $\pm$1-5%.[434] A similar procedure for tryptophan (p-dimethylaminobenzaldehyde, λ_{max} 590 nm)[435] has featured in earlier volumes of this Specialist Periodical Report. Voltammetric[436] and enzyme electrode methods[437] have been described for estimation of tryptophan.

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2
Peptide Synthesis

BY I. J. GALPIN
Appendices compiled by
C. M. GALPIN

1 Introduction

In the past year a large number of syntheses have been reported employing both solution methods and solid-phase synthesis; in the latter case both polyamide- and polystyrene-based syntheses have been widely used.

Solution methods have generally been employed for the preparation of small peptides in large quantity, although the synthesis[1] of thymosin α_1, a 28-residue peptide, by the Hoffmann La Roche group and the 53-residue epidermal growth factor synthesis[2,3] are good examples of the preparation of large peptides by solution methods. In both cases fragment condensation played an important part in the synthesis.

Frequently, solid-phase synthesis is used to prepare peptides for immunological studies, and the development of the so-called 'Tea-bag approach'[4] allows the synthesis of, for example, 248 different 13-residue peptides in four weeks.

The Proceedings of the 9th American Peptide Symposium held in Toronto[5] and the Proceedings of the 23rd Symposium on Peptide Chemistry held in Kyoto[6] have been published, and as in previous volumes in this series no attempt has been made to review the collective proceedings of these meetings. A reference text entitled 'Amino acids and peptides'[7] has been published which provides an excellent reference for easy access to many peptides, proteins and amino acid derivatives. Compilations of lists of amino acid derivatives[8] and more unusual amino acid derivatives[9] have appeared, and these publications provide a useful addition to other sources of references.

This chapter follows the pattern established in earlier years and reference is made to most published papers in this field of research; however, only those of particular interest are discussed, with the remainder appearing in the Appendices.

2 Methods

2.1 Protective Groups

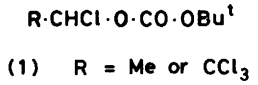
2.1.1 Established Methods of Amino-Group Protection

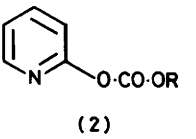
A general review has appeared[10] which is particularly directed towards organosilicon reagents, covering protection of a number of functional groups including the amino group.

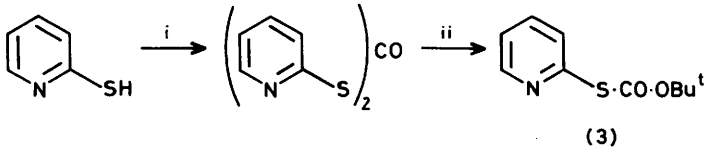
Both the butyloxycarbonyl and benzyloxycarbonyl protecting groups are widely used and it is therefore interesting to note that in a solvent titration study[11] it was found that employing butyloxycarbonyl group protection alleviated solubility problems whereas benzyloxycarbonyl protection tended to aggrevate problems. The self-association which was observed in dichloromethane could be disrupted by DMSO, DMF or HMPA. A point which relates to this is the observation that hydrophobic peptides containing, for example, a large proportion of valine or leucine tend to aggregate with the formation of β-sheet structure at the octapepide or nonapeptide level.[12,13] This β-sheet formation can be considerably disrupted by the introduction of α-aminoisobutyric acid, which tends to cause the formation of helical structures with the concomitant increase in solubility in organic media.

β-Sheet structure may also be disrupted by replacement of the NH group of amide bonds. In this context it is interesting to note that it has been found that the amide proton in the protected dipeptide Boc-Pro-Gly-OMe may be replaced by a butyloxycarbonyl group if the dipeptide is treated with 1.1 equivalents of di-t-butyl-pyrocarbonate in the presence of dimethylaminopyridine using acetonitrile as solvent.[14] This would allow the facile synthesis of peptides containing blocked amide bonds, which could possibly lead to increased solubility. Also, it is of interest that both amide protons of the primary amide benzyloxycarbonyl proline amide could be replaced with Boc groups; such butyloxycarbonyl groups could easily be removed by treatment with 33% trifluoroacetic acid in dichloromethane for 45 minutes.

Di-t-butylpyrocarbonate has become the most popular method of introducing the Boc group; however, efforts are still being made to find new reagents which provide a cheaper alternative to this compound. An example of this is provided by the synthesis of 1,2,2,2-tetrachloroethyl-tert-butylcarbonate, which is a compound of the general structure (1).[15] The compound is prepared by reaction of the corresponding chloroformate with

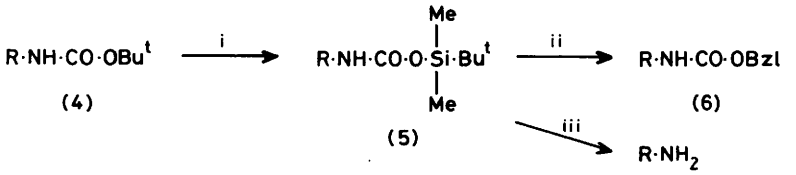






Reagents: i, $COCl_2/NEt_3$; ii, $Cu(OBu^t)_2$

Scheme 1



Reagents: i, $Bu^tSi(Me)_2 \cdot SO_2CF_3$/2,6-lutidine; ii, $(Bu)_4N^+F^-$/Bzl·Br; iii, F^-/H_2O

Scheme 2

tert-butanol. Compounds with R ▫ methyl and trichloromethyl were investigated, and it was found that the mixed carbonate in both cases was stable at room temperature for several years, being a medium boiling liquid. It was found that when R is a methyl group the compound is not particularly useful for introducing the Boc group; however, replacement with trichloromethyl gives a mixed carbonate which gives a much better yield of the corresponding Boc derivative, being comparable with di-t-butyl-dicarbonate.

Mixed carbonates of the type represented by structure (2) have been synthesised where R = tert-butyl or benzyl.[16] These mixed carbonates have been used for the introduction of both butyloxycarbonyl and benzyloxycarbonyl protection. A superior reagent (3) may be prepared by the route shown in Scheme 1. It is claimed that this mixed thiocarbonate gives higher yields than the corresponding carbonate.

A new method for the introduction of urethane protecting groups has been described[17] in which chloroformates are heated under reflux with the amino acid requiring protection in ethyl acetate. This Scheme requires no addition of base and it is claimed that good yields may be obtained in some cases. Reasonable yields were reported for the introduction of the trichloroethoxycarbonyl and allyloxycarbonyl protecting groups, but only moderate yields were observed with benzyloxycarbonyl.

A useful manipulation (Scheme 2) whereby butyloxycarbonyl groups may be converted into other N-alkoxycarbonyl groups has been described.[18] The transformation involves treatment of the Boc derivative (4) with tert-butyl-dimethylsilyl-trifluoromethane sulphonate in the presence of 2,6-lutidine. The resulting silyl derivative (5) is then treated with tert-butyl-ammonium fluoride and benzyl bromide to give the corresponding benzyloxycarbonyl derivative (6). Treatment of (5) with fluoride ion in water results in the liberation of the free amino group, thus providing a new means of cleaving the Boc protecting group. Conversions to a number of alkoxycarbonyl derivatives are described and yields are generally in the region of 80%.

The acid-labile 2-nitrobenzene-sulphenyl protecting group has been widely used in peptide synthesis; however, the reagent used for its introduction, 2-nitrobenzene-sulphenyl chloride, is known to be a little unstable and to undergo decomposition. As a means of combating this problem 2-nitrobenzene-sulphenyl saccharin (7) has been prepared.[19] The reagent (7) is easily prepared by reaction of sodium saccharide with the corresponding

sulphenyl chloride. The reagent may be easily crystallised and is stable for an extended period. The Nps derivative of an amino acid is prepared by reaction of the reagent (7) with the amino acid in the presence of sodium hydroxide, dioxan being used as the solvent.

The properties of the 9-fluorenyl-methoxycarbonyl protecting group have been investigated[20] and an X-ray structural analysis has indicated that the bond length from the sp^3 methylene carbon to oxygen is somewhat longer than one would normally anticipate, and this may be an explanation for the fact that the protecting group may sometimes be removed by hydrogenolysis in the presence of palladium. The preferred conformations and modes of self-association of such derivatives were also studied.

2.1.2 New Methods of Amino-Group Protection

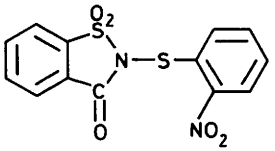
The cinnamyloxycarbonyl group has been proposed[21] as it may be removed under rather specialised conditions. The protecting group is introduced by reaction of the reagent (8) with an amino acid in the presence of triethylamine, water or aqueous DMF being used as a reaction medium. The protecting group is cleaved by treatment with $Pd(Ph_3P)_4$ in the presence of formic acid, pyridine and hydroxysuccinimide with THF as solvent. Heating under reflux cleaves the protected group, and the benzyloxycarbonyl and butyloxycarbonyl protecting groups are claimed to be stable under these conditions. In the absence of HONSu a π-allyl complex is formed, which may give rise to cinnamyl cations. Thus, the hydroxysuccinimide is added as a scavenger. The 2-(triphenyl-phosphonium)-1-methyl-ethoxycarbonyl protecting group has been developed.[22] This protecting group and two related protected groups indicated in structure (9) have been evaluated as modifications of the 2-(triphenylphosphonium)ethoxycarbonyl protecting group. The protecting group of type (9a) is more stable than the Peoc group by a factor of 4. These protecting groups are readily removed at pH 8 by treatment with sodium bicarbonate in aqueous methanol. The protecting groups of general type (9) do not suffer from an observed defect which is found with the Peoc group, which is the addition of the liberated amino group to the alkene which is formed on β-elimination during deprotection. The protecting group does suffer from a minor disadvantage, however, in that it contains an asymmetric centre and thus all L-amino acid derivatives would be diastereomers.

Phosphinic acid derivatives have been studied in detail as alternatives for amino-group protection.[23] A number of phosphinamides based on the unit (10) have been studied, including systems which contain identical or different R groups. The derivatives are generally acid labile and the diphenylphosphinyl protecting group is readily cleaved by six equivalents of methanol in HCl. The dimethyl derivative is the most labile, being cleaved at approximately 3.5 times the rate of the corresponding diphenylphosphinyl system, whereas the diethylphosphinyl group is cleaved five times more slowly than the diphenylphosphinyl group. These protecting groups should provide a viable alternative for butyl-based protecting groups as on cleavage they do not give rise to cations which can take part in deleterious side reactions.

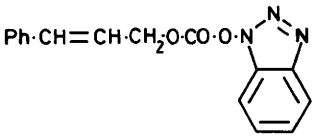
2.1.3 Carboxyl Protection

A simple mild esterification procedure for use with N-protected amino acids has been developed.[24] In this procedure N-protected amino acids are reacted with a chloroformate in the presence of triethylamine. The resulting mixed carboxylic/carbonic anhydride of general structure (11) is then treated with a catalytic amount of dimethylaminopyridine. This results in loss of carbon dioxide and conversion to the corresponding ester. The method has been used for the preparation of methyl, ethyl, benzyl and nitrophenyl esters; however, it was found to be unsatisfactory for trichloroethyl esters. Both benzyloxycarbonyl and butyloxycarbonyl amino acids were studied. A simple method for the preparation of long-chain alkyl esters of amino acids has been published.[25] In this procedure the amino acid is stirred as a melt with methane sulphonic acid in the presence of a long-chain alcohol, such as octadecanol, the resulting ester being obtained in yields ranging from 40 - 90%. The reaction between amino acids and alkyl p-toluene sulphonates has also been investigated,[26] and it was found that the reaction between amino acids and alkyl p-toluene sulphonates in alcohols proceeded by general acid-catalysed esterification, the acid being produced by alcoholysis of the corresponding toluene sulphonate. It was found that the reaction proceeded by general acid catalysis and that the contribution from transesterification was a minor one.

A new method has been developed for the preparation of diphenylmethyl esters of N-protected amino acids.[27] In this



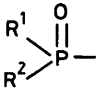
(7)



(8)

$$PhR^1R^2\overset{+}{P}\cdot CH_2\cdot CHMe\cdot O\cdot CO-$$

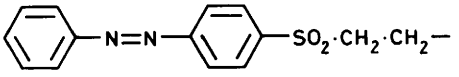
(9) a; $R^1 = R^2 = Ph$
b; $R^1 = R^2 = Me$
c; $R^1 = Me,\ R^2 = Ph$



(10)

$$R^1CO\cdot O\cdot CO\cdot OR^2$$

(11)



(12)

$$Me\cdot O\cdot CH_2\cdot CH_2\cdot O\cdot CH_2-$$

(13)

procedure the N-protected amino acid is reacted with diphenyl-diazo-methane which is formed in situ from benzophenone hydrazone by reaction with phenyl iodine (III) diacetate, which acts as an oxidant. This reagent is preferred to the use of peracetic acid or other oxidants as it does not seem to give rise to side reactions.

Carboxamidomethyl esters have been used in the synthesis of model peptides,[28] these esters being prepared by reaction of the N-protected amino acid with chloroacetamide in the presence of caesium carbonate. The carboxyamidomethyl esters are stable during hydrogenolysis or treatment with trifluoroacetic acid or diethylamine and can be easily removed by treatment with 0.5 molar sodium hydroxide. They cannot, however, be selectively removed in the presence of other alkali-labile ester protecting groups.

A number of other carboxyl protecting groups have been used in rather specialised applications, including a 2-thiosulphato-ethyl protecting group,[29] which has been used to increase solubility in aqueous media for use with chymotrypsin-catalyzed coupling.

The coloured C-terminal protecting group (12) has been used in the synthesis of haemagglutinin fragments,[30] the protecting group providing a useful coloured handle which aids monitoring by h.p.l.c. The 2,6-dimethoxybenzyl ester protecting group has also been used,[31] as it may be removed under mild oxidative conditions. Oxidative removal may be achieved by treatment with 2,3-dichloro-5,6-dicyanobenzoquinone or ceric ammonium nitrate.

2.1.4 Side-Chain Protection

Allyl esters have been suggested[32] for the temporary protection of the ß-carboxyl function of aspartic acid. The ester function may be introduced using allyl bromide in the presence of caesium carbonate and may be removed by treatment with palladium or rhodium complexes under acidic or neutral conditions. When this protecting group is used no aminosuccinyl formation occurs during deblocking and the protection is orthogonal to both benzyl-oxycarbonyl and butyloxycarbonyl protecting groups. Removal, using Tris-triphenyl-phosphine rhodium chloride in ethanol/water at 70°C or tetrakistriphenylphosphine palladium in the presence of morpholine or dimidone, is achieved rapidly.

The 2-methoxyethoxymethyl (Mem) protecting group (13) has been used for protection of the hydroxyl function of hydroxyproline and serine.[33] The protecting group is introduced by reacting the corresponding chloride with the amino-protected amino acid ester in the presence of di-isopropyethylamine over five hours. The Mem protecting group is considerably more stable towards TFA than tert-butyl ether protection, as it requires two hours to achieve complete removal rather than the five minutes which is required for the removal of tert-butyl protection. The protecting group is, however, completely stable to hydrogenolysis over palladium, thus allowing removal of benzyloxycarbonyl protection in the presence of this group.

The tert-butyloxycarbonyl group has also been used for the protection of hydroxyl functions, and it has been noted that when the protecting group is introduced by reaction with di-tert-butyl-di-carbonate yields can be considerably increased by employing potassium carbonate in the presence of 18-crown-6.[34] This procedure may also be used for protection of thiols.

In a synthesis of human calcitonin gene related peptide the 1-adamantyl group was used for the protection of cysteine.[35] It was noted that during the synthesis no sulphoxide formation occurred and that the protection could be smoothly removed by treatment with trifluoromethane sulphonic acid/thioanisole/trifluoroacetic acid or by treatment with thallic trifluoroacetate at $0^{\circ}C$ over one hour.

A novel regime for the protection of thiols and their reliberation is illustrated in Scheme 3.[36] The urethanyl N-methyl-γ-aminobutyric acid derivative (14) is reacted with a suitable cysteine derivative (15), for example benzyloxycarbonyl cysteine methyl ester, to give the thioester (16). Formation of the thioester (16) from the component compounds (14) and (15) could not be achieved using dicyclohexylcarbodiimide or active ester coupling, but was satisfactorily achieved using a symmetrical anhydride. The protecting group may be cleaved by treating the thioester with an acidic reagent to remove the urethane protecting group X, thus liberating a salt (17) which on neutralisation cyclises with the expulsion of the thiol. It is possible, however, that such a protecting group would suffer from a disadvantage in that the thioester (16) would be susceptible to attack from other nucleophiles during synthetic manipulation.

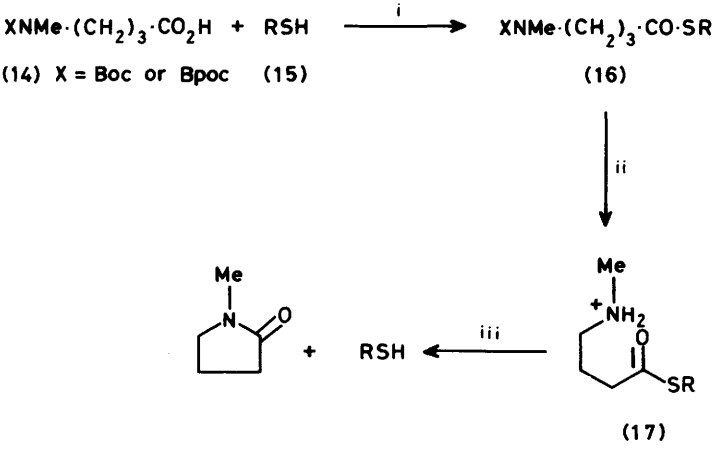
The carboxamide group of asparagine and glutamine is not

frequently protected, although often insolubility may arise because of the presence of free carboxamide groups. This insolubility may be considerably reduced by protection of the carboxamide, and recently the protection with urethane-type protecting groups has been described.[37,38] In a novel reaction bis-urethane protected ornithine or diaminobutyric acid may be converted to a carboxamide-containing derivative by treatment with ruthenium tetraoxide. The ruthenium tetraoxide, which is generated by reaction of ruthenium dioxide hydrate with 10% aqueous sodium metaperiodate, brings about oxidation of the terminal methylene group to a carbonyl function; thus the terminal urethane-protected amino group is converted to a urethane-protected carboxamide. In the case of butyloxycarbonyl protection, deprotection may be achieved by treatment with trifluoroacetic acid. Standard coupling methods may be used for coupling urethane-protected carboxamide derivatives and no side reactions or racemisation are observed.

In work on CCK-octapeptide sulphate the incorporation of tyrosine sulphate was studied.[39] It was found that the sodium salt of tyrosine sulphate could be directly incorporated or that the tyrosine residue could be sulphated after incorporation. Sulphation was investigated using the pyridine/SO_3 complex, DCCI/sulphuric acid and pyridinium acetyl sulphate. All gave the required product; however, pyridinium acetyl sulphate gave the best yield with a minimum of side reactions. The use of DCCI-mediated O-sulphation, using sulphuric acid in dry DMF, has also been studied by other workers.[40] In addition to DCCI they also studied water-soluble carbodiimides and their work indicates that all hydroxy groups are converted to O-sulphates and all thiol functions are converted to S-sulphonates; typtophan showed no reaction. The method has been used for radiolabelling with ^{35}S-sulphuric acid and when peptides are labelled in this manner trifluoromethane sulphonic acid can also be used to control the acidity of the medium.

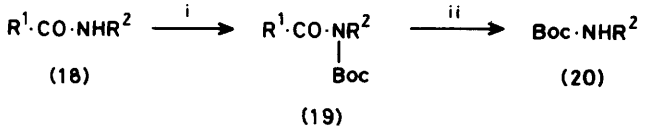
2.2 General Deprotection

The use of trifluoromethane sulphonic acid for the removal of benzyl-based protecting groups has now become widely used. Several papers which use trifluoromethane sulphonic acid in combination with thioanisole and trifluoroacetic acid have been published, including those on human GIP,[41] Neuromedin B and C [42]



Reagents: i, symmetrical anhydride; ii, HCl/dioxan or TFA/anisole; iii, organic base

Scheme 3



Reagents: i, Boc_2O/DMAP; ii, nucleophile, *e.g.* $NH_2·NH_2$

Scheme 4

and human gastrin releasing peptide (hGRP).[43] The general use of methane sulphonic acid and trifluoromethane sulphonic acid in the removal of a number of protecting groups is discussed in a review on brain, gut and skin peptides;[44] in this review removal of 2,4,6-trimethylbenzene sulphonyl protection from arginine and tryptophan is also considered. The use of trifluoromethane sulphonic acid in combination with meta cresol and ethanedithiol is discussed in a paper on Galanin.[45] In this work the 2,4,6-trimethylbenzene sulphonyl group is again used for the protection of tryptophan and it is claimed that alkylation of indole is suppressed. In this work and in the review mentioned above,[44] it was noted that the cycloheptyl ester function was used for the protection of the side-chain carboxyl group of aspartic acid and under the deprotection conditions mentioned β-peptide formation was minimised.

Frequently methionine is protected as its sulphoxide and deprotection needs to be carried out at the end of the synthesis, and phenylthiotrimethylsilane[43] or selinophenol[46] have been used for this purpose. In the case of phenylthiotrimethylsilane, tetrabutylammonium bromide was found to act as a catalyst.

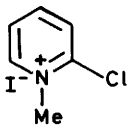
The use of transition-metal complexes for catalysing the removal of allyl esters has been mentioned above,[32] and in a similar application palladium has been found to catalyse the removal of the allyloxycarbonyl group from allyl carbamates.[47] The removal, which is carried out in formic acid, is highly efficient and no racemisation is observed.

A novel method for the removal of formyl, acetyl and benzoyl groups from amides has been published.[48] The method involves reaction of the protected amine (18) with di-t-butyl-dicarbonate in the presence of dimethylamino pyridine as indicated in Scheme 4. The resulting Boc derivative (19) is then reacted with a nucleophile such as diethylaminoethylamine, morpholine or hydrazine, giving the Boc derivative (20) as the product.

Several enzymes have recently been isolated which are able to hydrolyse urethane linkages; thus it has been found that benzyloxycarbonyl protection may be removed by the action of a urethane hydrolase.[49-51] The butyloxycarbonyl protecting group may on some occasions also be removed.[51]

2.3 Formation of Peptide Bonds

Many new reagents and methods have been reported for the



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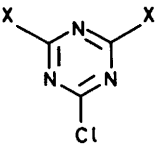

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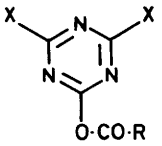

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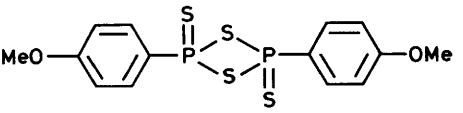

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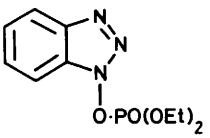

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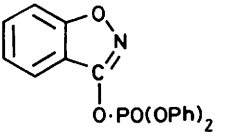

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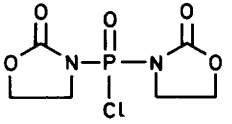

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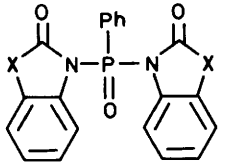


(28) X = O or S

synthesis of peptide bonds. The use of symmetrical and mixed anhydrides remains very popular, and reagents for use in a 'one-pot' context are also widely employed, active esters generally being the least popular method.

2-Chloro-1-methyl-pyridinium iodide (21) has been used as a coupling agent in peptide synthesis.[52] The reagent may be used in a 'one-pot' context and racemisation may be suppressed by the addition of hydroxysuccinimide. Presumably the reagent reacts by nucleophilic attack of the carboxylate at the two position of the pyridinium ring.

2-Chloro-4,6-disubstituted-1,3,5-triazines (22) have also been employed as condensing agents.[53] It has been known for some time that trichlorotriazine reacts with carboxylic acids to give acid chlorides; however, when two of the chlorine atoms are replaced with either methoxy or ethoxy groups activated intermediates of the type shown in (23) are obtained. Here again nucleophilic attack at the activated chlorine atom provides the driving force for the reaction. The intermediate (23) may be used to prepare esters, amides or anhydrides; thus the reagent 2-chloro-4,6-dimethoxy-1,3,5-triazine gave yields of di- and tripeptides of between 80 and 90%. Purification is facilitated as the weakly basic triazole ring allows extraction into aqueous acid. No racemisation was detected in the Anderson or Izumiya test, and the similarity between the intermediate (23) and O-acyl ureas which are intermediates in dicyclohexylcarbodiimide coupling was noted.

Racemisation levels using Lawesson's reagent (24) have been studied by h.p.l.c.,[54] and it was found that in 2+1 fragment condensation racemisation was very low, being in the order of 0.1%.

Benzotriazol-1-yl diethyl phosphate (25)[55] and the related 1,2-benzisoxazol-3-yl diphenyl phosphate (26)[56] have been synthesised. Both reagents can be used in a one- or two-step procedure for the preparation of amides and esters. The Young's test for racemisation showed that the reagent (25) gave no racemisation in DMF at $0^{\circ}C$; the reagent (26) also showed low racemisation but no stringent racemisation tests were employed.

The related phosphorous-containing compounds bis(2-oxo-3-oxazolidinyl)phosphinic chloride (27),[57] 3,3'-(phenylphosphinylidene)-bis(2-(3H)-benzoxazolone) (28a)[58] and 3,3'-(phenylphosphinylidene)bis-(2-(3H)-benzothiazolone) (28b)[58] have been prepared. These compounds were all effective coupling reagents, and compound (27) was found to be particularly useful for coupling

N-alkylated amino acids giving the minimum racemisation. The reagent (27) was used in combination with a variety of bases, but triethylamine and diisopropylethylamine gave products with the highest optical purity.

The pivaloyl mixed-anhydride method has been used for many years; thus it is of interest to note that it is claimed[59] that the reagent may be used for the introduction of hydroxyamino acids with free hydroxyl groups without complicating side reactions. Dialkyl-pyrocarbonates have been reported as being useful as activating agents for the formation of peptide and ester linkages.[60,61] Di-tert-butyl-pyrocarbonate was found to be particularly useful when used in the presence of pyridine and gave products with high optical purity.

The factors which influence urethane formation during mixed anhydride couplings have also been investigated.[62] In this work urethane formation during peptide-bond formation was studied by proton n.m.r. In a straightforward mixed-anhydride procedure using isobutyl-chloroformate for activation with N-methyl morpholine as the base, coupling of Boc-Val-OH to H-Pro-OBzl gave material which contained up to 60% urethane. If, however, the active ester is generated in the presence of hydroxybenzotriazole the amount of urethane formed is dramatically reduced and in the case mentioned above the required dipeptide now becomes the major product (80%). Similar results were observed when Boc-Val-OH was coupled to larger peptides. As hydroxybenzotriazole lowers the pH of the reaction medium additional N-methyl morpholine needs to be added in order to maintain a pH at which the coupling proceeds satisfactorily.

A related study[63] of factors affecting urethane formation and racemisation has been carried out, employing diphenylphosphinic mixed anhydrides. In a test coupling benzyloxycarbonyl glycyl phenylalanine was coupled to proline methyl ester, the products being monitored by proton n.m.r. As one would anticipate, the diphenylphosphinic mixed anhydride showed no 'wrong-way' opening, and it was found that the levels of racemisation encountered were comparable with couplings carried out using isobutylchloroformate, employing dichloromethane or DMF as solvent. Diphenylphosphinyl chloride gave the highest yields when used in combination with DMF as solvent, and it was noted that hydrophobic peptides were formed in particularly high yield.

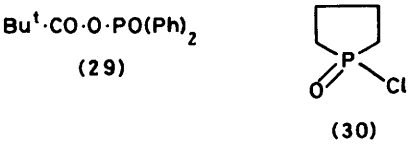
In other studies on the diphenylphosphinic mixed anhydrides[64,65] it was confirmed that phosphinic/carboxylic mixed

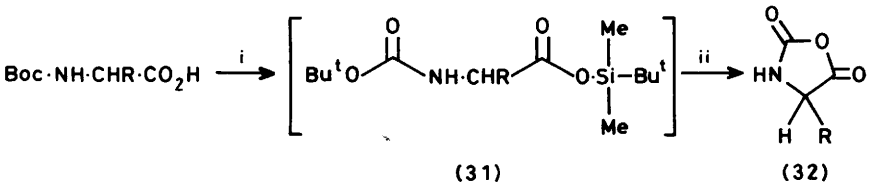
anhydrides, for example (29), showed no tendency to disproportionation, as indicated by ^{31}P n.m.r. The mixed-anhydride formation was instantaneous and acylation was rapid. Regioselective opening was always observed and a highly ordered transition state was indicated. This work[65] indicated that non-polar solvents were favoured and acylation was slower in DMF than in ethyl acetate; bulky side chains such as isopropyl were found to speed up the reaction. Diphenylphosphinyl chloride and the phospholane (30) were both satisfactory for peptide synthesis, although use of the diphenylphosphinyl chloride was preferred.

Two new routes to N-carboxy anhydrides have been published. The first[66] involves warming the amino acid in question with trichloromethyl chloroformate in THF. This known reaction involves decomposition of trichloromethyl chloroformate to phosgene in the warm solution; this then reacts with the amino acid to give the N-carboxy anhydride. This decomposition reaction is generally slow, but it may be catalysed by the presence of activated charcoal; under these conditions the reaction is complete in 30 minutes at 55°C. In order to ensure complete conversion to the N-carboxy anhydride 40% excess trichloromethyl chloroformate needs to be used.

An alternative route to N-carboxy anhydrides[67] involves the reaction sequence shown in Scheme 5. Here the Boc amino acid is reacted with tert-butyl-dimethylsilyl chloride in the presence of triethylamine; this gives the intermediate (31), which on treatment with a few drops of oxalyl chloride in DMF gives the required N-carboxy anhydride (32). During the reaction both carbon dioxide and carbon monoxide are evolved, and the reaction is thought to involve the intermediacy of an acid chloride. This is particularly interesting as the reaction proceeds without any evidence of racemisation. The side-chain protected Boc-N-carboxy anhydrides of ornithine and diaminoproprionic acid were prepared by reaction with the corresponding bis-Boc derivative.

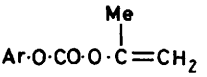
The use of acyl chlorides in peptide synthesis was also demonstrated in a rapid synthesis of thyrotropin releasing hormone.[68] In this work a urethane-protected amino acid dicyclohexylamine salt was treated for one minute with thionyl chloride in pyridine/dichloromethane. The resulting acyl chloride was then used to acylate the amino component in the presence of dimethylaminopyridine over twenty minutes. An excellent yield of product was obtained, although low yields were encountered if the

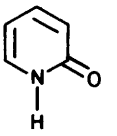


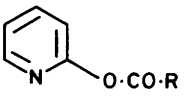


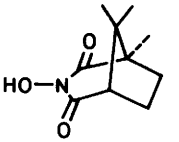
Reagents: i, NEt_3/Bu^{t}Si(Me)$_2$Cl; ii, COCl·COCl/DMF

Scheme 5









Boc-Leu$_3$-Aib·OH

(37)

H-Leu$_3$-OBzl

(38)

pyridine was omitted. It is interesting to note that only 6% racemisation was observed in the synthesis of Z-His(Bzl)-Pro-NH_2. In connection with the optical purity of such acid chlorides, it is worth noting that trifluoroacetyl amino acid chlorides have been used as chiral reagents in syntheses employing the Friedel-Crafts reaction.[69] In this work the trifluoroacetyl amino acid chloride was generated using oxalyl chloride and reaction with various benzene derivatives was carried out. In these reactions it was claimed that the configuration of the alpha-carbon atom was conserved.

Traditional active esters are still widely used and isoprenyl chloroformate has been recommended[70] for the preparation of mixed aryl carbonates for use in the preparation of active esters. Mixed carbonates of the type (33) were prepared in 90% yield by reaction of the sodium or potassium phenoxide with the chloroformate. The product was then reacted with a butyloxycarbonyl amino acid in the presence of N-methyl morpholine to give the corresponding active ester. A number of active esters were prepared in this way including nitrophenyl, 2,4-dinitrophenyl and 2,4,6-trichlorophenyl esters. 2-(1H)-Pyridone (34)[71] has been used to prepare active esters. The active esters were produced in this case by reaction of the N-protected amino acid with 2-(1H)-pyridone in the presence of DCCI and pyridine. These two pyridyl active esters gave good yields and high optical purity when used in coupling reactions. 2-(1H)-Pyridone was also used for the preparation of compounds of general structure (35), which were obtained by reaction with chloroformates in the presence of a base. Compounds of the general structure (35) were used to introduce the Boc, Z, and Adoc protecting groups.

An optically active active ester has been prepared from (+)-N-hydroxy-camphorimide (36).[72] When an optically pure amino acid ester of (36) was coupled with a racemic amino acid ethyl ester hydrochloride considerable enantioselectivity was observed. The yields were very variable, as was the degree of enantioselectivity, and the variability was found to depend considerably on steric factors.

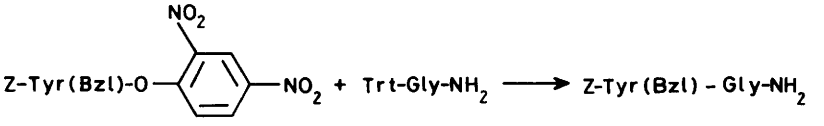
In the coupling of the protected peptides (37) and (38),[73] DCCI in the presence of para-nitrophenol was used as a coupling reagent. Initially it was assumed that the nitrophenyl ester was formed; however, in fact the coupling proceeded through a substituted 4,4-dimethyl-5-(4H)oxazolone. This oxazolone was rapidly formed due to the conformational effect of the terminal

amino isobutyric acid residue, and it was found that addition of HOBt catalysed the aminolysis by the amino component (38).

Couplings which may be carried out in the absence of tertiary bases have been investigated.[74,75] It has been known for some time that trityl and Bpoc protecting groups may be easily cleaved by acidic reagents such as trifluoroethanol. In this work use is made of the fact that deprotection may be achieved with the help of the carboxyl component or its activated derivatives; thus, the couplings shown in Scheme 6 could be carried out without prior deprotection, providing that trifluoroethanol was used as the solvent. The trifluoroethanol had the effect of cleaving the trityl group from the amino component (40) and the coupling with the activated ester (39) then followed immediately. Bpoc derivatives could be treated similarly, although ortho-nitrophenyl and hydroxysuccinimide active esters could not be used. Tetrazole (42) could also be used as an acidolic reagent,[45] cleavage of a trityl protecting group being shown in Scheme 7. The trityl derivative (41) is treated with tetrazole in trifluoroethanol to give the salt of the amino component (43); this salt can then be directly acylated by an active ester without further addition of tertiary amines. The tetrazole did not act as a catalyst in acylation, and hydroxybenzotriazole could be used as an additive in fragment coupling; however, the tetrazole could be used alone during the step-wise build-up of a fragment when urethane protection was employed.

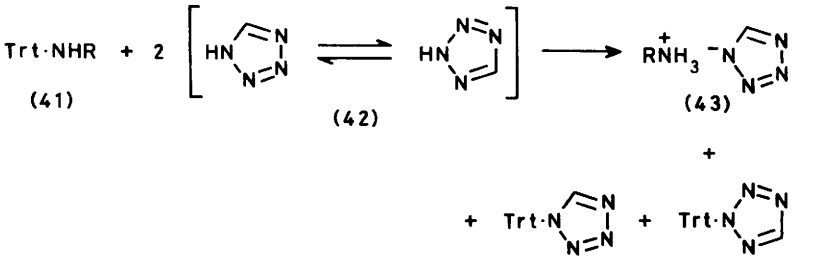
The use of dimethylaminopyridine as a catalyst in DCCI or water-soluble carbodiimide-mediated cyclisations has been investigated.[76] In DMF or dichloromethane using 20 equivalents of the diimide and 5 - 10 equivalents of dimethylaminopyridine, it was found that in the formation of cyclic pentapeptide analogues of thymopentin higher yields and purity could be obtained than when the azide method was used. This strong activation may lead to inversion at the carboxyl terminus when the linear peptide contains no D residue at the amino or carboxyl terminus.

Two reports of peptide-bond formation involving intramolecular reactions have appeared.[77,78] The essence of the first procedure is illustrated in Scheme 8; here the iminophosphorane (44), which is obtained from ethyl-2-azido-propanoate, may be reacted with benzoic acid to give the 5-co-ordinate phosphorous intermediate (45). This collapses to the acylated product (46). In this reaction the driving force is the intermolecularity of the reaction, and it shows little sensitivity to steric effects.

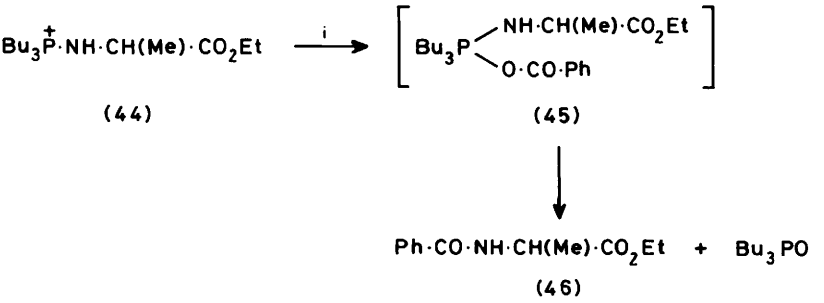


(39) (40)

Scheme 6



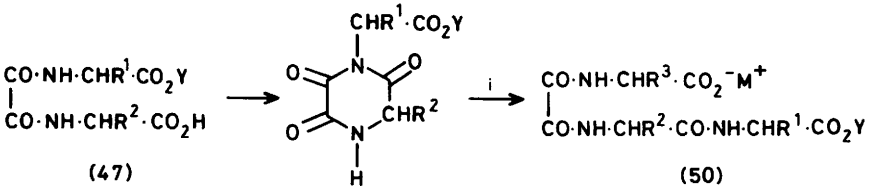
Scheme 7



(44) (45) (46)

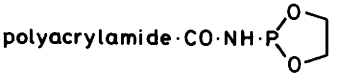
Reagent: i, $Ph{\cdot}CO_2H$

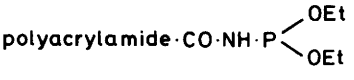
Scheme 8

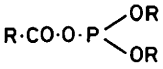


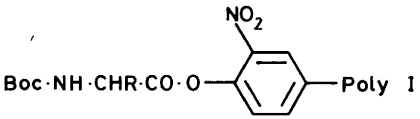
Reagent: i, $NH_2CHR^3 \cdot CO_2^- M^+$ (49)

Scheme 9









NH_2·peptide·Poly II

(55)

Boc·NH·CHR·CO·X

(56)

Peptides may also be formed by this procedure and the Anderson test shows that coupling by this method leads to no racemisation.

In a second intramolecular peptide-bond-forming reaction sequence (Scheme 9),[78] oxalyl derivatives of the type (47) may be converted into a cyclic intermediate (48) under the influence of acidic or basic catalysis. The derivative (48) may then be reacted with an amino acid (49) to give a new oxalyl derivative (50). This oxalyl derivative is of the same general type as structure (47) and thus may be used to propagate the sequence.

Polymeric N-acyl phosphoramidites of the structures (51) and (52) have been used in peptide-bond formation.[79] These reagents react with N-protected amino acids to give activated components of the type (53). The activated carboxyl component (53) may then react with an incoming amino component with formation of a peptide bond. The residual polymer may be regenerated by a reaction with a suitable chlorophosphite.

A mediator methodology which utilises two polymeric systems known as the 'Shadchan system' has been developed.[80] The method relies upon transfer of an N-protected amino acid from one insoluble polymer (the donor) to a polymeric acceptor by means of a soluble mediator X; this is achieved by reaction of an insoluble bank of active ester (54) with a soluble mediator X such as imidazole. This produces a soluble activated component (56) which is carried through the cyclic system and allowed to acylate the polymeric amino component (55). A stoichiometric supply of active ester may be ensured and the system is self-monitoring and allows a high yield of protected resin-bound peptide.

2.4 Racemisation

Many coupling procedures are now available which are able to minimise racemisation. The use of hydroxybenzotriazole and similar compounds as additives in fragment condensations is now routine and racemisation in such couplings is generally minimal. Recently isonitrobenzylnitrile, pentafluorophenol and the Lewis acids zinc chloride and zinc fluoride have been used to suppress racemisation;[81] racemisation was estimated in this case by application of the Anderson test.

The influence of imidazole on racemisation in active ester couplings has been studied.[82] It was found that the presence of imidazole in active ester couplings tended to increase racemisation and decrease the yields. In this context it was noted that there are no histidine active esters in the literature

in which the imidazole ring remains unprotected.

In a detailed study of racemisation during the coupling of bis-protected histidine active esters,[83] it was found that racemisation decreased with increasing electron withdrawal of the N^{τ}-protecting group. Several pentachlorophenyl esters of protected histidine derivatives were studied, and it was revealed that Z-His(Bzl)-OPcp showed racemisation even in the absence of base. In this case racemisation proceeded through both intra- and intermolecular processes. The relative rate of racemisation of trichlorophenyl active esters in THF is shown in Table 1.

TABLE 1 Relative rate of racemisation of trichlorophenyl active esters in THF

Z-Cys(Bzl)-OTcp	270.0
Z-Asp(OMe)-OTcp	19.0
Z-His(Bzl)-OTcp	7.8
Z-Phe-OTcp	6.7
Z-Glu(OMe)-OTcp	3.3
Z-His(Tos-OTcp	2.8
Z-His(Z)-OTcp	2.1
Z-Ala-OTcp	1

Racemisation of N^{τ}-substituted histidine derivatives was not particularly high and by far the most racemisation was encountered with a cysteine derivative. The rate of coupling decreased in the order OPcp>OTcp>ONp and was largest in polar solvents.

A racemisation test has been developed in which the trifluoroacetyl dipeptides (57) and (58) are coupled to the dipeptide (59).[84] The product of coupling is analysed by g.l.c. analysis on OV17. The test gave good results and DCCI coupling in the absence and presence of various additives was studied. The usual trends were found and HONb was found to be very similar to HOBt as an additive, being superior to HONSu.

In another g.l.c.-based racemisation test[85] trifluoroacetyl proline chloride is coupled to the isopropyl esters of amino acids (the amino acids being produced by acid hydrolysis of the peptide undergoing investigation). In this case the diastereoisomeric products are separated on a chiral g.l.c. column; in general good results were obtained. However it was noted that some racemisation of the trifluoroacetyl proline chloride occurred during the derivatisation. This is in contrast to the findings reported above.[68,69] One explanation may be that in this

racemisation test triethylamine was used during the coupling to the amino acid isopropyl esters.

A racemisation test based on reaction of amino acids with the reagent (60) has been reported.[86] Amino acids produced by hydrolysis are used to displace fluoride ion from the compound giving the possibility of diastereoisomers in the product. These diastereoisomeric products can be analysed by reverse-phase h.p.l.c. under well defined conditions. The aromatic ring contained in compound (60) gives rise to a high absorption coefficient and thus the method represents a very useful test of racemisation.

2.5 Repetitive Methods of Peptide Synthesis

The majority of solid-phase syntheses are probably still carried out on polystyrene-based resins, although considerable investigation of the nature of the linkage to the resin has been carried out. Simple benzhydrylamine resins have been widely used for the preparation of peptide amides, and in a synthesis of ovine corticotropin releasing factor[87] a 34% final yield was obtained after HF deprotection. At intermediate stages several of the Kaiser tests were not negative and so blocking with acetic anhydride was carried out. On several other occasions large excesses of the acylating components were used to ensure complete reaction. In another synthesis using the benzyhydrylamine resin[88] trifluoromethanesulphonic acid/thioanisole/trifluoroacetic acid was compared with HF/anisole for resin cleavage. Both methods gave a comparable yield after purification by preparative h.p.l.c.

The p-methyl-benzhydrylamine resin has been used for the synthesis of fragments of complement C3d [89] and Cercropin B.[90] In the latter paper it was found that the stability of the resin linkage was similar to the unmethylated resin and that the final rate of cleavage from the resin by the low-high HF procedure was increased.

Work on a multidetachable benzhydrylamine resin has been carried out.[91,92] The protected amino acyl resin (61) was prepared by reacting the phenolic compound (62) with a substituted carboxymethyl polystyrene using DCCI in the presence of dimethyl-aminopyridine. The Boc group is removed from the product, leaving a free benzhydrylamine function which is then coupled to the incoming amino acid. The resin is more chemically defined than the traditional benzhydrylamine resin and is not so prone to deleterious side reactions.

Tfa-Pro - Phe-OH

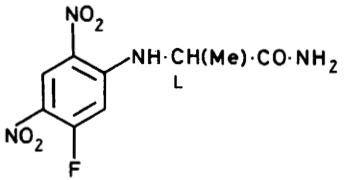
(57)

Tfa-Pro - Ala-OH

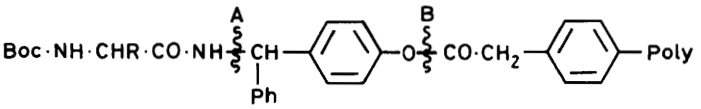
(58)

H-Val - Pro-OMe

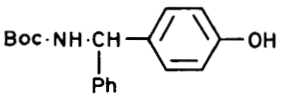
(59)



(60)



(61)



(62)

In an excellent synthesis of human gastrin I[91] a 60% overall yield from the first residue was achieved using this resin. This is particularly interesting, as it has been claimed by other workers that the gastrin sequence was inaccessible by procedures employing HF for deprotection. When the benzhydrylamine linkage shown in (61) was used, and deprotection was carried out by strong acid under S_N2 conditions (low-high HF deprotection), the difficulties which are usually associated with HF may be minimised. A number of varied HF procedures were tested and it was found that optimal conditions were obtained using a two-step deprotection, firstly treating with HF/dimethylsulphide/cresol/thiocresol (25:65:7.5:2.5), for two hours at 0°C, and secondly treating with hydrazine at 25°C followed by treatment with trifluoromethane sulphonic acid/trifluoroacetic acid/cresol (2.5:87.5:10) for one hour.

A benzhydrylamine linker of the type shown in (62) could clearly be used with other resins containing an acidic carboxyl group. Cleavage at A may be achieved by HF alone and cleavage at B may be achieved by treatment with sodium hydroxide in the presence of hydrogen peroxide at pH 10.6. Treatment with TFA/dichloromethane (1:1), followed by HF/DMS (1:1), followed by 5% hydrazine in DMF would produce the peptide bearing a benzhydrylamide at the carboxyl terminus. This can subsequently be removed by treatment with 1% trifluoromethane sulphonic acid in TFA. Such a resin clearly allows a considerable number of alternatives and permits purification after resin cleavage prior to final deprotection.

The 4-(aminoacyloxymethyl)phenylacetamidomethyl copolystyrene (1% divinyl benzene) resin has also been used on several occasions and a good example is provided by the synthesis of thymosin α_{11}.[93] In the synthesis of this 35-residue peptide by manual solid-phase peptide synthesis employing symmetrical anhydrides for coupling, an excellent product was obtained after final deprotection with anhydrous HF.

Syntheses of somatostatin[94] and [Glu_1] human gastrin 17[95] on a traditional solid-phase support have been reported. In these syntheses fragment condensations were used to build up the final product and Fmoc protection was used during the assembly.

In another solid-phase synthesis hydrophobic fragments of signal peptide were prepared.[96] In this case, however, cleavage from the resin was achieved by transesterification using anhydrous methanol in the presence of triethylamine, heating under reflux

for six to eight hours. Boc groups were removed subsequently with trifluoroacetic acid at 0°C and, following this, benzyl-based protection was removed from side chains using trifluoroacetic acid/meta-cresol/thioanisole.

Preparation of N-alkyl amides by solid-phase synthesis has also been explored,[97] using the modified resin shown in (63); ethyl and 1,1,1-trifluoroethyl substituents have also been investigated in place of the methyl group. It is claimed that the modified resin provides greater flexibility than when using aminolysis of a traditional benzyl ester linkage, although extended treatment with HF was needed to bring about complete cleavage.

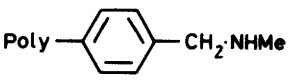
A three-dimensional orthogonal protection scheme for use with a modified polystyrene resin has been described.[98] The system depends upon the type of derivative shown in (64), which is prepared by reaction of a dithiasuccinoyl amino acid with tert-butyl-4-hydroxymethyl-3-nitrobenzoate in the presence of DCCI. The compound (64) is treated with trifluoroacetic acid to remove the tert-butyl group and the product subsequently coupled to an amino methyl polystyrene resin using DCCI. The chain may be extended by thiolytic cleavage of the dithiasuccinoyl group and on completion of the peptide assembly this method may be used for final cleavage of the protecting group. Acid-labile tert-butyl-based protection may be cleaved with trifluoroacetic acid and the resin linkage may be cleaved by photolysis at 350 nm. It was noted that at the dipeptide stage dithiasuccinoyl protection was superior to other methods as with tert-butyloxycarbonyl some diketo piperazine formation occurred, and with Fmoc protection this reaction was very rapid. The related resin (65) has been used in a preparative method for the actual synthesis of diketo-piperazines.[99] Dipeptides were assembled on this resin and then deprotected to give the trifluoroacetate of the resin-bound dipeptide. This was then treated with diisopropylethylamine, which brought about cyclisation of the free base to a diketo-piperazine in high yield.

Investigations into the attachment of Fmoc amino acids to polymers have been carried out. It was found[100] that Fmoc amino acids were best attached to a p-alkoxybenzyl alcohol resin by reaction with DCCI in the presence of hydroxybenzotriazole. When a low reaction temperature was maintained, dipeptide formation and racemisation were minimised. Fmoc amino acids could also be attached to polymers by the linker (66).[101] The Fmoc amino acid was esterified to the linker (66) by treatment with DMF/di-neopentyl

acetal. This ester could be purified and then coupled to an amino methyl support. It is claimed that this method is advantageous, as extra steps involving the intermediate deprotection of the handle are eliminated, also an additional methylene group converts what would be an acetyl group to a proprionyl derivative and in this case electronic factors increase the rate of acidolysis of the anchor bond by 2 - 3 fold; higher yields are also obtained.

Polyacrylamide-based solid-phase peptide synthesis is now well established and the linkage agent (67) has been used in a test synthesis of the (65 - 74) fragment of acyl carrier protein.[102] In this work Fmoc amino acid pentafluorophenyl esters were used in DMF using HOBt as a catalyst. Acylation was very efficient and final cleavage from the resin with 95% trifluoroacetic acid gave 96% cleavage. The product, which had a very reasonable h.p.l.c., was obtained in good overall yield, and low losses were encountered at each coupling step; in contrast the corresponding hydroxysuccinimide active esters were found to be less efficient, giving rise to more problems.

The protection of sulphydryl groups during solid-phase synthesis using Fmoc amino acids has been investigated.[103] In this work the acetamidomethyl, tert-butyl and tert-butylthio protecting groups were compared. They were all satisfactory as evidenced by the synthesis of a linear oxytocin, the choice between tert-butyl and acetamidomethyl being mainly influenced by the general hydrophobicity of the sequence in question. Selective disulphide-bond formation was found to be possible using the acetamidomethyl group in conjunction with the tert-butylthio protecting group, the latter being removed by the action of tributylphosphine. This combination of protecting groups was used to advantage in an elegant synthesis of Conotoxin G1.[104] In this synthesis selective disulphide-bond formation was required as aerial oxidation of the tetrathiol was not very satisfactory. One minor disadvantage of the ^{t}butylthio protecting group is that it may give rise to β-elimination; for this reason the acetamidomethyl-protected cysteine residue was introduced first in the presence of dimethylaminopyridine. Arginine was protected by the 4-methoxy-trimethylphenylsulphenyl protecting group, and histidine was incorporated as its bis-Fmoc derivative. After the incorporation of bis-Fmoc histidine, acylation was possible at each stage, although piperidine should eventually deacylate the imidazole function. Both acetamidomethyl and tert-butylthio protecting groups were stable to



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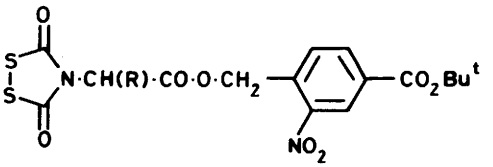

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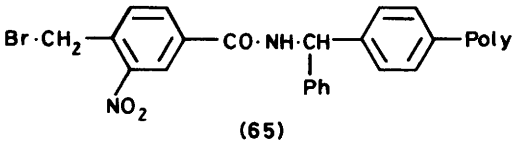
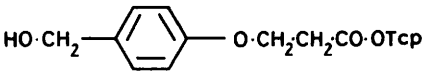


(65)



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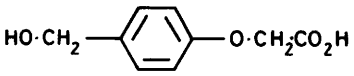
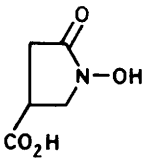


(67)

Br·CH$_2$·CO·NH·CH$_2$·CH$_2$·NH·CO·Poly

(68)



(69)

Boc-(Leu$_3$ - Pro$_2$ - Gly)$_n$-OH

(70) n = 1, 2, or 4

H-Phe-(soluble polystyrene)

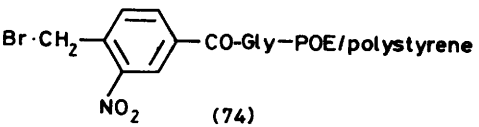
(71)

H-Leu $_3$ - Phe-(copolystyrene 1% DVB)

(72)

H-Leu $_9$ - Phe-(copolystyrene 1% DVB)

(73)



(74)

prolonged acidolysis; however, in the presence of 5% thioanisole deleterious deprotection of the cysteine side-chain protection occurred, the acetamidomethyl function being cleaved to the extent of 13% and the tert-butylthio group being 54% cleaved. Tributylphosphine was eventually used to remove the tert-butylthio protection, and after aerial oxidation at high dilution the remaining disulphide bond was formed by iodine oxidation.

In a synthesis of β-endorphin analogues containing a disulphide bond on a conventional polystyrene support[105] 3,4-dimethylbenzyl protection was used for cysteine, being cleaved by HF in the presence of anisole. Also, in a synthesis of human insulin-like growth factor II, ethyl carbamoyl was used for cysteine protection.[106] This protecting group was finally removed by treatment with hydrazine over 40 minutes, total deprotection being carried out in the usual way by treatment with hydrogen fluoride.

An ultra-high-loading gel for use in solid-phase peptide synthesis has been developed.[107] The resin, poly-N-(2-(4-hydroxyphenyl)ethyl)acrylamide may be used at near maximal loading, the solvated gel network consisting mainly of protected peptide. The method was used to prepare fragments of dynorphin and β-endorphin, the fragments being assembled by the use of diisopropylcarbodiimide, which was crucial to success. DMF/hydrazine was used for final resin cleavage, the resulting hydrazides being useful as intermediates in further fragment condensations.

A new phenacyl-type handle which may be used in many types of polymer-supported synthesis has been reported.[108] Coupling to the parent resin of bromopropionyl phenoxy acetic acid using potassium chloride or a caesium salt method gives a linker which may be cleaved by photolysis or by the action of nucleophiles.

A polyacrylic resin with a glycolamide linker of the type (68) has been evaluated in a synthesis of histone H4 (14 - 21).[109] The first Boc amino acid may be attached as its cesium salt and subsequent couplings may be performed by symmetrical anhydrides using intermediate TFA deprotection with diisopropylethylamine as the base; cleavage may be carried out by methanolysis in the presence of triethylamine. It is claimed that the resin is superior to the traditional benzhydrylamine resin, although some losses were observed at the dipeptide stage, presumably arising from diketopiperazine formation. This could be minimised by the careful use of diisopropylethylamine as the base, and relatively clean products were observed on h.p.l.c.

A p-nitrobenzophenone oxime polymer has been used for the construction of an amphiphilic peptide model for the fragment of apolipoprotein A1.[110] Small fragments could be cleaved from the resin and then re-added as fragments, purification being permitted at intermediate stages. Cleavage from the resin was effected by treatment with N-hydroxypiperidine, which gave the corresponding ester, which was then treated with zinc in acetic acid for one hour to give the corresponding carboxylic acid.

A related 'active ester' resin has been prepared by reaction of 1-hydroxy-5-oxo-3-pyrolidine carboxylic acid (69) with an amino methyl polystyrene polymer.[111] Linkage to the polymer was achieved using DCCI and unreacted amino methyl groups were acetylated to prevent side reactions at a later stage. Acetylation of the N-hydroxy group also occurred, but this could be cleaved by treatment with an amine. The resin was loaded by reaction of a Boc amino acid with DCCI in the presence of HOBt, and the resulting Boc amino acid polymer could then be reacted with the amino component. The resulting dipeptide is obtained in solution and the polymeric N-hydroxy polymer may be used in subsequent activation cycles for the addition of other amino acids.

Work on liquid-phase peptide synthesis, in which the supporting polymer remains soluble, has been continued. The peptide (70)[112] was coupled to the soluble polystyrene-bound amino component (71), or the amino component (72) or (73), which were bound to the insoluble co-polystyrene cross-linked with 1% divinyl benzene. With the soluble polymer, coupling efficiency decreased very little as n was increased in compound (70); however, with the cross-linked insoluble amino components (72) and (73), coupling efficiency was considerably retarded. This was attributed to the fact that the permeability was severely impaired and the fact that β-sheet formation was occurring.

A graft co-polymer of polyoxyethylene/polystyrene (74) has been prepared in the hopes that the benefits of the polyoxyethylene liquid-phase approach may be carried over to the manipulations involved in traditional solid-phase synthesis.[113] The bromomethyl-nitrobenzoyl-linked polyoxyethylene/polystyrene graft co-polymer was used for the preparation of insulin B(21-30). The peptides were assembled by standard methods and could be cleaved by photolysis in a methanolic suspension over 22 hours. It was found that manipulations were easier using the graft co-polymer than when the straightforward liquid-phase approach was used, and in general the approach was similar to that employed with a polystyrene

support, except that in alcoholic solvents considerable swelling of the resin was observed.

A general method for the rapid solid-phase synthesis of large numbers of peptides was mentioned in the Introduction.[4] The method involves sealing portions of the support resin into polypropylene bags. The bags are then labelled and subjected to the first coupling cycle. After completion of the first cycle modification may be made by removing some of the bags before running the second cycle; the bags would then be recombined and other permutations made during subsequent stages of further coupling cycles. By this method 10 - 20 mgs of, for example, 248 different thirteen-residue peptides were assembled in four weeks using a polystyrene support. Final cleavage was carried out in the traditional manner with hydrogen fluoride in the presence of anisole. The average purity of product was 84% although the range extended from 70 - 94%. The method allows easy preparation of many peptides with closely similar sequences which can be used in investigations of antigen-antibody interactions.

Analytical control during solid-phase peptide synthesis has been studied by using Fast Atom Bombardment (FAB) mass spectrometry.[114] It was found that the resin could be analysed whilst still bound to the support and that many of the characteristic fragments that would be observed for the free peptide could be observed.

A solid-phase Edman approach has also been used for the analysis of resin-bound peptides.[115] The phenylthiohydantoin derivatives of many protected amino acids were synthesised and characterised and several model peptides were analysed whilst still bound to the resin.

2.6 Enzyme-Mediated Synthesis and Semi-Synthesis

A review of the use of proteolytic enzymes in peptide-bond formation has been published.[116] This work describes synthesis of aspartame, enkephalin, dynorphin, insulin and eledoisin.

The most widely used enzyme is probably chymotrypsin and several publications describing its use have appeared.[117-121] Factors influencing the kinetics such as nucleophile concentration and temperature[118] and pH and solvent dependence[119] have been reported. Both elastase and chymotrypsin were used in work connected with the synthesis of oxytocin fragments (1 - 6) and (7 - 9).[121] Both elastase and chymotrypsin were studied, and it was confirmed that no side-chain protection is required and

that no racemisation is observed during coupling; products were analysed by h.p.l.c. The concentration of amino component appears to be critical and 50% molar excess of the amino component gives optimal yields. Methanol and DMF may be added as cosolvents and up to 50% of the organic solvent may be used. In the work on the synthesis of the oxytocin fragments,[121] benzyloxycarbonyl and butyloxycarbonyl protection were both used and carboxy groups were protected as the ethyl ester, phenyl hydrazide or amide. Coupling between Boc-Ile-OH and H-Gln-NH-NH-Ph gave a poor yield with most of the enzymes; this is believed to be due to the fact that the isoleucine derivative is a poor substrate and is not accommodated well at the Pl' site of most proteases. Ethyl ester protection was, on some occasions, removed by treatment with papain whilst attempting to carry out peptide-bond formation; however, this may be suppressed by using ethanol as the cosolvent in place of DMF. In this work only thermolysin was found to catalyse the formation of prolyl peptide bonds.

Papain has been used on several occasions and it was found that a basic medium was required when using this enzyme for the synthesis of leucine- and methionine-enkephalin.[122] Papain, which had been immobilised onto a column of silica gel, has also been evaluated in enzymatic peptide synthesis.[123] The effects of pH, temperature and reagent concentration were studied. In an attempt to increase solubility of derivatives for use in enzymatic peptide synthesis amino acids and dipeptides have been esterified to polyethylene glycol.[124] The polyethylene glycol esters were used as substrates for various proteases in amide-bond formation. Free and immobilised chymotrypsin were studied and it was found that these esters gave excellent results with the free enzyme, but in the case of the immobilised enzyme hydrolysis of the ester linkage occurred. The sodium thiosulphatoethyl esters[29] have also been used to enhance solubility, and they were successfully used in chymotrypsin-catalysed coupling.

Enzymatic synthesis using the serine proteinases subtilisin and elastase has been reported.[125] The peptide methyl esters were used as substrate and aqueous/organic media were used at a pH between 7.8 and 8.1.

Trypsin has been used for transpeptidation in the preparation of single-chain des-B30 insulin.[126] In this work a peptide bond was formed between lysine B29 and glycine A1, with concomitant removal of the alanine at position B30. Oxidation of reduced single-chain des-B30 insulin and porcine proinsulin has been

studied.[127] A pH range of 8.6 - 9.2 and a temperature range of between 4°C and 37°C were investigated. It was found that the recovery of the single-chain des-B30 insulin was substantially greater than porcine proinsulin; thus it was confirmed that the single-chain des-B30 insulin folds more efficiently than the proinsulin.

In a traditional semisynthesis of a linear gramicidin,[128] gramicidin A/C was deformylated by a treatment with 4 molar HCl in dioxan/methanol over one hour at 40°C. Edman degradation was then used to remove the amino-terminal valine residue, and position-one analogues were generated by coupling formyl leucine or formyl D-valine with DPPA.

Human somatotropin analogues have been prepared using combination of synthetic fragments with the natural 1 - 134 sequence.[129] Three carboxyl fragments of human growth hormone 150 - 187, 152 - 187 and 154 - 187 were synthesised by solid-phase synthesis. These fragments were then complemented with the natural amino-terminal 1 - 134 fragment, giving recombinants with high activity.

A semisynthetic analogue of bovine pancreatic RNase containing a unique nitrotyrosine residue has been prepared.[130] Native ribonuclease was treated with pepsin to remove the 121 - 124 carboxy-terminal sequence. The 1 - 120 portion was then treated with carboxypeptidase A, which removed two further residues to give the 1 - 118 portion. Chemical synthesis of the 14-residue 111-124 sequence was then carried out and the tyrosine at position 115 was nitrated. Complex formation was then carried out between the native 1 - 118 and synthetic 111 - 124 portions, and it was found that binding between the fragments was not affected by nitration of the tyrosine at position 115.

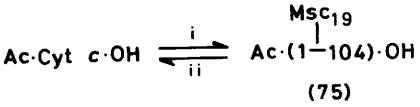
Semisynthetic studies on hen egg-white lysozyme[131-133] have shown that both methoxycarbonyl sulphenyl chloride and O-nitrophenylsulphenyl chloride are able to cleave peptides at methionine in a similar manner to cyanogen bromide. Treatment of hen egg-white lysozyme with methoxycarbonyl sulphenyl chloride lead to the formation of a covalently linked 1-12/106-129 fragment. It was also found that cyanogen bromide treatment of hen egg-white lysozyme could be used to prepare the $Hse^{12,105}$ analogue of hen egg-white lysozyme. Two alternative routes were discussed. The first route involved cleavage of the peptide backbone with generation of homoserine lactone residues at positions 12 and 105. These were then utilised in the reformation of amide bonds.

The other approach involved using a minimum of cyanogen bromide for the digestion, in which case chain fragmentation does not occur, but the methionine residues are replaced by homoserine. The same analogue was obtained from both routes and it was found to be enzymatically inactive.

Semisynthetic work on cytochrome c has also been carried out.[134,135] The enzyme was treated with methylsulphonylethyloxycarbonyl-N-hydroxysuccinimide, giving the protected derivative (75) as shown in Scheme 10. Digestion with cyanogen bromide then gave cleavage at residues 65 and 80. The 81 - 104 fragment may then be extended by the addition of methionine to give a new 80 - 104 sequence (76). This may then be coupled to a synthetically modified 66 - 79 sequence (77), giving an analogue sequence 66 - 104 (78). Removal of the Msc protecting groups and coupling with the 1 - 65 homoserine lactone (79) then gives the modified cytochrome c (80), the details of this synthetic manipulation being shown in Scheme 11.

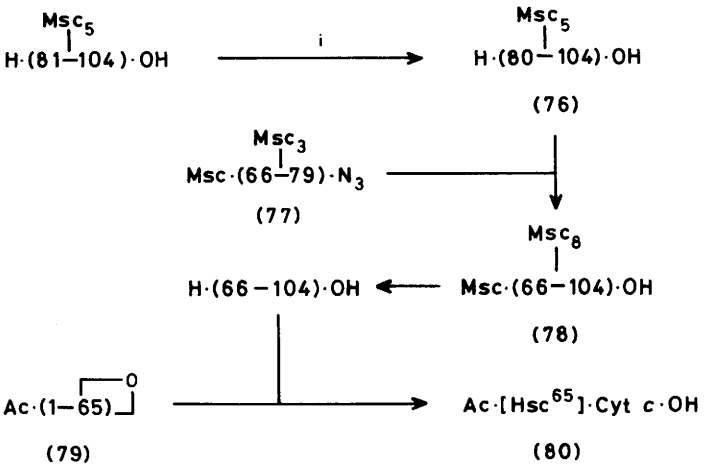
3 Synthesis

The syntheses of thymosin α_1[1] and thymopoietin[136] are good examples of the use of fragment condensation in the synthesis of large peptides. In the synthesis of thymosin α_1 tert-butyl-based side-chain protection was used, with benzyloxycarbonyl protection being used for the α-amino groups. Considerable use was made of the mixed-anhydride method employing isobutyl chloroformate for activation, and the products were well characterised by FAB mass spectrometry. The fragments were combined by the DCCI/HOBt procedure and some traces of racemisation were detected at points of fragment coupling. In the synthesis of thymopoietin[136] seven fragment condensations were carried out using the azide method. In this case side-chain protection was benzyl based and was removed at the end of the synthesis by treatment with HF/anisole/thioanisole/meta-cresol. A similar fragment condensation approach, using azides, was used in the synthesis of mouse epidermal growth factor.[2,3] In this case fifteen protected fragments were synthesised, with the trichloroethoxycarbonyl hydrazide being used for carbonyl-group protection; this was then converted to the hydrazide and in turn to the corresponding azide. Final deprotection after fragment condensation was carried out with trifluoromethane sulphonic acid/thioanisole/trifluoroacetic acid. The product was purified by DEAE ion-exchange chromatography and h.p.l.c., and



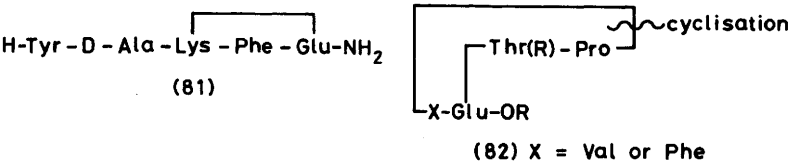
Reagents: i, Msc·ONSu; ii, base

Scheme 10



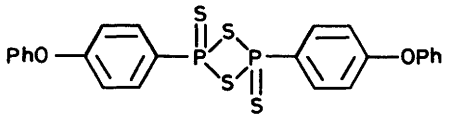
Reagents: i, Boc-Met-OPfp, TFA

Scheme 11



(81)

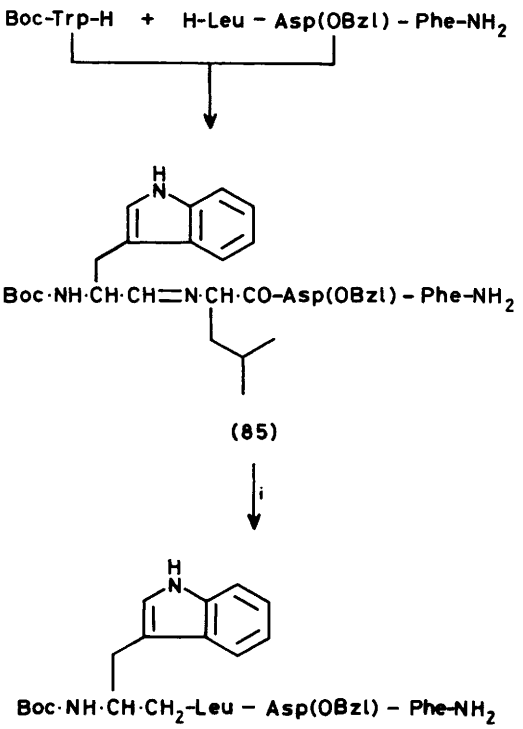
(82) X = Val or Phe



(83)

H·CO-Met - Leu - Phe-OR

(84) R = Me or Bu^t



(85)

Reagent: i, $NaBH_3CN$

Scheme 12

was found to be fully active in the bioassay.

Cyclic peptides have again been important and often hormone and neuropeptide analogues may be produced which have considerably elevated activity. An example of this is the cyclic opiate peptide (81).[137] This synthesis was carried out on a Merrifield polymer using Fmoc protection for the α-amino functions. Acid-labile protection was used for side chains and the cyclisation was carried out on the resin prior to final cleavage with hydrogen fluoride. The cyclisation could be carried out with DCCI/HOBt in dichloromethane, but the reaction in this case was slow and incomplete; in DMF, however, the reaction was complete. It was thought that DMF enhanced chain flexibility and in some cases intersite linkage was also observed.

An unusual side-chain-branched cyclic structure (82) has also been synthesised.[138] Cyclisation was carried out at the point indicated in structure (82), and it was found that, using pentafluorophenyl esters with dioxan/pyridine as solvent in the presence of dimethylaminopyridine and trifluoroethanol, a yield of 46% could be obtained. Only traces of the dimeric product were obtained, although use of hydroxysuccinimide active esters or DPPA at low temperatures gave an increased yield of the cyclo dimer. The dimethylaminopyridine was found to suppress dimer formation and 1.3% racemisation was observed.

Cyclic bradykinin analogues have also been prepared [139] in which one or two ω-aminododecanoic acid residues were incorporated. Pentafluorophenyl esters were in this case used for cyclisation and diisopropylethylamine was used as the base.

Modification of the peptide backbone is now becoming commonplace, and the reagent (83) has been used to prepare thionated analogues of chemotactic peptides.[140] If the temperature and time of thionation are carefully controlled, a number of thionated products may be obtained from the formyl tripeptide (84). At $0^{\circ}C$ over one hour, only the formyl group was thionated; however, over three days a mixture of thionated products was produced.

Analogues of tetragastrin in which amide-bond carbonyl groups are replaced by methylene groups have been reported.[141] These compounds are prepared through a Schiff base (85), Scheme 12, which is formed by reaction of a protected amino aldehyde with a free peptide. The amino aldehyde was generated by a new method involving lithium aluminium hydride reduction of an N,O-dimethylhydroxamate.[142] Such a reduction was also used in

the preparation of aldehydic peptides as inhibitors of renin.[142] The Schiff base (85) may be reduced with sodium cyanoborohydride to give the methylene isosteric replacement. Other replacements of a similar nature were made in the gastrin tetrapeptide, and it was revealed that the backbone carbonyl groups have an important role in maintaining gastric activity.

Synthesis of retro-inverso peptides has been widely studied,[143-146] and in the majority of cases the diamino alkane component is generated by treatment with iodobenzene-bis-trifluoroacetate. It was confirmed that no racemisation occurred during this reaction;[114,145] however, after coupling with, for example, a half ester of benzyl malonic acid only diastereoisomeric products were obtained. After elaboration of the remainder of the peptide chain the diastereoisomeric peptides may often be separated; however epimerisation of the malonyl proton is very rapid in most cases. It is interesting to note that in work on retro-inverso cyclic enkephalins[145] it was found that epimerisation was very slow and optical purity was maintained over two days. In a similar linear example epimerisation occurred very easily and was complete within six or seven hours. It was concluded that the resistance to racemisation in the cyclic peptide was due to the constrained geometry in which the benzyl malonate carbonyl group is forced into a non-linear arrangement and therefore cannot epimerise readily.

In glycopeptide synthesis protecting-group strategy is critical, and in the preparation of the glycopeptide partial sequence 80-A-84-A of human fibroblast interferon [147] allyl esters played an important part in the synthesis. Their stability to acids allowed manipulations involving the Boc group for α-amino-group protection and ultimately the ester function could be removed by treatment with tris-triphenylphosphonium rhodium chloride at 70°C in aqueous ethanol.

In another glycopeptide synthesis[148] 2-(hydroxymethyl)-9,10-anthraquinone was used as a temporary protecting group. This ester protecting group was used in conjunction with the Fmoc α-amino protecting group which allowed elaboration of a threonine glycoside. The glycosidic linkage between the protected threonine derivative and tetraacetyl galactose was formed using mercuric bromide as a catalyst. The $\underline{N}^{\alpha}$-Fmoc protection could be removed with piperidine, allowing DCCI/HOBt coupling to be used for chain extension. The ester function was removed by treatment with $Na_2S_2O_4$ and the free glycopeptide

was liberated by subsequent treatment with morpholine and hydrazine hydrate.

Human glycophorins A^N and A^{MC} have been studied.[149] In these glycopeptides the peptide chain is joined to the sugar through an amide bond which connects it to the 2-amino function of the 2-amino-2-deoxy-α-D-galactopyranosyl residue. In this type of linkage, protection is not as critical, as Fmoc protection may be used for the amino terminus and an active ester such as the hydroxysuccinimide or nitrophenyl active ester may be used for coupling to the amino group. Chain elaboration then takes place after removal of the Fmoc α-amino protecting group.

4 Appendix I : A List of Syntheses Reported in 1985

The syntheses are listed under the name of the peptide to which they relate, as in previous years.

Peptide	Ref.
4.1 Natural Peptides, Proteins, and Partial Sequences	
Adrenocorticotropic hormone (ACTH)	
α-MSH/ACTH (4-10) analogue	150
ACTH (11-24) dimer	151
Cyclic ACTH analogues	152
Tritiated ACTH analogue	153
Adrenorphin	
Adrenorphin analogues	154
Alamethicin	
Alamethicin	155
AM Toxin	
Abu^1, Leu^1 and Phe^1 AM Toxin I	156
Angiotensin	
Angiotensin II analogues substituted at positions 1 and 8	157
Angiotensin II position-8 analogue	158
Angiotensins II and III containing Tyr(Me) and D-Trp	159
Antibiotic heptapeptide (K582A)	
Analogues of the antibiotic heptapeptide K582A	160
Anti-writhing peptides	
Anti-writhing peptides related to neurotensin and tuftsin	161
Antrimycin	
Dehydropeptides related to antrimycin	162

4.2 Sequential Oligo- and Poly-peptides

4.3 Enzyme Substrates and Inhibitors

Ketovinyl and hydroxyethylidene dipeptide isosteres 471
Peptidyl fluoromethyl ketones 472
Maleoyl peptides 473
Maleoyl peptides 474
Maleoyl peptides 475
Methotrexate analogues 476
β-Methyllanthionine derivatives 477
Model peptides of defined hydrophobic periodicity 478
Nicotinamide-cyclopeptide 479
β-Hydroxynorvaline diastereoisomers 480
Fragments of basic nuclear proteins 481
Amino acid/nucleotide derivatives 482
Nucleopeptides 483
Nucleoside/amino acid derivatives 484
Oligoleucines containing α-aminoisobutyric acid 12
Oligoleucines containing α-aminoisobutyric acid 485
Hg^{II} complexes of cysteine-containing oligopeptides 486
Synthetic peptide antigens 487
Di- and Tri-peptides containing double-bond isostere 488
Aspartyl-D-alanyl tripeptide sweeteners 489
Retro-inverso peptide sweeteners 490
Aminoethyl phosphonic acid derivatives 491
Phosphonic and phosphinic analogues of ornithine 492
Phosphonopeptides 493
N-phosphoryldipeptides 494
Unsymmetrically substituted 2,5-piperazinediones 495
A synthetic antigen for Plasmodium malaria
(Asn-Ala-Asn-Pro)$_n$ (n = 3,4) 496
Sequential basic polypeptides with branched and linear side chains 497
Basic ribosomal protein fragments 498
Tetrapeptide stearyl esters 499
Trifluoroacetylamino acid amides 500
3-(^{3}H)-5-(4'-azobenzene arsanic acid)-L-tyrosine 501
Z-Gla-Arg-OMe 502

5 Appendix II : Amino Acid Derivatives Useful In Synthesis

The list of derivatives is divided into two groups, the coded and the uncoded amino acids. The following unusual abbreviations are employed: OCam, carboxamidomethyl ester; Coc, cinnamyloxycarbonyl; Dep, diethylphosphinyl; Dmp, dimethylphosphinyl; Mem, 2-methoxymethyl. *1 Measured between 19° and 28°C.

Compound	M.p./°C	α_D^{*1}	Conc./g 100 cm^3	Solvent	Ref.
5.1 Coded amino acids					
Alanine					
Coc-Ala-OH	74.5-75	-8.4	2	EtOH	21
Z-Ala-OCam	58-64	-13.5	2.1	DMF	28
Aspartic acid					
N-Benzyloxy-L-Asp(OBu^t)-OBu^t	78-80	-5.6	6	CH_2Cl_2	467
N-Benzyloxy-L-Asp(OMe)-OMe	116-117	-2.7	7	MeOH	467
Coc-Asp-OH	135	+1.5	3.95	EtOH	21
Cysteine					
Fmoc-Cys(Acm)-OH	150-154	-27.5	1	EtOAc	103
Fmoc-Cys(Bu^t)-OH	74-76	-87.0	1	EtOAc	103
Fmoc-Cys(SBu^t)-OH	135-136	-24.0	1	DMF	103
Glutamine					
Coc-Gln-OH	119-122	-9.3	1.07	EtOH	21
Glycine					
Boc-Gly-OCam	64-67	-	-	-	28
Coc-Gly-OH	129.5-130.5		-	-	21
Z-Gly-OCam	105-108	-	-	-	28
Histidine					
Boc-His(Bzl)-OPcp	131-132	-2.25	1.78	$CHCl_3$	83
Boc-His(DNP)-OTcp	105-107	+8.49	2.12	DMF	83
Coc-His(Bzl)-OH	217-218	+11.5	1.05	DMF	21
Z-His(Bzl)-OMe-HCl	48	-14.8	2.77	MeOH	83
Z-His(Bzl)-OPcp	110-112	+8.23	2.71	THF	83
Z-His(Tos)-OMe	72-74	+3.91	1.15	DMF	83
Z-His(Tos)-ONp	125-126	+3.97	2.9	THF	83
Z-His(Tos)-Pcp	149-151	+4.686	2.134	THF	83
Z-His(Tos)-Tcp	157-158	-3.56	1.96	THF	83
Z-His(Z)-ONp	106-107.5	-16.6	2.048	THF	83
Z-His(Z)-OPcp	123-125	+18.12	3.15	DMF	83
Isoleucine					
Coc-Ile-OH	127-128	+10.8	1.02	EtOH	21
Leucine					
Coc-Leu-OH	143-144	-3.6	1.1	DMF	21
Dep-Leu-OH	158	-	-	-	23
Dmp-Leu-OH-DCHA	140-141	-	-	-	23
Lysine					
Nps-Lys(Boc)-ONSu	175-182	+4.5	1	DMF	293
Methionine					
Boc-Met-OCam	78-81	-29.2	2.7	DMF	28

Coc-Met-OH	82.5	-14.9	1	EtOH	21
Phenylalanine					
Boc-Phe-OCam	113-116	-16.6	2.95	EtOH	28
Coc-Phe-OH	97-98	+5.1	2	EtOH	21
Proline					
Coc-Pro-OH	119-121	-16.2	1.06	DMF	21
Serine					
Boc-Ser(Bzl)-OBzl	-	-40.2	2	EtOH	400
Boc-Ser(Bzl)-ODpm	115-116	-8.7	2	$CHCl_3$	27
Coc-Ser-OH	95-97	+12	1.08	MeOH	21
Fmoc-Ser(Bzl)-ODpm	139-140	-1.7	2	$CHCl_3$	27
Fmoc-Ser-ODpm	99.5-101	-3.9	2	$CHCl_3$	27
Troc-Ser(Bzl)-ODpm	114-115	-3.3	2	$CHCl_3$	27
Troc-Ser-ODpm	128-129	-6.3	2	$CHCl_3$	27
Threonine					
Coc-Thr-OH	101-102	+2.6	3.51	EtOH	21
Fmoc-Thr-ODpm	156-157	-21.3	2	$CHCl_3$	27
Troc-Thr-ODpm	65-66	-21.2	2	$CHCl_3$	27
Valine					
Coc-Val-OH	101	+3	1.66	EtOH	21
Dep-Val-OH	114-145	-	-	-	23

5.2 Uncoded Amino Acids

β-Alanine (β-Ala)					
Coc-β-Ala-OH	97-98	-	-	-	21
Fmoc-β-Ala-OH	130-133	-	-	-	9
γ-Aminobutyric acid (Gaba)					
Boc-Gaba-OH	53-55	-	-	-	9
Boc-(Me)Gaba-OH	60.5-61.5	-	-	-	36
Bpoc-(Me)Gaba-OH	123.5-125	-	-	-	36
2-Amino-4-(3-pyridyl)butyric acid [Ala(3Pm)]					
PhCO-D-Ala(3Pm)-OMe	141-142	+15.2	0.99	DMF	368
Aspartic acid (Asp)					
N-Benzyloxy-D-Asp(OBu^t)-OBu^t	73-75	+6.0	6	CH_2Cl_2	467
N-Benzyloxy-D-Asp(OMe)-OMe	115-116	+2.0	7	MeOH	467
γ-Carboxyglutamic acid (Gla)					
Z-Gla(OBu^t)$_2$-OH	89-90	-12.05	1	MeOH	503
Z-Gla-OPh	137-139	-30.3	1	MeOH	503
Dehydroproline (Δ-Pro)					
Boc-Δ-Pro-OH	94-96.5	-272.5	1	MeOH	9
Boc-D-Δ-Pro-OH	93-94	+27.1	1	MeOH	9

Homocysteine (Hcy)					
Boc-Hcy(Bzl)-OBzl	57-58	-28.0	1	MeOH	465
β-Homo-phenylalanine (β-HPh)					
Boc-β-HPh-OH	99-101	-14	2	DMF	9
Boc-D-β-HPh-OH	96-98	+26.8	2	DMF	9
Z-β-HPh-OH	116-119	-31.9	1	MeOH	9
β-Homo-proline (β-HPr)					
Boc-β-HPr-OH	99-102	-39.1	2	DMF	9
Boc-D-β-HPr-OH	86-88	+37.3	2	DMF	9
Z-β-HPr-DCHA	145-148	-17.9	1	MeOH	9
Z-β-HPr-OH	Oil	-	-	-	9
Z-β-HPr-NH_2	129-130	-27	1	MeOH	9
Trans-4-Hydroxyproline (Hyp)					
Fmoc-Hyp-OH	161-162	-47.7	2	DMF	9
Methionine (Met)					
Boc-(Et)Met-OH	46-55	+57.8	1	$CHCl_3$	504
Ornithine (Orn)					
Boc-D-Orn(Z)-ONSu	123-126	+22.3	1	DMF	160
Phenylalanine (Phe)					
Boc-(Et)Phe-OEt	58-61	-131.5	1	$CHCl_3$	504
Boc-(Et)Phe-OH	64-66	-126.2	1	$CHCl_3$	504
Boc-Phe(p-N_3)-ONSu	143-144	+5.9	8.3	Dioxan	220
$Cl^-H_2^+$-(Et)Phe-OEt	-	+42.7	1	MeOH	504
Z-(Me)Phe-OH	Oil	-	-	-	9
Z-(Me)Phe-OH-DCHA	116-117	-18.7	2	DMF	9
Phenylglycine (Phg)					
$(Z\text{-Phg})_2O$	165	-	-	-	65
Pipecolic acid (Pip)					
Boc-Pip-OH	126-127	+20.5	1.15	H_2O	9
Boc-D-Pip-OH	129	+56	1	AcOH	9
Fmoc-Pip-OH	145-149	-23.3	1	MeOH	9
Fmoc-D-Pip-OH	144-146	+24.7	1	MeOH	9
Z-D-Pip-OH	112-113	-57	2	DMF	9
Z-Pip-OH	112-113	+57	2	DMF	9
Proline (Pro)					
Boc-D-Pro-OH	130-132	+56.8	1	MeOH	9
Fmoc-D-Pro-OH	108-109	+35.8	2	DMF	9
Tyrosine					
Boc-(Et)Tyr(Bzl)-OH	113-118	-101.2	1	$CHCl_3$	504
Boc-(Et)Tyr-OH	75-80	-124.4	1	$CHCl_3$	504
$Cl^-H_2^+$-(Et)Tyr(Bzl)-OH	-	+25.4	1	MeOH	504

$Cl^-H_2^+$-(Et)Tyr-OH	-	+52.2	1	MeOH	504
Tyrosine sulphate [Tyr(SO_3H)]					
Boc-Tyr(SO_3Na)-OH	285-290	-52.4	1	DMF	39
Valine (Val)					
H-(Bu^tO-CO-CH_2)Val-OEt	Oil	-18.0	1	MeOH	443
Z-(HO-CO-CH_2)Val-OEt-DCHA	109.5-110	-40.1	1	$CHCl_3$	443

6 Appendix III : Purification Methods

Methods for the purification of protected peptides and proteins are given; the list also includes purification of free peptides and separation of diastereoisomers.

Technique	Ref.
6.1 High-Performance Liquid Chromatography	
H.p.l.c. analysis of angiotensins	505
Ion-pair h.p.l.c. of aspartame and related compounds	506
H.p.l.c. of amino acid phenylthiohydantoins	507
H.p.l.c. of amino acid phenylthiohydantoin	508
H.p.l.c. of some sulphonyl chloride amino acid derivatives	509
H.p.l.c./mass spectrometry of amino acids	510
H.p.l.c./mass spectrometry	511
H.p.l.c. on chiral stationary phases	512
H.p.l.c. of amino acid enantiomers on a chiral phase	513
H.p.l.c. determination of secondary amino acids	514
Fluorescent labelling of amino acids for h.p.l.c.	515
H.p.l.c. cation exchangers	516
H.p.l.c. on a chiral stationary phase	517
H.p.l.c. separation of enantiomers using a chiral Cu eluent	518
H.p.l.c. on a chiral stationary phase	519
H.p.l.c. using a chiral hydantoin-based ligand	520
H.p.l.c. of cyclosporin A	521
Peak broadening on h.p.l.c. of cyclosporin	522
H.p.l.c. of enkephalins and metabolites	523
H.p.l.c. of glucagon derivatives	524
H.p.l.c. of glucagon derivatives	525
H.p.l.c. of Glp, Glu and Gln	526
H.p.l.c. gradient elution	527
H.p.l.c. gradient elution	528
H.p.l.c. on hydrophobic columns	529
H.p.l.c. of insulin derivatives	530
H.p.l.c. of Insulin A and B chains	531

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3

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

BY J. S. DAVIES

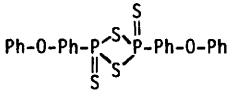
1 Introduction

The format of the inaugural Chapter in this series last year highlighted two main themes - peptide-backbone modifications in structural-activity studies and the role of physico-chemical methods in the interpretation of peptide conformations. In the papers published during 1985 there seems to have been less activity in the field of peptide-bond surrogacy but continued interest in a variety of ways for restricting the flexibility of the peptide backbone. High-field n.m.r. studies, both 1D and 2D, supported by NOE studies, amide NH temperature dependence etc. and computer graphics, have this year made a significant contribution to the understanding of conformations in the solution phase.

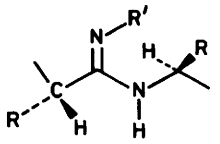
Although there might have been a change in productivity under the various categories of titles, there does not seem to be as yet any justification in changing the sub-themes used last year.

2 Peptide-backbone Modifications

2.1 ψ[CSNH] Analogues. - Thioamide bonds have been incorporated[1] into all the amide positions of the chemotactic peptide N-formylmethionyl-leucyl-phenylalanyl ester, using the reagent

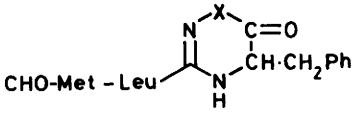


Biological activity is suppressed by thioamide insertions in all positions on the peptide backbone except when the sulfur is in the N-terminal formyl group. The thioamide bond in these analogues has also been used[1] as a precursor of some novel amidoxime, cyanoamidine and amidrazone analogues of type (1), where R' can be OH, OCH_3, CN or OAc. Activity is restored in the amidoximes but not with the cyanoamidine linkages except at the formyl group. Restricting the flexibility by bridging as in (2), X = NH or O, gives an analogue with strong

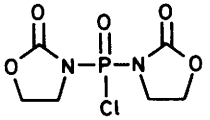


(*Z*) (*E*)

(1)



(2)



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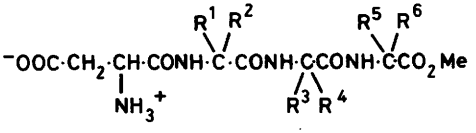
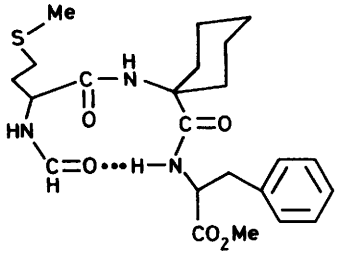
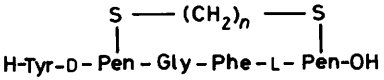


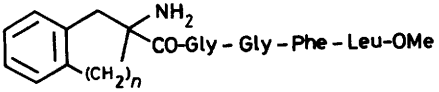
(10)



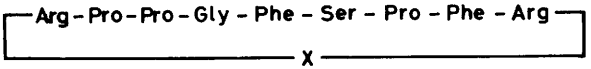
(11)



(12) n = 2 or 3



(13) n = 1 or 2



(14) X = $-CO(CH_2)_{11}NH-$

(15) X = $-CO(CH_2)_{11}NH\cdot CO(CH_2)_{11}-$

(16) X is absent

activity. Regiospecific incorporation[2] of a thioamide bond to form Boc-Gly ψ[CSNH]-Ala-AibOMe(3) (Aib = aminoisobutyric acid) was possible using the Lawesson reagent because of the steric hindrance of the Aib residue. Boc-Gly-Ala-ψ[CSNH]-AibOMe(4) was obtained by using this reagent as a coupling agent. X-Ray diffraction studies showed that the type III β-turn of the original all-oxygen analogue is preserved in (4) but (3) is in a partly extended form. The Aib residue seems to induce the formation of a β-turn with a thiopeptide inside the ring formed by the intramolecular bond. When the Lawesson reagent is used in coupling, h.p.l.c. studies[3] show only a trivial amount (0.5%) of racemisation is evident.

2.2 ψ[NHCO] Retro-Inverso Analogues. - The importance of the Gly^3-Phe^4 amide bond of the enkephalins in receptor binding has been highlighted from work[4] on the partial retro-inverso analogue Tyr-D-Ala-g Gly(RS)-mPhe-$LeuNH_2$ (5) and its cyclic counterpart Tyr-cyclo[-D-A_2bu-g Gly(RS)-mPhe-Leu-] (6). High yields of (6) were obtained by cyclisation of its linear precursor using an insoluble inorganic base (KH_2PO_4) without the need for high dilution. All analogues had about 10% of the Leu-enkephalin activity. A retro-inverso isosteric replacement[5] for the Phe-Ala scissile bond of the bradykinin potentiating peptide BPP_{5a} has been made using standard procedure. [g Phe^3(S)-mAla^4]BPP_{5a} proved to be a moderate inhibitor of angiotensin converting anzyme (ACE) but more potent than BPP_{5a} as a hypotensive in normotensive rats. The postulated internal cleavage sites between residues Phe^7 and Lys^9 of somatostatin have been the subject[6] of retro-inverso modifications. Reversal between position 7 and 8 gave [g Phe-m (RS)-Lys]-somatostatin, and using h.p.l.c. all four diastereoisomers could be obtained pure. From the biological-activity results it would seem that the Phe-Trp region is more tolerant to change than the Trp-Lys region, which is consistent with a type II' β-turn at Trp^8-Lys^9 being important. Retro-inverso analogues[7] of dermorphin involving substitution over the 3 to 5 region exhibited low *in vitro* and *in vivo* opioid activities. However, good sweet-tasting characteristics have been found in retro-inverso versions of the dipeptide L-Asp-D-Ala-NHR. The R-form of L-Asp-NH-CH(CH_3)-NHCOR' with R' ranging from t-butyl to substituted cyclopentyl derivatives represent a new class of amino acid base sweeteners.[8]

2.3 ψ[CONR] N-Alkylated Analogues.- The 600 MHz ^{1}H n.m.r. spectra of [N-MeGly^7]-oxytocin and [N-MeAla^7]-oxytocin in D_2O reveal[9] the presence of two slowly interconverting conformers due to cis-trans conformers about the bond between residues 6 and 7. The trans isomer is energetically favoured but in binding studies with neurophysin at pH 3 both conformers seem to be equally tightly bound to the protein. Problems with difficult peptide couplings using N-alkylamino acids seem to have been overcome[10] in the cyclosporin A synthesis through the use of the phosphinic chloride reagent (7). It gives very useful yields and low racemisation but cannot cope with the gem-dimethyl groups of aminoisobutyric acid residues.

2.4 ψ[CH_2NH] Amino Methylene Analogues. - Coupling between an appropriate N-terminal aldehyde component and an amino group followed by $NaBH_4CN$ reduction has yielded[11] several amino methylene analogues of tetragastrin with different biological effects. t-Boc-Trpψ[CH_2NH]-Leu-Asp-Phe-NH_2(8) and t-Boc-Trp-Leuψ[CH_2NH]-Asp-Phe-NH_2 (9) showed the same affinity as the parent tetragastrin analogue for gastrin receptors but t-Boc-Trp-Leu-Asp-ψ[CH_2NH]-Phe-NH_2 (10) had a lower affinity. Analogue (8) stimulated acid secretion in the anaesthetised rat, while (9) was an antagonist. Compound (10) did not show any agonist activity so the amide bond between Leu-Asp must be essential for acid secretion.

2.5 Replacement of L-Residues by D-Residues.- Substitution of a D-Met(O) residue in position-2 of adrenorphin H-Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val-NH_2 and into the similar position in [Arg^6, Phe^7]-enkephalin gives enhanced analgesic activity[12] and is an improvement on incorporating D-Ala in the same position. The relative potencies in vitro of [D-Ala^2]-enkephalinamide and [D-Ala^2, D-Leu^5]-enkephalinamide have been reported[13] to be 540:19000, respectively. Worth noting is the fact that the D-residues could be incorporated using direct coupling of enzymatic and azide methods for peptide-bond formation. Two papers[14] have reported the consequence of inserting D-residues in the β-turn part of the antibiotic gramicidin S. Synthesis and c.d. analysis of [D-Val$^{1,1'}$] and [D-Val$^{1,1'}$, L-Phe$^{4,4'}$]-gramicidin S show a distinct change in conformation on insertion of the D-residue, which probably explains the lack of biological activity of the analogues. [L-Pro$^{4,4'}$, D-Phe$^{5,5'}$]-Gramicidin S is also inactive, but just inserting one D-residue as in the [L-Pro^4, D-Phe^5] analogue did not affect the activity. Substitution of a

D-Phe residue in position 7 of the highly potent cyclic melanotropin Ac-Ser-Tyr-Ser-Cys-Glu-His-Phe-Arg-Trp-Cys-Lys-Pro-Val-NH_2 shows increased potency by more than a factor of 10, but does not show prolonged activity.[15] In a detailed study[16] of the effect of the side chains of each amino acid in the L-α-aspartyl- tetrapeptide esters (10) on taste it is found that to give a sweet taste the second residue must have a D-configuration and a small side chain, although sweetness was often accompanied by a bitter taste. With increasing length of peptide, receptor interaction is more difficult. Lack of binding of the all-D-model ribonuclease-S-peptide to the S-protein is given[17] as an explanation for its inactivity against the substrate cytidine-2'3'-monophosphate, although the c.d. spectrum showed that the D-form possessed α-helical structure.

2.6 α, α-Dialkylated Glycine Analogues. - Following up on a series of previous papers on the synthesis of cyclopropyl-phenylalanine derivatives information has now been revealed[18] on the result of their incorporation into Leu^5-enkephalin. The results of the incorporation of cyclopropyl analogue ∇Phe in position 4 are given in Table 1. This shows clearly that the receptors prefer only one of the isomers to all the others. When Aib, 1-aminocyclopentane carboxylic acid (Acc^5) and 1-aminocyclohexane carboxylic acid (Acc^6) are substituted in turn into the 2-position of the chemotactic peptide analogue HCO-Met-X-Phe-OMe, only the 2-Acc^6 analogue (11) is more active (80 times) than the parent molecule.[19] N.m.r. studies and model building on

Table 1

	Rat brain binding IC_{50} (nm)	Tail Flick Latency concn. (i.c.v.) 10^{-8}M	10^{-7}M
(-)-∇^{E}Phe-Enk	13,300	5	92
(+)-∇^{E}Phe-Enk	8,900	0	35
(-)-∇^{Z}Phe-Enk	30.7	20	62
(+)-∇^{Z}Phe-Enk	1.2	42	63

this analogue clearly favour a β-turn conformation. Crystallographic and n.m.r. data[20] on Boc-Aib-Acc^6OMe (12) and Boc-Aib-Acc^6NHMe (13) show that (13) adopts a β-turn conformation but the Aib residue in (12) adopts a partially

extended conformation. As a follow-up to work on dipeptide aspartame analogues, synthesis and structure-taste relationships have been carried out[21] on L-Asp-D-Ala tripeptides. α,α,-Diethylglycine, Aib and Acc methyl esters have been used as the C-terminal unit to reveal that the sweet/bitter taste is dependent on Acc ring size. Three-to six-membered rings render sweet taste reactions while higher ring sizes are bitter or tasteless.

3 Conformationally Restricted Bridged Analogues

3.1 Somatostatin. - Earlier studies have already revealed high activity amongst cyclic octapeptide analogues. An even more potent inhibitor of growth hormone secretion has now been found[22] in the form of H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2. A detailed n.m.r. study[23] has been carried out on a cyclic analogue, cyclo[-D-Trp-Lys(Z)-Thr-Val-Pro-Gly-Phe-],of the active sequence in [Phe^6, Thr^{10}]-somatostatin, but deprotection of the derivative revealed no biological activity.

3.2 Enkephalins. - In further attempts to form side-chain to side-chain bridges between an α,ω-diamino acid and the carboxyl group of Glu or Asp of the type H-Tyr-D-Lys-Gly-Phe-Glu-NH_2, links can now be made[24] directly on the Merrifield resin. A second major component obtained[25] from the crude product taken from the resin was identified as a dimer $(H\text{-}Tyr\text{-}D\text{-}Lys\text{-}Gly\text{-}Phe\text{-}Glu\text{-}NH_2)_2$, equipotent to [$Leu^5$]enkephalin in assays for μ-opioid receptors with 1/10th potency at the δ-receptors. A cyclic analogue containing a Leu-enkephalin sequence has been analysed[23] using n.m.r. techniques, but showed no biological activity. In comparing the biological activities of the cyclic enkephalin analogues[26] H-Tyr-cyclo(-N-Xxx-Gly-Phe-Leu), where Xxx is varied between L-Orn, D-Orn, L-Lys or D-Lys, it is found that the D-Lys^2 analogue is about 10 times more active in the guinea-pig ileum assay than the D-Orn^2. High-field n.m.r., with assignments made by 2D techniques, COSY, SECSY and NOE effects, suggests that the ornithine analogues have a rather rigid conformation, while the Lys analogues with their added flexibility include a β-turn. Success with the development of [D-Pen^2,Pen^5]-enkephalin analogues (last year's report) has initiated a more general approach[27] to include a larger bridge size by subjecting Na/liqNH_3 solutions of the linear precursors to reaction with dibromoalkanes to yield the series (12). The conformation of the highly active cyclic analogue H-Tyr-cyclo[-D-A_2bu-Gly-Phe-Leu-] has been probed[28] by ^1H n.m.r. and computer

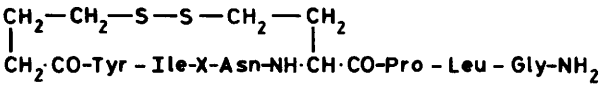
simulations. Two transannular H-bonds, LeuNH→GlyCO and D-A_2bu side-chain NH→either D-A_2buCO or Phe CO, are present in $^2H_6$-DMSO solutions. Introducing restricted mobility only at the N-terminal residue through analogues (13) does not produce significant analgesic activity[29]. Azide coupling under high dilution has yielded[30] ⌐Tyr(2,6Cl_2Bzl)-Gly-Gly-Phe¬.

3.3 Bradykinin. - The range of biological activities in an interesting series of cyclo analogues of bradykinin have been correlated with ring size[31]. Cyclo analogues (14) and (16) exhibit prolonged hypotensive activity in anaesthetised rats *in vivo*. Compound (15) was found to be inactive, and the conclusion is drawn that hypotensive activity within this series primarily depends on the size of the bridging link.

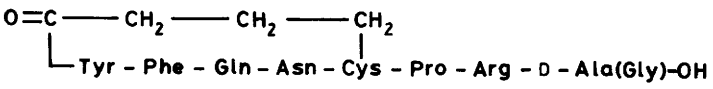
3.4 Carba Analogues of Oxytocin and Vasopressin. - Analogues of oxytocin such as (17) have been prepared[32] by solid-phase techniques requiring the cyclo-condensation of S-carboxypropyl cysteine or S-carboxyethylhomocysteine residues using DCC/HOBt either on or off the resin. The Thr analogue exhibits high *in vitro* and *in vivo* uterotonic and *in vivo* galactogogic activities. Solid-phase synthetic techniques including cyclisation on the resin have also featured[33] in the synthesis of carba analogues of arginine vasopressin (18) which possess high biological activity.

3.5 Other Cyclic Analogues. - Disulfide bridges have been introduced[34] across the 15-26 and 16-26 positions in human β-endorphin using solid-phase techniques. Both [Cys^{15}-Cys^{26}, Phe^{27}, Gly^{31}]- and [Cys^{16}-Cys^{26}, Phe^{27}, Gly^{31}]-β-endorphin show over twice the opiate receptor binding activity of β-endorphin. The quest for receptor binding information has also initiated[35] an examination of conformationally restricted analogues of substance P(SP). In the guinea-pig ileum assay, [Cys^5-Cys^{11}]-$SP_{5-11}NH_2$ and [Cys^6-Cys^{11}]$SP_{5-11}NH_2$ were inactive while [Cys^5-Cys^6, Nle^{11}]SP was a weak agonist, so a pseudocyclic structure for SP_{5-11} may not be important in receptor recognition.

The active sequence of thymopoietin II, Arg-Lys-Asp-Val-Tyr (now called thymopentin), has been modified to give a more rigid cyclic analogue[36] having the structure cyclo(-Arg-Lys-Xxx-D-Val-Tyr-) where Xxx = Asp or Glu. A full range of high-field n.m.r. techniques on protected derivatives confirm the existence of a βII'/γ-structure in DMSO solutions. On deprotection the cyclic analogues show high activity in the phythammaglutinine and plaque-forming cell



(17) X = Thr or Glu



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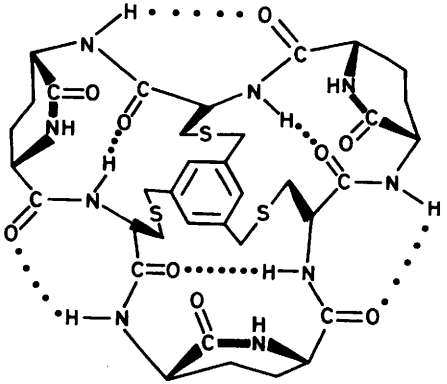

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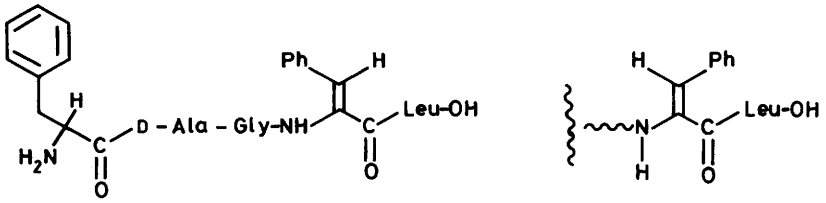


(*Z*) (*E*)

(20)

assay. In a related n.m.r. study[23] the cyclic heptapeptides cyclo[-Ala-Ile-Val-Ser(Bzl)-Xxx-Phe-Gly-] where Xxx = Pro or Aib have been investigated. The effect of ring size in the activity profiles of cyclic analogues of morphiceptin and dermorphin has been studied[37]. The rigid cyclic monomer H-Tyr-D-Orn-Phe-Asp-NH_2 (13-membered ring) was shown to be one of the most selective μ-receptor ligands reported, while in contrast H-Tyr-D-Lys-Phe-Glu-NH_2 (15-membered ring) showed no receptor preference. The all-L-form of cyclo(-Pro-Val-Pro-Val-) retards[38] the stem growth of rice while the L-D-L-D form promotes root growth. The conformational forms of these cyclic compounds in solution are believed to have the amide bonds in cis-trans-cis-trans conformations with the L-D-L-D analogue tending to be all trans in trifluoroethanol-d_3. A previously synthesised β-turn-inducing dipeptide analogue, 3-amino-2-piperidone-6-carboxylic acid (Acp) analogue, has been incorporated[39] into the cyclo-nonapeptide cyclo(-L-Cys(Meb)-LL-Acp-)$_3$ where Meb = p-methoxybenzyl. Removal of the S-blocking group followed by a ring-forming reaction with 1,3,5-tris(bromomethylene)benzene gives an unusual series of rigid cage compounds of type (19), which can bind 3 water molecules. Comparisons are made with cyclo(-Gly-L-Cys(Bzl)-Gly-)$_3$ and cyclo(-L-Pro-Gly-L-Cys(Meb)-)$_3$.

4 Dehydroamino Acid Analogues

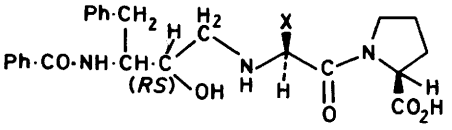
The synthetically more difficult (E)-isomer of dehydrophenylalanine has been incorporated[40] into Leu^5-enkephalin, giving [D-Ala^2, Δ^E-Phe^4, Leu^5]-enkephalin (20), but this showed a drastically decreased potency for δ- and μ-receptors (260- and 150-fold loss of affinity respectively), when compared with the (Z)-isomer. This preference for the Δ^Z-Phe form has also been explored[41] by comparing the conformation of cyclo(-Gly-Pro-Δ^Z-Phe-D-Ala-Pro-) (21) with that of cyclo(-Gly-Pro-D-Phe-D-Ala-Pro-). ^1H and ^{13}C n.m.r. data show that the β-turn conformation preferred for the latter in previously published work must be retained in (21) since the n.m.r. parameters change very little from one to the other. Increased activity by substitution of Phe by Δ^Z-Phe is not universal, however. In a preliminary report[42] on the synthesis and biological activity of tetrapeptide analogues of dermorphin, H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-HN_2, the replacement of Phe^3 by D-Phe or by Δ^Z-Phe was not tolerated and gave inactive analogues. Comparisons have been made[43] between the ease of synthesis of protected α,β-dehydroamino acids.

While condensation between amides of t-Boc-amino acids and pyruvic acid leads to t-Boc-dipeptides of dehydroalanine, the same condensation using phenylpyruvic acid would not yield the dehydrophenylalanine analogue. Removal of the Z-protecting group from Δ-Ala dipeptides gave more side reactions than in peptides containing Δ-Phe, but the problems can be overcome preparatively by using ammonium formate in the presence of Pd/C to remove the protecting group. Previously published methodology has yielded[44] configurationally defined C-terminal dehydrotripeptide sequences of the trimycins and cirriatomycins.

5 Enzyme Inhibitors

In a perspective review[45] an assessment has been made of the development of transition-state analogue inhibitor hypothesis based on pepstatin inhibitors.

5.1 Angiotensin converting enzyme (ACE) Inhibitors. - An amino alcohol group inserted[46] in the scissile amide-bond position of the known substrate of ACE, N-Bz-Phe-Ala-Pro-OH, gives rise to a new class of potent inhibitors having structure (22). A 1:1 mixture of the (RS)-diastereoisomer proved to be potent (I_{50} = 35 nM), and on separating the isomers it was found that the (R)-form was 400-fold more inhibitory than the (S)-form, while removal of the OH group or the introduction of a methyl group to make it a tertiary alcohol was deleterious to activity. A neighbouring amine group was a requirement for activity with an argininyl or lysyl side chain at position X in (22) giving satisfactory potency. There has been an interest[47] in knowing whether an L-pyroglutamyl analogue (23) of the potent ACE inhibitor enalapril possesses similar properties to the parent structure. Pharmacological assays establish that (23) parallels enalapril in inhibiting ACE but only has half the potency. Replacement of the CO group of substrate analogues with a $CHCO_2H$ group as in (24) has given[48] several analogues with inhibitory potency equal to that of enalaprilat. Ketovinyl and hydroxyethylidene isosteres can be prepared[49] using Grignard additions of vinyl magnesium bromide to chiral α-amino aldehydes such as Boc-L-phenylalaninal. An analogue (25) of the ACE inhibitor Bz-Phe-Gly-Pro-OH was prepared in this manner. Amongst a series of 1-benzazepin-2-one and indoline derivatives synthesised[50] as potential ACE inhibitors, compounds (26) and (27) were amongst the most active in the series. The advantages of having a hydrophobic substituent in the α-position to the



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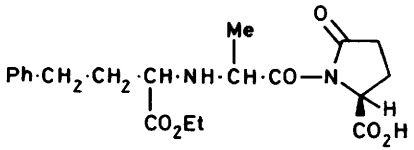

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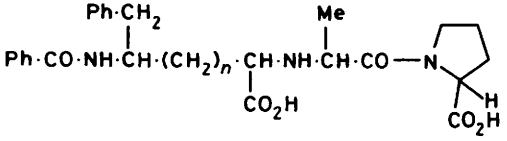
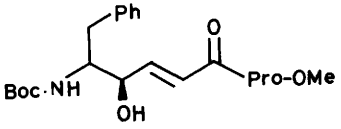


(24) n = 0 or 1



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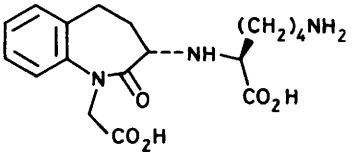

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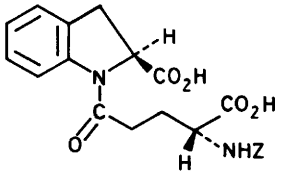

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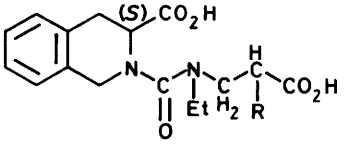
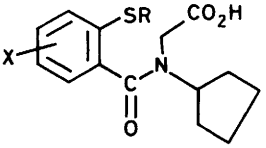


(28) R = C_8H_{17} to $C_{12}H_{25}$



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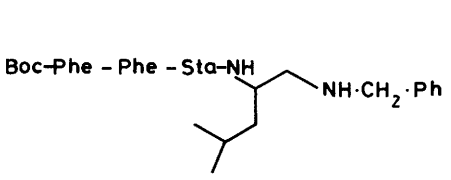

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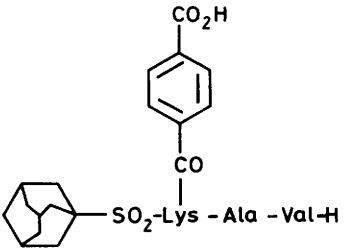

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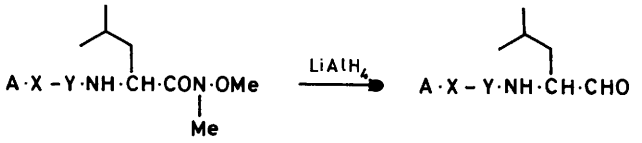
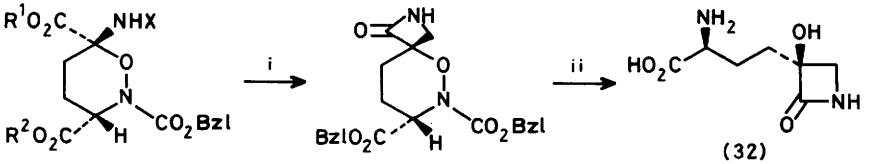


A = Boc or Z
X - Y = Phe - Phe, Val - Val, Trp - Val, Tyr - Val, or Phe - Leu

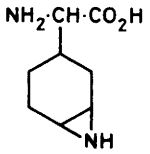
Scheme 1



(32)

Reagents: i, Ph_3P – di-2-pyridyl disulfide – MeCN; ii, H_2/Pd–C

Scheme 2



(33)

Pmp - D - Tyr(Et) - Phe - Val - Asn - Cys - X

Pmp = β,β - cyclopentamethylene - β - mercaptopropionic acid

(34) X = Pro - Arg - Gly-NH_2
(35) X = Pro-NH-$(CH_2)_5$-NH_2

side-chain COOH group have been confirmed[51] in the potent ACE inhibiting properties of analogues in the series (28). In a series of captopril analogues (29) based on mercaptoaroyl amino acids the need[52] for a β-relationship between mercaptan and amide carbonyl was found necessary for potency. Replacement of the SH group by NO_2, OH or CO_2H proved non-productive, but substitution of the aromatic ring by small polar groups resulted in the N-3-chloro-2-mercaptobenzoyl derivative having the most activity (I_{50} = 0.28 μm).

A conformational study[53] using X-ray techniques and n.m.r. studies of the bicyclic octahydropyridazo [1,2-a]pyridazine diones as models for alanylproline and captopril shows that the molecules exist in a rigid chain-twist boat conformation, mimicking a relatively high-energy conformation of Ala-Pro, not optimal for binding.

5.2 Renin Inhibitors. - The key role of renin in the blood-pressure-regulating renin - angiotensin cascade has justified an intense effort to devise successful inhibitors. Tactical themes emerging seem to fall into three main themes: peptide analogues of segments of the natural substrate; peptides combining the substrate sequence of the natural inhibitor pepstatin; peptides with groups in place of the scissile bond of the substrate. A pecking order of potency is slowly emerging in the series based on statine [(3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid] containing peptides. At the beginning of the year (see last year's report) the most potent was based on Boc-Phe-Phe-Sta-Leu-Phe-NH_2. This has now been superceded[54] by Boc-Phe-Phe-Sta-Leu-$NHCH_2Ph$ (200 x more potent), which in turn has been beaten by (30), which is a further three orders of magnitude more potent (K_1 versus human kidney renin, 2.6 x 10^{-11}M). Analogue (30) was also shown to have a strong affinity for an unknown component in human plasma, which renders it undetectable by h.p.l.c. Molecular modelling studies have successfully predicted[55] that substitution of the cyclohexyl analogue of statine, (3S, 4S)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA), should improve potency. The analogue N-isovaleryl-His-Pro-Phe-His-ACHPA-Leu-Phe-NH_2 was an even better renin inhibitor, with over 50 times the potency of analogue (30). Several new aldehydic peptides have been synthesised[56] as tetrahedral transition-state analogues. The synthetic route and typical structures are summarised in Scheme 1 and include analogues that were more inhibiting than pepstatin. A new source of renin inhibitors has emerged[57] as a result of the synthesis of protected dipeptide amides derived from amino glycols. Thus,

Boc-Phe-Leu-NHCH(CH_2Ph)CH(CH_2OH)OH and its His^2 analogue both inhibit human renin *in vitro*.

5.3 Other Types of Inhibitors. - In the development of new analgesics, an alternative approach to building in enzyme resistance into the enkephalin structure is to develop potent and specific inhibitors of enkephalinase. N-Phosphorylated dipeptides have been synthesised[58] for this purpose by reacting dipeptide benzyl ester benzene sulfonates with dibenzylphosphoryl chloride followed by hydrogenolysis. N-Phosphorylated-Phe-Ala-NH_2 prepared in this manner gave a potentiation and prolongation of the analgesic effect of Met-enkephalin. Studies on the inhibition of elastases at a molecular level have resulted[59] in the synthesis of protected dipeptides which are reversible and specific inhibitors of human leucocyte elastase. A typical structure of an adamantyl sulfonyl derivative is summarised in (31). A series of peptides based on the proteinase inhibitor chymostatin have been tested[60] for their toxicity and ability to suppress protein degradation in the isolated mouse diaphragm. The di- and tri-peptide aldehydes used, e.g. Z-Arg-Leu-Phe-H and their semicarbazone derivatives, show very similar inhibitory activities. An interesting synthetic route[61] summarised in Scheme 2 has been developed for the synthesis of (±)-tabtoxinine-β-lactam (32), which is a potent inhibitor of glutamine synthetase. Studies on glutamine analogues as inhibitors of glucosamine-6-phosphate synthetase have initiated a successful scheme for the synthesis[62] of the N-benzyloxycarbonyl derivative of 3,4-iminocyclohexyl glycine (33). This glutamine analogue exhibits a weak inhibitor activity with regard to glucosamine synthetase. Although not strictly in the field of enzyme inhibition, it is interesting to note[63] that inhibitory effects on the cross-linking reaction of α_2-plasmin inhibitor to fibrin in the presence of Factor XIIIa can also be effected by the N-terminal pentapeptide sequence of α_2-plasmin inhibitor, H-Asn-Gly-Glu-Gln-Val-OH.

The potential of using computer simulation to analyse the interactions of three haloenol lactone suicide inhibitors with α-chymotrypsin has been investigated[64]. To study the process, a 115 non-hydrogen atom model of the active site of chymotrypsin was constructed with co-ordinates taken from X-ray data. A systematic conformational analysis and energy minimisation of the mechanism were performed at 3 stages - Michaelis complex stage, acyl enzyme alkylation and suicide compound bis-adduct. The method confirms the

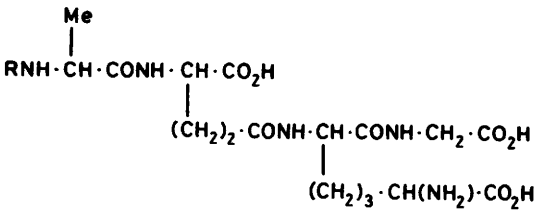
established mechanism involving an intermediate acyl enzyme which alkylates the His-57 residue inactivating the enzyme.

6 Side-Chain Interactions Studied by Residue Substitution or Deletion, and Related Modifications

The considerable contribution of the Prague Laboratory of Peptide Chemistry to the development of vasopressin analogues with high and specific antidiuretic activity has been reviewed.[65] The work highlights the development of the arginine vasopressin analogue DDAVP and includes a philosophical statement worth quoting: "It is better to follow an imperfect road leading to the target, than a perfect one which does not lead anywhere". In an attempt to design vasopressin receptor antagonists, the class of antagonists previously described by Manning and co-workers have been taken as modifiable template molecules. Thus the X portion of the sequence in (34) has been replaced[66] by a lysine analogue as in (35), which retains good activity and confirms the hypothesis that a minimum requirement for activity is a basic group outside the ring.

The possible existence of intramolecular interactions involving the Tyr and His residue in angiotensin II has been investigated[67] by measuring activities of the functional groups in the molecule. The findings are consistent with the presence of a Tyr-hydroxyl-His-carboxylate charge relay system in angiotensin II in an aqueous environment. The requirement for an OH function in the N-terminal position of FK-156 has been confirmed[68] by the synthesis and resulting adjuvant activities of the analogues (36)-(38).

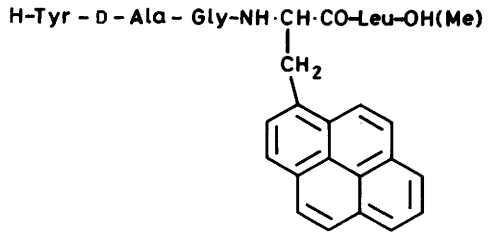
The N-terminal tetrapeptide of dermorphin, H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2, has been recognised already as the minimum sequence for opioid activity. The H-Tyr-D-Arg-Phe-Gly-OH analogue of the tetrapeptide showed[69] five times the potency of morphine, while replacement of Gly^4 by Sar or D-Ala enhanced stability to peptidases. Only amino acids with small side chains could be tolerated as analogues in position 4. Extending the length of the Leu^5-enkephalin peptide sequence by one residue does not appear[70] to make a significant effect on opioid activity. Although detailed pharmacological results have not yet appeared, considerable attention has been focussed[71] on improving the hydrophobic properties of the enkephalin through the synthesis of a series of amides Tyr-D-Met-Gly-Phe-Pro-$NH(CH_2)_n CH_3$, where n is 7, 9, 11 or 13. A photoreactive enkephalin analogue, [D-Ala^2,p$N_3$$Phe^4$-$Met^5$]-



(36) R = HO·CH$_2$·CO—

(37) R = Ph·CHOH·CO—

(38) R = HO·CH$_2$·CHOH·CHOH·CHOH·CHOH—



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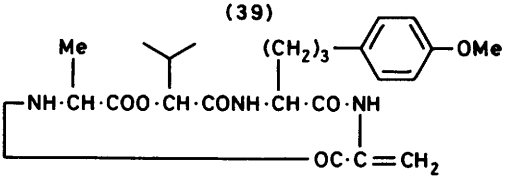
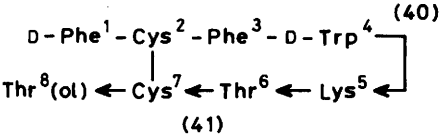


(40)



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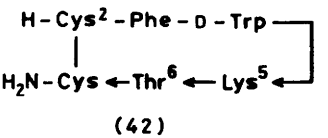

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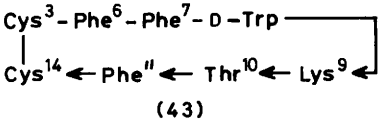

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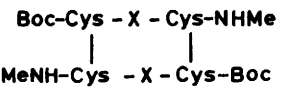
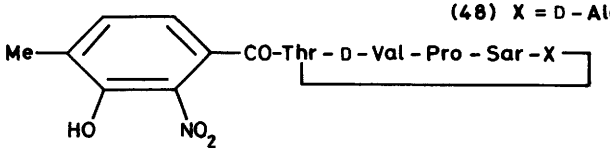


(47) X = L-Ala

(48) X = D-Ala



(49) X = MeVal or MeAla

enkephalin, shows promise[72] in photoaffinity labelling of the receptor site as it has four times the binding efficiency of the parent enkephalin. The fluorescent amino acid L-1-pyranylalanyl(Pya) has also been incorporated[73] into [D-Ala, Leu^5]-enkephalin, giving (39) and the analogue substituted in position 5, and they both show strong fluorescent intensity and strong binding affinity to opiate receptors. The Pya^4-enkephalin shows high specificity and selectivity for μ-receptors while the Pya^5 analogue has specificity for δ-receptors. Three synthetic analogues of human β-endorphin (β_hEP), namely [Gln^8Gly^{31}]- β_h-EP-Gly-Gly-NH_2, [$Arg^{9,12,24,28,29}$]-β_hEP and [$Cys^{11,26}Phe^{27}Gly^{31}$]-$\beta_h$-EP, have been found[74] to possess potent inhibiting activity to β_h-EP-induced analgesia. The terminal methionine site in substance P, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2, appears to be very important for activity. Analogues [Arg^{11}]-SP and SP(1-10)-Arg-OH both showed[75] diminished potency. The smallest fragment of the C-terminal tetrapeptide of gastrin exhibiting antagonist activity has been found[76] to be Boc-L-Trp-L-Met-Asp-NH_2. In AM Toxin-I (40) it has been found from studies[77] on the analogues [L-2-aminobutanoic $acid^1$] -, [L-Leu^1]- and [L-Phe^1]-AM Toxin I that considerable variation in the size of side chain at position 1 is allowed, for the induction of necrotic activity for apple leaves. A calcitonin analogue MCT-II having the potential to form an amphiphilic α-helix from residue 8-22 with a continuous surface of aliphatic Leu side chains has been synthesised[78]. MCT-II has minimal sequence homology to any natural calcitonin but its biological activity is similar to that of salmon calcitonin. Details of the syntheses of a protected nonapeptide of the mycobacillin 8-13-1-3 sequence and an open-chain tridecapeptide analogue of the 4-13-1-3 sequence have appeared.[79] Tryptophyllin-7 analogues have also been synthesised[80].

The development of new tools for studying receptor localisation and dynamics in tissue slices and target cells has been investigated[81] using α-melanotropin conjugates. Introduction of a 5-bromopentanoyl unit instead of the acetyl group of the natural hormone followed by treatment with sodium thiosulphate gave N-deacetyl-N-[5-(sulfothio)valeryl]α-melanotropin. This was then used to prepare tobacco mosaic virus/α-melanotropin conjugates containing up to 330 disulfide-linked peptide molecules/virion. The conjugates proved to be superpotent agonists for tyrosinase stimulations in Cloudman S-91 melanoma cell cultures but were inactive for cyclic AMP accumulation. The possibility of using micelle-like polymers of 10-undecenoate as carrier for

small peptide hormones has been investigated through binding studies[82] of the polymer with tryptophan peptides.

7 Conformational Information Derived from Physical Methods

The application of high-field n.m.r. techniques continues to be the sector which is expanding in scope and usage. It is almost routine for practitioners in this field now to use 2D techniques, augmented by COSY and other programmes to make signal correlations. Conformational information then follows from chemical-shift/temperature studies on the amide hydrogens and on NOE difference spectroscopy of protons on the peptide backbone. In the next section on n.m.r. studies it will be assumed that these approaches will be the main ones used in the papers, and no specific reference will be made to them in each context.

7.1 N.M.R. Studies. - A potent analogue of somatostatin SMS 201-995 (41) containing the essential pharmacophore in a restricted conformation has been studied[83] at 500 MHz in both aqueous and d_6-DMSO solutions. In water the molecule is rather flexible with no evidence for a β-turn structure involving Thr^6. Two β-turns stabilised respectively by Cys^2-D-Trp^4 and Phe^3-Lys^5 H-bonds are believed to be responsible for the large upfield shift observed for the Lys^5 γ-protons. A less flexible situation exists in d_6DMSO, with a predominance for a β-turn type II' conformation involving residues Phe^3 to Thr^6. A similar study[84] on the basic sequence SMS 201-456 (42), which only shows low biological activity, highlights a fundamental difference in conformation between SMS 201-995 (41) and SMS 201-456 (42). In the former the β-turn conformation is stabilised by the additional intramolecular H-bond between D-Phe^1 and Thr^8(ol). Conformational studies[85] at 500 MHz in water on the cyclic octapeptide somatostatin analogue (43) with an intermediate ring size confirm that the analogue's conformation remains very flexible. The somatostatin molecule itself, Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys, has also been studied[86] at 500 MHz to detect any side-chain interactions which might be present in aqueous solution. Ring current efforts have been found in the 2-phenyl protons of Phe^6 and Phe^7 but no shifts in Phe^{11} protons, suggesting that a folded structure favouring Phe^6-Phe^{11} ring interactions can be excluded.

The synthesis of stereospecifically deuterated glycine analogues of the eosinophil chemotactic tetrapeptides H-Val-(S)-[α^2H_1]-Gly-Ala-Glu-OH has

allowed[87] the assigments of the ^{1}H resonances, which confirm a type I β-turn can be adopted by all the analogues in the series investigated in DMSO solutions. Quite considerable differences were noticed in the extent of conformational averaging, yet the biological activity remained equivalent in all analogues. Thus the conformation of the peptides when bound to the receptor must be the important one for activity. An excellent example of the details now possible from homo- and heteronuclear n.m.r. techniques augmented by ^{15}N-n.m.r., all measured at 300 MHz in $CDCl_3$ and d_6-benzene solutions, has been reported[88]. Two techniques using coupling constants across amide bonds have been applied to obtain all the assignments: (i) a ^{1}H, ^{1}H-COSY spectrum optimised for small coupling constants to detect long-range couplings from NMe groups to both α-protons, (ii) a ^{1}H, ^{13}C-COSY spectrum optimised for C,H long-range couplings to the eleven CO groups yields coupling to both α-protons attached to that amide bond. Three cyclic pentapeptides cyclo(-D-Phe-L-Pro-Gly-D-Ala-L-Pro-) (44), cyclo(-D-Phe-Gly-Ala-Gly-Pro-) (45) and cyclo(-D-Phe-Gly-Val-Gly-Pro-) (46) have been analysed[89] by high-field n.m.r. techniques both in the solid state and in solution. Compound (44) appears to be the same in both states. At first glance there are almost no similarities between the solid and solution spectra of (45). There is a large difference in $\Delta\delta_{\beta\gamma}$ proline shifts for solution and the solid phase. In solution, molecule (45) is probably undergoing averaging between two conformations with Pro in either a β- or γ-turn, but in the solid the Pro appears in a γ-turn. The phenyl rings of crystalline (45) and (46) undergo relatively slow ($10\text{-}10^{2}$Hz) re-orientation about the C_β-C_γ-axis, although the ring in (44) is static on this time-scale. The cross-polarisation magic-angle spinning method used in solid-state n.m.r. has also revealed[90] that ^{13}C-chemical shifts derived from ^{13}C-enriched [1-^{13}C]glycine residues placed in homo polypeptides are sensitive to whether the conformations are α-helix, β-sheet, 3-helix or ω-helix.

Both dermorphin and its [L-Ala^{2}] analogue have been investigated[91] in aqueous and DMSO solutions by means of ^{1}H, ^{13}C-n.m.r., c.d. and u.v. spectroscopy. pKa's for the α-amino groups and the phenolic groups have been determined and the inference made that both peptides exist in d_6-DMSO as rapidly interconverting conformers, with a β-sheet conformation stabilised by intermolecular association being present at the high concentration required for n.m.r. measurements. Strong H-bonding between the amide proton of Phe^{7} and the CO group of Pro^{4} is suggested[92] from the 350 MHz n.m.r. of physalaemin, Glp-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2. High-resolution ^{1}H n.m.r. at

300 MHz of human gastrin and its des-Glu[1] analogue reveals[93] that in gastrin the ratio of cis to trans conformers around the Gly[2]-Pro[3] bond is 3:7. Addition of Na^+ or Ca^{2+} causes only minor changes in the spectrum but the paramagnetic Co^{2+} produces a number of changes reflecting a probable strong binding of the Co to Glu residues with weaker bonding to Asp-16. The conformation of the 1:1 complex of Val[1]-gramicidin A with Cs atoms has been determined[94] in methanol/chloroform solutions using 2D ^{1}H-n.m.r. A right-handed antiparallel double-helical dimer $\uparrow\downarrow\pi\pi_{LD}^{7.2}$ with 7.2 residues per turn incorporating 2 Cs atoms is implied by the data. ^{13}C-N.m.r. spectroscopy has been utilised[95] in the analysis of various segments during the unequivocal synthesis of alamethicin. There is no indication either of long 3_{10}-helical segments or of β-structures in chlorinated or alcoholic solvents.

The conformational behaviour of polyoxyethylene (POE)-bound model peptides Boc(L-Ala)$_2$-X-Y-(L-Ala$_2$)NHPOE (X-Y = L-Pro-Gly or Gly-L-Ile) as well as the repetitive hexapeptide of elastin, Boc-L-Val-L-Ala-L-Pro-Gly-L-Val-Gly-A-NHPOE-M (where A = photosensitive 3-nitro-4-(bromo methyl)benzoyl group), has been studied[96] by ^{1}H-n.m.r. and c.d. spectroscopy. β-Turn structures have been proposed involving Pro and Gly in the (i + 1) and (i + 2) positions respectively. N.m.r. studies have also confirmed[97] a C_2 symmetric structure possessing 4 intramolecular bonds for the 22-membered cyclic bis(cystine peptides) (47) and (48). N.m.r. data and results from i.r. and X-ray investigations have been used[98] in detailed β-folding analysis of the model dipeptides t-BuCO-L-Pro-Xaa-NHMe. In solution the β-I turn allows an attractive interaction between the Xaa NH bond and the polar or aromatic part of the Xaa side chain. For D-Xaa residues a β-II-turn conformation exists in solid and solution.

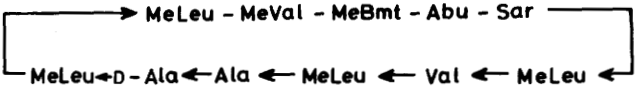
The actinomycin-related peptide lactones (49) have been subjected[99] to n.m.r. and X-ray analysis, and two conformers (A) and (C) have been found to exist in various solutions. The complex situation has been rationalised in terms of associated conformational pairs $A_2 \rightleftharpoons 2C$ in dry $CHCl_3$, conformer C in wet $CHCl_3$ and A in dry acetone. Replacement of MeVal by MeAla, which lowers biological activity, is not shown up as a conformational change. All the ^{13}C-resonances for the tripeptide glutathione (L-γ-glutamyl-L-cysteinyl-L-glycine) have been assigned[100] and their behaviour in the pD range 0.7-12.3 investigated. It is possible to assume that the conformation of glutathione at physiological pH is similar to that at pD 3.25. Protein-peptide interaction studies are often hampered in n.m.r. work by overlap between peptide and

protein resonances. It now appears[101] that by using the per-deuterated peptides of Phe-PheNH$_2$ and Leu-PheNH$_2$ the identity of bovine neurophysine-I residues at the hormone-binding site can be probed. Analysis of changes induced in the protein's spectrum reveals unique differences between the two peptides in their effects on the Tyr-49 ring in the protein, which confirms the proximity of this aromatic ring to the side chain of the bound peptide. The assignment of the aromatic ^{1}H-n.m.r. resonances of the 4 tyrosine residues of bovine-zinc insulin has been made[102] using double-resonance techniques and examination of specific derivatives nitrated at tyrosine A.14 and A.19. The n.m.r. data are consistent with the interpretation of Blundell and co-workers of the monomer and dimer obtained in insulin crystals. Preliminary n.m.r. evidence[103] indicates that the peptide backbone in the antifreeze glycopeptide (Galβ1-3-Gal$\underline{\text{N}}$Acα1-Thr-Ala-Ala)$_{\underline{n}}$ lies in an extended helical conformation.

The cation complexation sites in [(4'-amino)Phe^4, Leu^5]-enkephalin when complexed with Eu^{3+} have been identified[104] by ^{1}H-n.m.r. as the Leu^5-carboxyl group and probably the Phe amide carbonyl group. The modes of Ca^{2+} and Eu^{3+} binding were also investigated by the spectrofluorometric method, which indicated that complexation increased the distance between the Tyr^1 and Phe^4 aromatic rings. Calcium and praseodymium complexes of the tetrapeptide Ac-Asp-Val-Asp-Ala-OH have been used[105] as models for the Ca^{2+} binding sites of proteins such thermolysin and calmodulin. The metal ions bind predominantly to both CO_2H groups of the two Asp residues in bidentate fashion. The preferred conformations of two cyclised dipeptides in d_6DMSO, cyclo(L-Ala-L-Ala-ε-amino-caproyl-), have been elucidated[106] by 2D n.m.r. and NOE effects and comparison with computed low-energy conformations. The predominant conformations are type III and a type II bend respectively for the two compounds.

^{17}O-N.m.r. chemical shifts of linear peptides with and without protecting groups in a variety of solvents have been found[107] to be 256-350 ppm downfield from external water. ^{17}O-Resonances of cyclic dipeptides appear at higher field relative to linear analogues (303-317 ppm compared with 327-337 ppm).

7.2 X-Ray and Related Techniques. - X-Ray data on cyclosporin A (50) in the solid state, which is in a twisted β-pleated sheet, with a β-bend at Sar-MeLeu^4 of type II' and the loop having a cis-peptide bond between MeLeu^9 and MeLeu^{10}, have been compared[108] to the situation in solution. The main difference between crystal and solution is the orientation of the side chains



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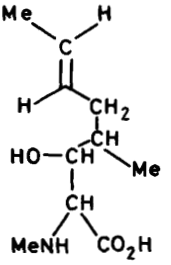
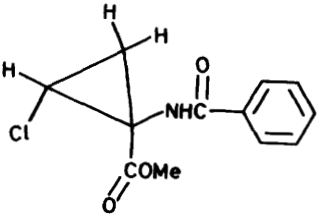


(51)

H_2^+-(L-Val)$_y$-(L-Ala)$_2$-(L-Val)$_3$-NH·PEGM
Cl^-- " -(L-Leu)$_2$- " -" · "
" -(L-Ile)$_2$- " -" · "

(52) $y = 0-5$



(53)

of the unusual amino acid MeBtm (51) and of MeLeu[10], which is explained as the breakdown of the intermolecular H-bond of the OH group of MeBtm on dissolution of the crystal. A crystal structure determination[109] of Tyr-D-Nle-Gly-Phe-NleS, where NleS = $MeCH_2CH_2CH_2CH(NH_2)SO_3H$, has been carried out to clarify the solid-state conformation of the enkephalins. The molecules in the crystal display similar 2'-β-bend conformations with an intramolecular H-bond between Tyr CO and NH of Phe. In another molecule a second H-bond exists between $TyrNH_3^+$ and the CO of Phe. The crystal structure of the sweetener L-Asp-L-PheOMe(aspartame) reveals[110] a columnar polymeric arrangement of molecules which might explain its unexpected low water solubility and fibre-forming tendency. The molecule is in the all-*trans* amide form with a positively charged N-terminus and a negative aspartyl side chain. It is highly hydrophilic on the aspartyl side and hydrophobic on the Phe side. Another set of model peptides[111] are organised in double layers: a hydrophobic layer containing the Me groups of Ala and a hydrophilic layer with side chains of Lys or Tyr. The X-ray data on AcLys-X-Tyr-Ala-Lys-NHEt (X = Ala, D-Ala), AcLys-Ala-Lys-Ala-LysNHEt and Ac-X'-Ala-LysNHEt (X'=Lys, Tyr) all show a cross β-structure with antiparallel organisation of the peptide chains.

X-Ray details[112] from cyclo(-D-Ala-Gly-Pro-D-Phe-)$_2$ reveal a C_2 backbone symmetry containing *trans* peptide bonds. Type II β-turns at L-Pro-D-Phe followed by type III' turns at D-Phe-D-Ala exist in the crystal. An n.m.r. analysis shows a 40° rotation of the plane of the Phe-Ala bond relative to the local α-carbon planes. The first cyclic pentapeptide with an LLDLL chiral sequence, cyclo(-Ala-Pro-Gly-D-Phe-Pro-), to be studied[113] by X-rays shows no intramolecular H-bonds and possesses a *cis*-Ala-Pro bond. In solution in DMSO n.m.r. studies show the same *cis* form but in $CDCl_3$ it changes to an all-*trans* form with probably a type II Pro-Gly β-turn. In the peptide lactones cyclo(-L-2-hydroxyisovaleryl-L-Pro-L-Pro-) and cyclo(-D-2-hydroxyisovaleryl-L-Pro-L-Pro-), prolyl residues possess approx. C_2 symmetry in the L-L-L form and C_S symmetry in D-L-L analogue[114]. All peptide bonds adopt *cis* conformation and the two lactones *trans*. In the cyclic dipeptide of dehydrophenylalanine [3,6-bis(phenylmethylene)piperazine-2,5-dione] the piperazine-dione ring is planar[115] while the cyclic dodecapeptide cyclo(-L-Val-L-Pro-Gly-)$_4$ contains two β II-turns.[116]

7.3 I.R. and C.D. Studies. - FT-IR and laser Raman investigations[117] on Leu[5]- and Met[5]-enkephalins in solution support the earlier evidence obtained from

n.m.r., X-ray and c.d. studies. Leu^5-enkephalin in aqueous solution exists in both type II β-turn and β-sheet structures whereas Met^5-enkephalin has a lesser tendency to form a β-sheet structure. The smallest possible sequence where a β-turn might exist, namely a dipeptide RCO-X-Y-NH-R', has undergone[118] i.r. spectroscopic analysis. An estimate of the β-folding ratio in CH_2Cl_2 solutions can be made by analysing the NHMe absorption band at 3450 cm^{-1}, knowing its molar extinction coefficient. In dipeptides of Ala, Gly, Pro, Ser and His quite good correlation is found between β-folding ratios and the difference in the n.m.r. chemical shifts of the C-terminal NH proton. When the observed Raman/i.r. bands of cyclo(D-Phe-L-Pro-Gly-D-Ala-L-Pro), which contains a β-turn, have been compared[119] with amide modes predicted from calculations, good correlation was obtained between the two approaches. I.r. absorption band studies[120] in the solid state of a series of N-t-Boc-N(π)-benzyloxymethyl homo-oligo-L-His methyl esters, ranging in size from di- to hepta-peptides, reveal that the highest oligomers adopt an intermolecularly H-bonded β-structure.

C.d. studies[121] on synthetic peptides representing the signal sequences of chicken lysozyme, Escherichia coli proteins and other proteins have been carried out in trifluoroethanol. α-Helical conformational features are discernible even in 12-residue fragments.

Conformational influences of making amino acid insertions into homo-oligopeptides consisting of L-valyl residues have been studied[122] using c.d./i.r. techniques and using polyethyleneglycol monomethyl ether (PEGM) of the peptide series (52). The tendency to form β-structures and to aggregate increases with increasing similarity in the spatial size of the side chains inserted. C.d. studies[123] show that only biologically active analogues of calcitonin were able to quantitatively solubilise dimyristorylphosphatidyl glycerol, and in this solubilized form the peptides have a higher helical content.

7.4 Computational Methods. - Molecular dynamics and energy minimisation techniques[124] applied to the molecule H-Cys-Tyr-Phe-Glu-Asn-Cys-Pro-Lys-Gly-NH_2(Lys-vasopressin) show a flexible peptide structure able to undergo spontaneous conformational transitions, even within the constraints of the cyclic ring structure. A new buildup and energy minimisation procedure for computing the backbone conformation of Met-enkephalin has been compared with a Monte Carlo approach,[125] and the agreement shown indicates that the

multiple-minima problem has been overcome. In the same work a Monte Carlo algorithm based on importance sampling has been tested. The energy profile for the interaction of Ca^{2+} with gramicidin A channel has been computed[126] and shows that there is a very low permeability, if any, of gramicidin A for Ca^{2+}. The solvent-accessible surface areas of the atoms in tripeptides around the minimum-energy conformations of β-bends types I, I', II and II' have been computed[127] as a first step in the study of solvent accessibility of secondary structures. Comparisons between this methodology with observations from the crystal structures of six cyclo-hexapeptides showed that all the β-bends could be identified by the computational method. In order to assist in the design of potent peptide hormones, conformational calculations have been carried out[128] on cyclopropyl amino acid derivative (53), whose relevance in conformational restriction has been reported elsewhere in this chapter. Selective substitutions on the C^{β}-atom may be used effectively to restrict ϕ or ψ angle values into a narrow range.

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4

Cyclic, Modified, and Conjugated Peptides

BY P. M. HARDY

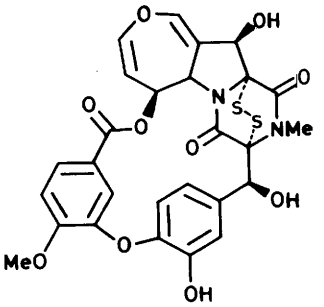
1 Introduction

The introductory remarks made in Vol.17 apply also to this report. The subdivisions of topics remain unchanged except for the omission of phosphonopeptides as a separate section and the conflation of the sections on α-carboxyl and side-chain conjugates. Noteworthy this year is the larger number of papers appearing on 2,5-dioxopiperazines and cyclic tetra-, penta-, and hexapeptides; their versatility in a number of roles appears evergreen. Overall the number of papers covered in this review remains similar to last year's.

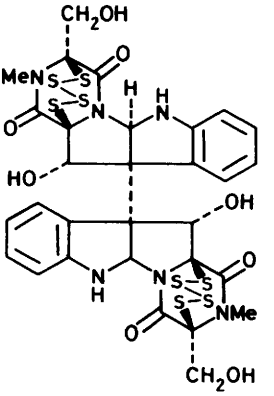
2 Cyclic Peptides

2.1 Dioxopiperazines (Cyclic dipeptides). - Three new naturally occurring cyclic dipeptides have been reported. One is a macrocyclic epidithiodioxopiperazine (1) from Emericella striata whose structure was determined by an X-ray examination of its methanol solvate. Derived biogenetically from two molecules of phenylalanine and a benzoate, it has strong antifungal activity.[1] Chetracin A, isolated from three Chaetomium species, is a symmetric dimer (2) containing tetrasulphide bridges. X-Ray work confirmed the n.m.r. assignment of the structure; the dioxopiperazine rings are in the boat conformation, but are more planar than is the case with disulphide bridges.[2] Nigrifortine (3), a metabolite of Penicillium nigricans, is the first recorded example of a prenylated dioxopiperazine fungal product in the form of an indolic symmetrical dimer. It has no apparent toxic effect when given intraperitoneally in mice at up to 40 mg/kg.[3]

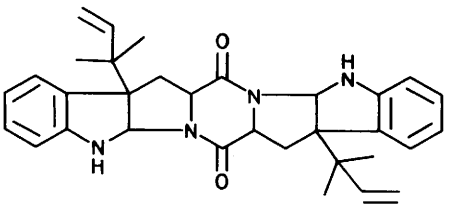
Two heterodetic cyclic dipeptides containing a 15-membered ring have been isolated from Streptomyces griseorubiginosus. These compounds, biphenomycins A and B (4), have potent antibiotic activity against Gram-positive bacteria.[4] Feeding mixtures of [2',4',6'-$^{3}H_{3}$]-m-tyrosine and [1-^{14}C]-phenylalanine to cultures of Gliocladium deliquescens gives good incorporation of ^{14}C but little



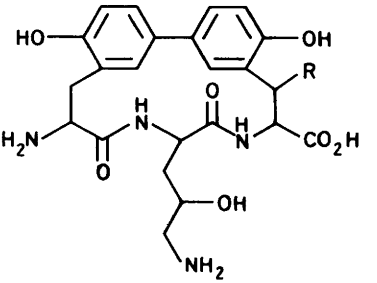
(1)



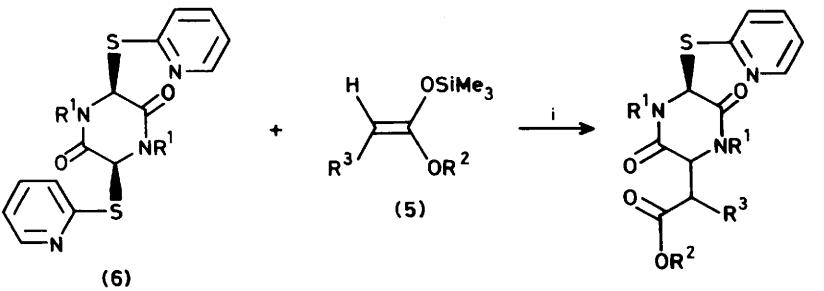
(2)



(3)



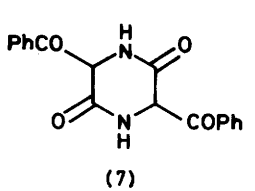
(4) A; R = OH
B; R = H



(6)

Reagent: i, M^+

Scheme 1



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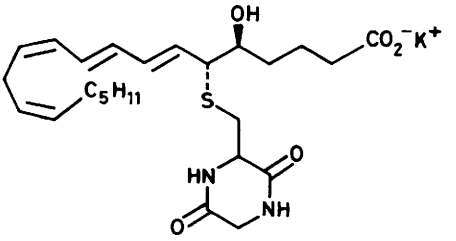

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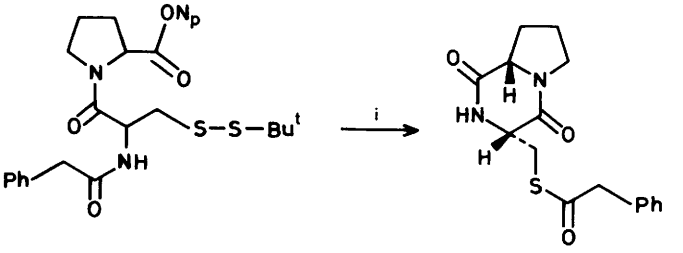
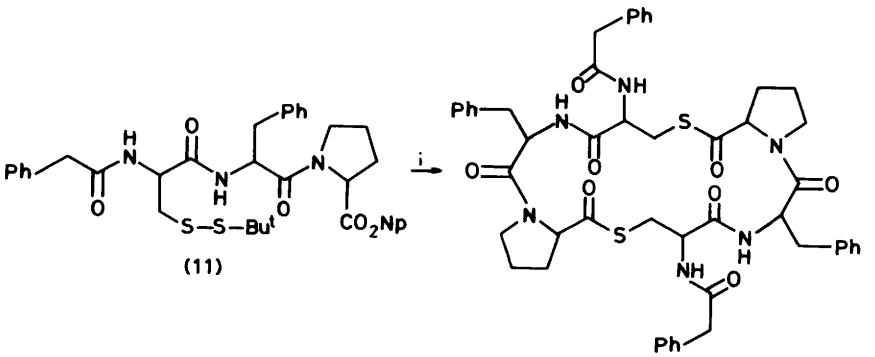


(9) (10)

Reagent: i, PBu^n_3

Scheme 2



(11) (12)

Reagent: i, PBu^n_3

Scheme 3

of ^{3}H into gliotoxin, and incorporation of [3'-^{3}H]-, [2'-^{3}H]-, and [4'-^{3}H]-phenylalanine occurs without loss or migration of tritium. These results show that neither m-tyrosine nor any other hydroxybenzene derivative can be an obligatory intermediate in the biosynthesis of gliotoxin.[5]

Cairomycin A (6-isopropyl-2,5-dioxopiperazine-3-acetic acid) has been synthesised by cyclisation of Asp(OBzl)-Val-OMe and subsequent catalytic hydrogenation. The product has the same inhibitory action on S.Aureus and E.coli as the natural material.[6] A novel general approach to the synthesis of unsymmetrical 3,6-disubstituted 2,5-piperazinediones features the coupling of ketene trimethylsilyl acetals (5) with the bis-(2'-thiopyridyl) derivative (6) in the presence of thiophilic metal salts (Scheme 1; first coupling shown). In no case was 3,6-bis-coupled product observed in the first stage. This method was developed because of the poor nucleophilicity of 2,5-dioxopiperazine enolate anions towards more highly functionalised electrophiles.[7] Cyclo(-Phe_2-) has been converted to the dibenzoyl derivative (7) by a selective $FeCl_3$-H_2O-u.v.-promoted photo-oxidation,[8] and a leukotriene D_4 analogue (8) containing a dioxopiperazine ring in place of the peptidyl fragment has been synthesised. It retains one-tenth of the biological activity of the parent, and since it is thought that in vitro ring opening is not taking place this suggests that cisoid geometry could be preferred at the Cys-Gly amide bond.[9]

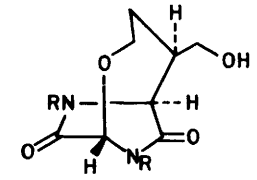
While the N-phenylacetyl-Cys-Pro derivative (9) on S-deprotection gives the S-phenylacetyl-dioxopiperazine (10) (Scheme 2), a corresponding reaction with a similarly protected Cys-Phe-Pro compound (11) gave the dimeric thiolactone (12) (Scheme 3). Replacement of the N-phenylacetyl group of (11) with an N-chloroacetyl group, however, leads to formation of the cyclol (13) on S-deprotection.[10] The synthesis of cyclic dipeptides from dipeptidyl derivatives of the resin α-(4-bromomethyl-3-nitro-benzoylamino)-benzyl-copoly(styrene-1% divinylbenzene) by treatment with 0.3M ethyldi-isopropylamine has been explored. Better (70-91%) yields than earlier polymer methods are claimed, with no detectable racemisation.[11] All four stereoisomers of cyclo(-His-Pro-) have been prepared from the trifluoroacetate salts of the corresponding Pro-His-OMe stereoisomers using potassium bicarbonate in refluxing methanol. Any excess of base over that required for neutralisation caused racemisation of both amino acids. The dioxopiperazines were

tested in rats to see if they significantly reduced food intake, but contrary to earlier reports none had any appreciable effect.[12]

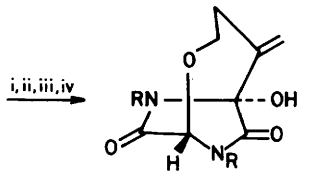
Full details of a completely regio- and stereo-controlled total synthesis of bicyclomycin (14) in twelve chemical steps have been published. The later stages of the synthesis are outlined in Scheme 4.[13] A brief outline of a seventeen-step chiral synthesis of bicyclomycin from N,N'-diacetyl-cyclo(-Gly_2-) has also appeared;[14] details of the synthesis of an intermediate (15) used in this synthesis have been reported.[15] The basic bicyclic nucleus (16) of bicyclomycin has also been prepared for the first time and found devoid of antimicrobial activity. On the basis of some synthetic analogues, it has been concluded that the entire structure of bicyclomycin is generally necessary for activity, although the racemic analogue (17) shows an effectiveness against Gram-positive organisms of the same order as bicyclomycin itself against Gram-negative microbes.[16] The mechanism by which bicyclomycin irreversibly forms covalent bonds to the inner-membrane proteins of E.coli may involve reaction of the olefinic bond with protein sulphydryl groups. Thiolate addition of bicyclomycin and some analogues has now been examined in detail, and the findings are consistent with the hypothesis that ring opening to an αβ-unsaturated ketone (18) is the first step; this then adds thiolate and regains the cyclic structure (Scheme 5). However, a lack of correlation between thiolate susceptibility and biological activity indicates that this reaction alone is not the whole story.[17]

Oxidative removal of the N-(4-methoxybenzyl) group from 2,5-piperazinediones with cerium(IV) diammonium nitrate under mild conditions has been further explored (cf. Scheme 4). Alkylidene derivatives, however, undergo oxidative addition.[18] The asymmetric synthesis of two amino acid esters with a 3,4-epoxy function via the bis-lactim ether route has been achieved; chloroacetone or ω-chloroacetophenone was added to the lithiated bis-lactim ether of cyclo(-Val-Gly-), followed by epoxidation (using hydroxide treatment) and 0.1M HCl hydrolysis. Only the 3R-addition products were obtained.[19] Reaction of the same metallated bis-lactim ether (19) with hexachloroethane gave a 94:6 mixture of the cis:trans isomeric chlorides (20) in ca. 90% yield. With sodium dialkyl malonates, (R)-β-(alkoxycarbonyl)aspartic diester derivatives (21) are subsequently obtained (Scheme 6).[20] The bis-lactim ether route has also been used in the enantioselective synthesis of

(13)



R = *p* - methoxybenzyl



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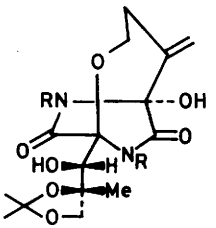
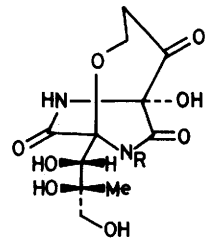
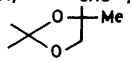
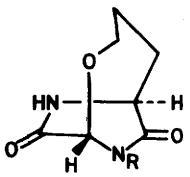




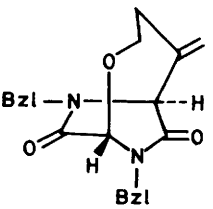
(14)

Reagents: i, CF_3SO_2Cl, NEt_3; ii, $NaBH_3SePh$; iii, 30% H_2O_2; iv, Bu^nLi, HMPA, $(Me_2N)_3P$ then O_2; v, Bu^nLi; vi,  ; vii, $(CF_3CO)_2O$, *p* - dimethylaminopyridine; viii, $Ce(NH_4)_2(NO_3)_6$

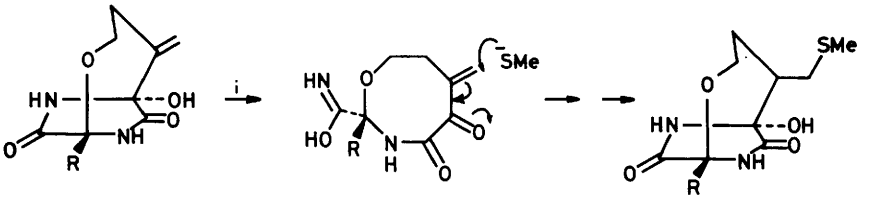
Scheme 4



(16)



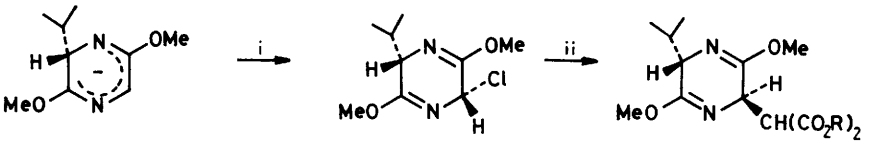
(17)



(18)

Reagent: i, pH 12·5

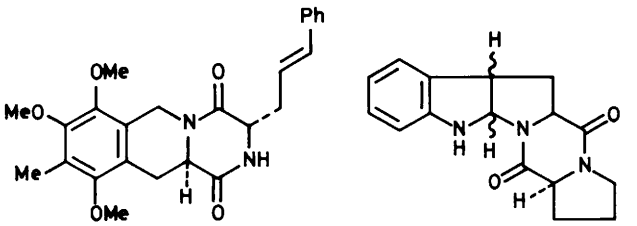
Scheme 5



(19) (20) (21)

Reagents: i, C_2Cl_6; ii, $\bar{C}H(CO_2R)_2$

Scheme 6



(22) (23)

(R)-α-MeTrp-OMe [21] and a monolactim ether alkylation used to construct a model (22) of naphthyridomycin.[22]

On treatment with trifluoroacetic acid or 85% phosphoric acid, cyclo(-Pro-Trp-) forms two diastereoisomers of the 'cyclic tautomer' (23); cyclo(-D-Pro-Trp-) and cyclo(-N-MePhe-Trp-) do not undergo this type of cyclisation, but cyclo(-N-Me-D-Phe-Trp-), cyclo(-Ala-Trp-), and cyclo(-D-Ala-Trp-) do. The cyclisation products of the Ala compounds were obtained as single isomers, the stereochemistry at the junction of the five-membered rings being unknown.[23] In the hydrolysis of p-nitrophenyl laurate, cyclo(-D-Leu-His-) and cyclo(-D-Val--His-) are specifically effective catalysts, being much more efficient than their diastereoisomers or imidazole.[24]

On treatment with N,N'-dicyclohexylcarbodiimide at 20°C, N-trityl glycine forms N,N'-ditrityl-2,5-dioxopiperazine; the crystal structure of this compound and its 1:1 complex with CH_2Cl_2 have been determined by X-ray.[25] The crystal structure of cyclo(-Aib-Phe-) shows a folded conformation with one of the methyl groups located close to the phenyl ring. The ^{1}H-n.m.r. spectrum in trifluoroacetic acid suggests that the folding pattern is maintained in solution.[26] Pyroergotamine, N-(pyruvoyl)-cyclo(-Phe-D-Pro-), has been prepared by treating the N-trimethylsilyl derivative of cyclo(-Phe-D-Pro-) with pyruvoyl chloride. The molecule adopts a boat conformation whose high degree of buckling approaches the values found in proline-containing homochiral dioxopiperazines.[27] X-Ray analysis of cyclo(-dehydro-Phe_2-) indicates that the heterocyclic ring is approximately planar, but the styryl group deviates from planarity. The presence of different conformations in the crystal structure suggests some conformational flexibility.[28] In cyclo(-dehydro-Ala_2-), as might be predicted, the molecules are planar and linked in ribbons by two hydrogen bonds through the amido groups.[29]

As part of a study on the biosynthesis of ethylene by plants, a detailed n.m.r. study of cyclo(-Aib-Ala or Phe-) and cyclo(-Acc-Phe or D-Phe-) (where Aib = 2-aminoisobutyric acid and Acc = 1-aminocyclopropane-1-carboxylic acid) has been made. Complete assignment of the proton signals of the Acc methylene groups allows for the non-destructive, non-isotopic diluting analysis of various biosynthetically derived deuterated Acc compounds formed from the corresponding deuterated S-adenosylmethionines.[30] ^{1}H-N.m.r. spectra of cyclic dipeptides of L-pipecolic acid and Sar or Gly

indicate the dioxopiperazine ring is slightly buckled. The degree of folding increases by 2-4° if L-γ-thio-pipecolic acid replaces pipecolic acid.[31] Conformational aspects of the pyrrolidine ring of dioxopiperazines containing Pro or Hypro residues have been re-examined by ^{1}H-n.m.r. and re-interpreted.[32]

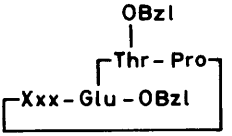
Electrostatic, inductive, and dispersion terms have been calculated for three conformations of cyclo(-Gly-Phe-). The folded form actually observed appears principally stabilised by quadrupole-quadrupole and dispersion interactions.[33] Assuming a standard backbone geometry, theoretical calculations of c.d. spectra in the π-π^* absorption region near 190 nm for a series of cyclic dipeptides containing Gly, Ala, Pro, and Val residues show fair to good agreement with experimentally measured values.[34] The effect of the hydrophobic interaction in aqueous media of tyrosine-containing cyclic dipeptides and 1-acyl-4-bromobenzenes containing various lengths of acyl chain have been investigated by the quenching of the fluorescence of the former by the latter.[35] The structure-taste relationship of the L-L and D-L forms of cyclo(-Leu-Trp-) is consistent with the model receptor proposed to account for the recognition of chirality in simple amino acids.[36]

2.2 Cyclic Tri- to Hexapeptides. - A calculated c.d. spectrum for a weighted average of low-energy conformers of the tuftsin cyclo analogue Thr-Lys-Pro-Arg closely resembles that obtained experimentally.[37] The solution conformations in DMSO-d_6 of cyclo(-Ala$_2$-ε-aminocaproyl-) and cyclo(-Ala-D-Ala-ε-aminocaproyl-) have been analysed by 2D n.O.e. The type III and type II β-bends respectively deduced agree with results obtained earlier from i.r., Raman, and c.d. spectra.[38] Two sidechain-to-sidechain cyclised opioid peptide analogues, Tyr-D-Orn-Phe-Asp-NH_2 and Tyr-D-Lys-Phe-Glu-NH_2, have been synthesised by cyclisation of the resin-attached tetrapeptides. The former, more rigid, compound proved to be one of the most selective μ-receptor ligands reported to date. Although the latter compound was highly potent, it was non-selective, as were both the cyclodimers which were formed in comparable amounts to the cyclic monomers during the treatment with N,N'-dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole.[39] The preparation of three other cyclic tripeptides (Tyr-D-Glu-Phe-Lys-NH_2, Tyr-D-Orn-Gly-Glu-NH_2, and Tyr-D-Ala-Lys-Phe-Glu-NH_2) and two cyclic tetrapeptides (Tyr-Lys-Gly-Phe-Glu-NH_2 and Tyr-D-Lys-Gly-Phe-Glu-NH_2) by similar methods has been reported, but no biological activities are given.[40]

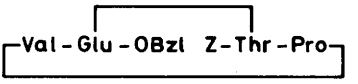
Five other reports concerning cyclic tetrapeptide analogues of enkephalin have appeared. Conformation studies in solution for the series Tyr-cyclo(-N^{ω}-Xxx-Gly-Phe-Leu-), where Xxx = Orn, D-Orn, Lys, or D-Lys, suggest a rather rigid structure for the Orn analogues, with a $Gly^3CO \leftarrow HNLeu^5$ γ^i-turn and a $Orn^2CO\text{-}HN^{\delta}Orn^2$ hydrogen bond. The lysine peptides appear more flexible, the D-Lys compound including a β-turn ($Gly^3CO \leftarrow HN^{\varepsilon}$D-$Lys^2$). The D-Lys analogue is *ca.* 10 times more active in the GPI assay than the D-Orn^2 one.[41] The conformation of Tyr-cyclo(-D-A_2bu-Gly-Phe-Leu-), where A_2bu = α,γ-diaminobutyric acid, has been the subject of theoretical calculation and an n.m.r. study. The results agree in supporting (in DMSO-d_6) an arrangement of two transannular hydrogen bonds, GlyCO ← HNLeu and A_2buCO or PheCO ← $NH^{\gamma}A_2$bu. The former is disrupted by the addition of water, but the overall conformation does not change.[42] The analogues Tyr-cyclo(-N^{ω}-D-A_2bu or Orn-Gly-Phe-Leu-) have also been the subject of a separate study, together with Tyr-cyclo(-$N^{\omega}A_2$pr-Gly-Phe-Leu-). It is concluded here that several conformations are favoured for all three compounds, all being compatible with a Gly^3-Phe^4 bend.[43]

The partial retro-inverso analogue Tyr-D-Ala-gGly-(R,S)-mPhe--Leu-NH_2 and its cyclic counterpart Tyr-cyclo[-D-A_2bu-gGly-(R,S)--mPhe-Leu-] have been synthesised. These diastereoisomeric mixtures proved separable by h.p.l.c., but those of the linear compound rapidly racemised under the conditions of testing. Activities were 6-14% of that of Leu-enkephalin, indicating the Gly^3-Phe^4 amide bond to be important in receptor binding.[44] In rat brain, [D-Pen^2, Pen^5]- and [D-Pen^2, D-Pen^5]-enkephalin are more δ-selective than the linear analogues [D-Thr^2, Leu^5]-enkephalyl-Thr^6 and [D-Ser^2, Leu^5]-enkephalyl-Thr^6, but the rather low affinity of the D-D isomer induced high experimental errors, cancelling the benefit of its good δ-selectivity.[45]

In solution in $CDCl_3$ cyclo(-Pro-Val-Pro-Val-) has a *cis-trans--cis-trans* backbone conformation which differs slightly from that in DMSO-d_6 or the crystalline state; it also retards stem growth of rice seedlings. Its diastereoisomer cyclo(-Pro-D-Val-Pro-D-Val-) has a similar backbone in DMSO-d_6, but an all-*trans* conformation in trifluoroethanol, and promotes root growth in rice seedlings.[46] Two cyclic tetrapeptides (24) and a cyclic lactone analogue (25) have been prepared to try and mimic a tetrapeptide region around the mutation site of sickle-cell haemoglobin. It is hoped such a



(24) Xxx = Val or Phe



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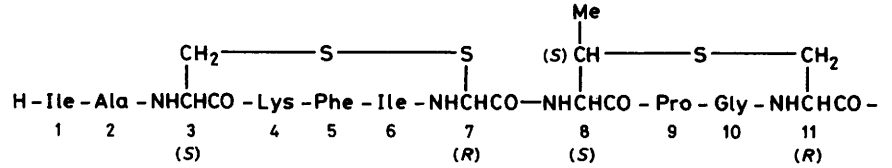

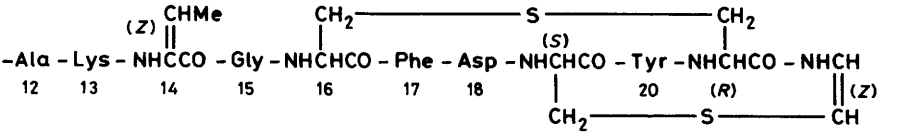

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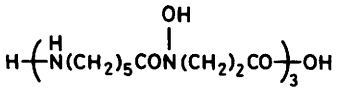

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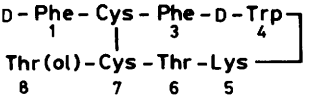

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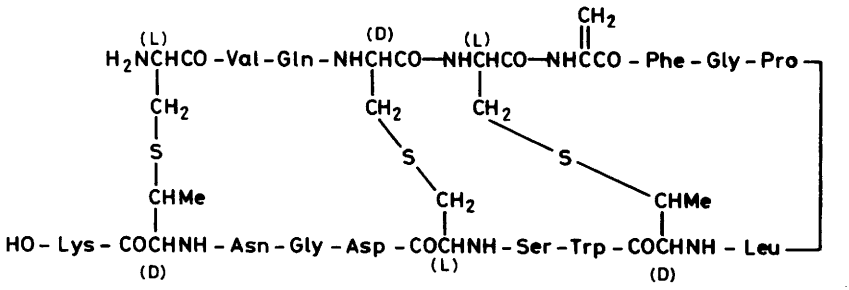


(29)

compound may bind to the acceptor site of haemoglobin and inhibit polymerisation. The best yields of cyclomonomer were obtained using pentafluorophenyl ester (of Pro) cyclisation; use of diphenylphosphoryl azide or 1-succinimidyl esters gave predominantly cyclodimer.[47]

The structure of epidermin (26), a ribosomally synthesised tetracyclic heterodetic polypeptide antibiotic, has been determined. A c.d. study indicates that this product of Staphylococcus epidermidis, unlike nisin or subtilin, has an extremely rigid conformation. Epidermin is highly effective against the pathogen Propanibacterium acnes occurring in acne disease and against staphylococci and streptococci.[48] An analogue of HC-toxin from the same source, Helminthosporium carbonum, contains the same amino acid sequence except that glycine replaces D-alanine. The sequence was determined by collision-activated decomposition following FAB ionisation. It is only about 1/35th as effective an inhibitor for root growth of maize seedlings as HC-toxin.[49] The synthesis of [2-aminobutanoic]-, [Leu]-, and [Phe]-AM toxin I has been reported. These analogues show considerable biological activity, including host specificity, indicating that necrotic activity is tolerant of variation of the size of the side chain in residue one.[50] In cyclo(-Pro-D-Leu-D-Tyr(Me)-Ile-) the predicted cis-trans-cis-trans backbone conformation is adopted in solution. The cis Leu-Tyr(Me) peptide bond contrasts with the trans one in the L-D diastereoisomer. In this paper five rules are proposed for predicting the conformations of cyclic tetrapeptides and tetradepsipeptides.[51]

Theoretical calculations on the cyclic somatostatin antagonist cyclo[-Ahep-Phe-D-Trp-Lys-Thr(OBzl)-], where Ahep = $NH(CH_2)_6CO$, indicate seven distinct conformational families that contain reverse turns. They differ in the positions of the turns in the primary sequence; frame-shifted turns are observed at each possible position.[52] Two cyclic pentapeptide thymopentin analogues, cyclo(-Arg-Lys-Xxx-D-Val-Tyr-), where Xxx = Asp or Glu, show a high activity in the phytohaemagglutimin and plaque-forming cell assay, being the most active of ten cyclic pentapeptide analogues investigated. 2D n.m.r. indicates the protected compounds to contain a γ-turn and a βII' turn.[53]

Cyclo(-Gly-Pro-D-Phe-D-Ala-Pro-) also adopts the γ-turn and type II β-turn combination in solution; substitution of D-Phe by dehydro-Phe causes little change in conformation, although the

yield on cyclisation (p-nitrophenyl ester method) drops from 37% to 5% (C-terminal Gly).[54] ^{13}C-Solid-state n.m.r. spectra of cyclo-(D-Phe-Pro-Gly-D-Ala-Pro) show the same single conformation[55] as seen in solution,[56] but cyclo(D-Phe-Gly-Ala-Gly-Pro) under some conditions appears to crystallise in two different forms; these may be in dynamic equilibrium in solution. The phenyl rings of the latter undergo a relatively slow ($10-10^2$ Hz) reorientation about the $C_\beta-C_\gamma$ bond axis, but the rings of the former are static on this time scale.[55] The vibrational modes of cyclo(D-Phe-Pro-Gly-D--Ala-Pro) have also been calculated and the predicted amide I, II, III, and V bonds found to compare well with those observed.[57] In crystals of cyclo(Ala-Pro-Gly-D-Phe-Pro) a cis Ala-Pro peptide bond exists and there are no intramolecular hydrogen bonds, and this form persists in DMSO-d_6. In $CDCl_3$, however, the conformation is all-trans with some ill-defined hydrogen bonding, possibly a type II β-turn for Pro-Gly within which is a weak γ-turn (GlyNH-Ala-CO).[58] Unequivocal sequence determination of cyclic penta- and hexapeptides has been achieved by tandem m.s. Production of gas--phase $[M + H]^+$ by FAB is followed by investigating either uni-molecular or collision-induced dissociation of mass-selected $[M + H]^+$ and $[MH-\text{amino acid residues}]^+$ fragments.[59]

As a model of an interior β-turn in lysozyme, cyclo(D-Tyr(Bzl)--Gly-Ile-Leu-Gln-Pro) has been made. In both $CDCl_3$:DMSO-d_6 (98:2) (the small percentage of DMSO is needed to prevent aggregation) and sulpholane a single conformation containing two linked β-turns is adopted, one having the same sequence (Gly-Ile-Leu-Gln) and geometry (type I) as the turn in the enzyme.[60] A linear tri-hydroxamic acid with alternating residues of 6-aminohexanoic acid and 3-(hydroxyamino)propanoic acid (27) has been prepared and cyclised (as its tris-benzyl derivative) to yield analogues of ferrioxamines E and G, the natural products having the hydroxamic acid moieties reversed. The cyclic analogue appears to take up Fe(III) rather slowly but more firmly than the linear derivative.[61]

Three novel vasopressin analogues of the type Dnp-D-Tyr(Et)-Phe-Val-Asn-Cys-Xxx, where Xxx = $ProNH(CH_3)_5NH_2$, $Lys-NH_2$, or $NH(CH_2)_5NH_2$, all show good antagonist activity in vitro, but the last two show somewhat reduced in vivo activity. These results indicate that both a terminal carboxamide and the proline residue are not essential for potent antagonist activity; a basic moiety attached directly to the ring constitutes an effective

pharmacophore.[62] The very potent cyclic analogue of somatostatin (28) has been studied by 500 MHz n.m.r. in aqueous solution. An equilibrium between conformations each containing two γ-turns is proposed. In DMSO-d_6 the molecule is less flexible than in water, one conformation with a type II' β-turn involving Phe^3 to Thr^6 predominating. The less active analogue Cys-Phe-D-Trp-Lys-Thr-Cys-NH_2 contains a similar type II' β-turn in DMSO, but there is a different orientation of the cystine bridge, which may account for the difference in biological activities.[63] A more conformationally restricted analogue, [D-Phe^5,Cys^6,Tyr^7,D-Trp^8,Pen^{11}]-somatostatin-(5-12)-octapeptide amide, displays both a high affinity (7800 times that of somatostatin itself) for the opiate receptor and μ-selectivity.[64] The solvent-accessible surface areas of the atoms in tripeptides around the minimum-energy conformations of the types I, I', II, and II' β-bends have been computed and used to correctly identify those occurring in six cyclic hexapeptides of known crystal structure.[65]

2.3 Larger Cyclic Peptides.- The conformations of four cyclic heptapeptides have been examined, two having the sequence cyclo-(-Ala-Ile-Val-Ser(Bzl)-Xxx-Phe-Gly-). Where Xxx = Pro, the Ser-Pro bond is *cis*, and, as is the case when Xxx = Aib, a conformation similar to a β-pleated sheet around Gly-Ala-Ile-Val is seen; the GlyCO is an acceptor for two hydrogen bonds from the NH_2 of Ile and Val. The enkephalin analogue cyclo(-Gly-Phe-Leu-Ala-Lys(Z)-Tyr-(Bzl)-Gly-) seems to exist in terms of a relatively rigid framework, while the somatostatin analogue cyclo(-D-Trp-Lys(Z)-Thr-Val-Pro-Gly--Phe-) appears inhomogeneous following the usual interpretations of u.v. data.[66] Ancovenin (29), a new peptide inhibitor of ACE, has been isolated from a *Streptomyces* species. It is more active than potentiator C, but less active than captopril. It is the third naturally occurring cyclic sulphide peptide to be isolated, the others being nisin and subtilin. The triply overlapped ring system is unique and made ancovenin a particularly difficult peptide to sequence.[67]

Complex formation by the octapeptide cyclo[-Gly-Lys(Z)-Sar-Pro-]$_2$ has been investigated. In acetonitrile the uncomplexed compound exists as a mixture of at least five conformations, the predominant one having two *cis* Lys-Sar peptide bonds and being C_2 symmetric; three types of cation complex were observed.[68] The crystal structure of cyclo(-D-Ala-Gly-Pro-D-Phe-)$_2$ shows only *trans* peptide

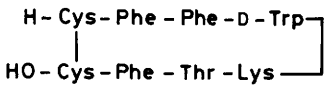
bonds, with a Pro-D-Phe type II and a D-Phe-D-Ala type III' β-turn. It differs from a solution conformation primarily in a 40° rotation of the plane of the Phe-Ala peptide bond relative to the local plane of the α-carbons.[69] The cyclic octapeptide somatostatin analogue (30) does not appear to adopt any predominant conformation in solution.[70] The dimeric cyclic enkephalin analogue (31) has been isolated as a second major component from a solid-phase synthesis of the corresponding cyclic monomer. In comparisons with [Leu^5]-enkephalin, (31) has similar μ-receptor activity but is only 1/10th as potent at the δ-receptor, an activity profile clearly distinct from that of the cyclic monomer.[71]

Two cyclic decapeptides (S2 and S3; cyclo[-Val or Abu-Orn-Leu--D-Phe-Pro-Abu-Orn-Leu-D-Phe-Pro-]) isolated as new members of the gramicidin S family have been synthesised, and proved identical with the natural materials.[72] [D-$Val^{1,1'}$]- and [D-$Val^{1,1'}$, $Phe^{4,4'}$]-gramicidin S (GS) have been prepared; their c.d. spectra in aqueous solution differ from that of gramicidin S, and they have no antibacterial activity, indicating the importance of the D-Phe-Pro-Val sequence.[73] [Pro^4, D-Phe^5]-Gramicidin S shows at least half the activity of GS, but [$Pro^{4,4'}$, D-$Phe^{5,5'}$]-GS is virtually inactive; the c.d. spectra of both differ from GS itself.[74] A new basic GS analogue, [D-$Dpr^{4,4'}$]-GS (where Dpr = α,β-diaminopropionic acid), has been converted by Hofman degradation into [$\Delta Ala^{4,4'}$]-GS. Both the latter and the di-β-Z derivative of the former show high activity against Gram-positive bacteria. The di-β-Z compound is also active against Gram-negative bacteria. Reduction of the ΔAla residues gave DL-Ala.[75] Tandem Fourier-transform mass spectrometry using laser desorption of gramicidin S and the linear peptide gramicidin D gives peaks providing complete sequence information for GS and for 12 out of the 15 amino acids for GD. Striking advantages are claimed for this approach compared to tandem mass spectrometers hitherto used.[76] C.d. spectral analyses of Dnp-Leu-Ala or D-Ala-Pro-D-Val-pNa (where pNa = p-nitroanilide) tetrapeptides related to the β-turns of the biologically inactive [D-$Val^{1,1'}$]- and [D-$Val^{1,1'}$, $Phe^{4,4'}$]--GS indicate that they have a very low preference for β-turn formation.[77]

The crystal structure of the Li complex of the biologically inactive perhydroanalogue of antamanide (which contains cyclohexylalanine in place of the four Phe residues) has been determined. The backbone encapsulates the Li ion in an almost identical manner

to antamanide itself, but, whereas in the latter the phenyl groups are folded against the globular backbone, in the former the cyclohexyl rings are extended away from the folded backbone. This results in the exposure of large portions of the polar backbone to the environment.[78] Cyclic nona- and decapeptides (32) containing the complete bradykinin sequence have been prepared by pentafluorophenyl ester cyclisation. The two smaller of the ring systems show prolonged hypotensive activity, the other analogue being inactive.[79] The structure-function relationships of these compounds and cyclo(-ε-kallidin-), α-Arg-cyclo[-ε-(Lys,[1] Gly[6])-bradykinin-], and cyclo[-ε-(Lys[1], Gly[6])-bradykinin-] have been further discussed.[80] Cyclo(-Pro-D-Leu-)$_5$ and cyclo(-Pro-Leu-)$_5$ have been synthesised and their ion selectivity in solvent polymeric membranes studied. The former behaves as an ionophore which selects Mg(II) over Ca(II) by a factor of 100, while the latter induces selectivity for Mg(II) over Li, Na, and K by factors of 400, 200, and 10 respectively, but selectivity of Mg(II) over Ca(II) is poor.[81] TEMP (2,2,6,6-tetramethylpiperidine-1-oxyl) and three of its derivatives have been used to enhance the spin-lattic relaxation rates of the cyclic decapeptide tyrocidine A in MeOH and DMSO solutions. As previously observed with gramicidin S, the relaxation is mainly controlled by the peptide conformation, being fully consistent with the presence of β-pleated sheet, βI turn, and βII' turn segments. The neutral parent free radical is suggested as the most suitable probe for investigating biomolecules as it shows less specificity in its interactions.[82]

Full details of the synthesis of the cyclic [11]-peptide cyclosporin A and some analogues, first reported last year, have now been published.[83] The primary metabolite of cyclosporin A in human and rabbit bile has been identified as an acidic compound in which the methyl group attached to the alkene link of the unsaturated amino acid component has been oxidised to a carboxylic acid. The product has no immunosuppressive activity.[84] A combination of different homo- and heteronuclear 2D n.m.r. techniques has enabled a complete assignment of all H, C, and 4 of the 11 N signals in the 300 MHz n.m.r. spectrum of cyclosporin A. The assignment is based solely on scalar couplings with a minimum of empirical arguments.[85] X-Ray analysis of cyclosporin A itself reveals a conformation very similar to that of the previously examined iodo derivative. In solution a similar backbone conformation is retained, although the side chains of the unusual amino



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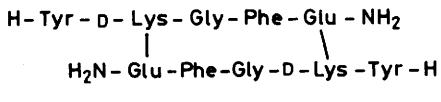

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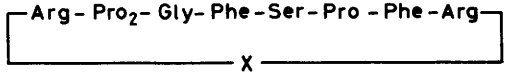
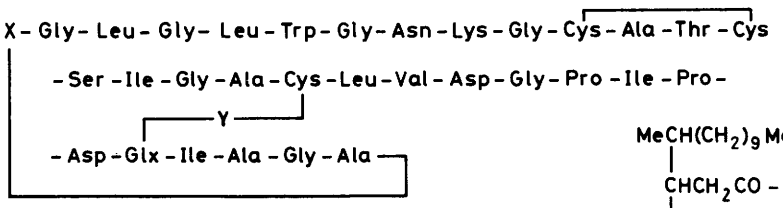
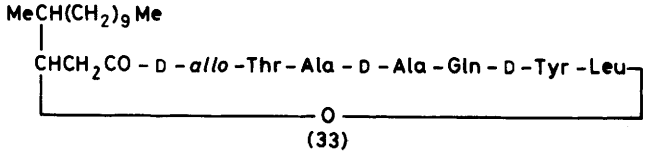


(32) X = $-CO(CH_2)_{11}NH-$, $-CO(CH_2)_{11}NHCO(CH_2)_{11}NH-$, or is absent



(32a)



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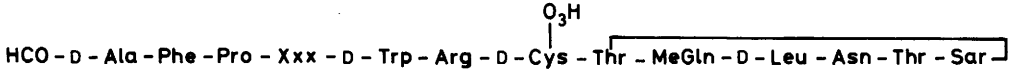


(34) A; Xxx = D-t-Leu-t-Leu

B; Xxx = D-Val-t-Leu

C; Xxx = D-t-Leu-Val

D; Xxx = D-Val-Val

acid MeBmt are oriented differently as intermolecular hydrogen bonds of its OH group break on dissolution of the crystal.[86]

Cyclo(-Val-Pro-Gly_2-)$_3$ has been prepared in a 90.6% yield by a p-nitrophenyl ester cyclisation of a linear [12]-peptide. In solution the dominant conformational feature of the molecule is a recurring type II β-turn with a $Gly^4NH \rightarrow COVal^1$ hydrogen bond. A comparable conformation was deduced by in vacuo energy calculations.[87] The crystalline state of cyclo(-Val-Pro-Gly-)$_4$, the cyclic tetramer of a repeat tripeptide of elastin, contains two type II β-turns. The only connections between cyclic peptide molecules are through water molecules.[88] Subtilosin A from Bacillus subtilis, active against Gram-positive bacteria, has a novel macrocyclic structure (32a; X and Y are unknown residues of molar masses 246 and 163 respectively). Production of this antibiotic is repressed by inhibitors of protein and RNA synthesis, suggesting that it is produced by the usual mechanism of protein synthesis and post-translationally modified.[89]

2.4 Cyclic Depsipeptides. - The crystal structures of the LLL- and DLL-diastereoisomers of cyclo(-2-hydroxyisovaleryl-Pro-Pro-) have been determined. All peptide bonds are cis and the lactone bond trans in both compounds, but the prolyl residues show C_2 symmetry in the LLL-form and C_5 symmetry in the DLL-isomer.[90] Actinomycin D and its novel [$MeAla^{5,5'}$] analogue have been synthesised by a new route. The antimicrobial activity of the latter is an order of magnitude lower than its parent, although X-ray analysis in the solid state shows a conformation similar to that of actinomycin D. In dry $CDCl_3$ these compounds both exist as two conformers, the ratio of which is concentration dependent, one being a dimer of the other. In wet $CDCl_3$ only the dimer is seen.[91] The structure of a new cyclodepsipeptide antibiotic (33) from Fusarium roseum has been determined, largely by m.s. This compound causes Penicillium digitatum cells to swell to 10 times their normal diameter and inhibits their germination.[92] The structure of discodermin A, first reported last year, has been revised (34); originally the Pro was thought to have the D-configuration.

Three more discodermins from the same source are also described (34). They all show antimicrobial activity and inhibit the development of starfish embryos.[93] The crystal structure of unhydrated enniatin B has been re-evaluated and refined but is still based on data collected in 1969.[94] Two antibiotics active

against yeasts and filamentous fungi but not bacteria, herbicolins A and B (35), have been isolated from the bacterium Erwinia herbicola. The main component herbicolin A has an additional D-glucose moiety linked in a 1-α-glycosidic bond to the 3-hydroxytetradecanoic acid. It is the first glycolipidodepsinonapeptide antibiotic to be characterised. The N-Me-aThr-Arg bond seems unusually labile to alkali.[95] A comparison of solution conformations of complexes of valinomycin with Mg and Sr with those earlier described for Ca and Ba suggests that cations with co-ordination numbers of six (Mg and Ca) form 2:1 ion sandwich and 1:1 carrier-cation complexes whereas those with higher co-ordination numbers (Sr and Ba) form 1:2 complexes also.[96] A consideration of the crystal structures of d(GC)-actinomycin D and d(CGTACG)-triostin A suggests that the perfect hydrophobic character of the inner surface of the cyclic depsipeptides is necessary for DNA-antibiotic interaction as it ensures that these interactions are directed, unambiguous, and screened from interference by solvent. It is further hypothesised that the antibiotics carry five or six apparent A/T sequences on the surface of the cyclic depsipeptide rings, presenting a deceptive terminal signal to RNA polymerase.[97]

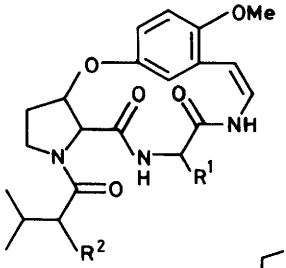
2.5 Other Cyclic Peptides with Modified Backbones. - From the bark of Zizyphus sativa two new peptide alkaloids have been isolated. Sativanine D (36a) is the first of its class to have an additional, imidazolidinone, ring in the side chain. Sativanine F (36b) is also novel in the possession of an N-formyl group.[98] Discaria febrifugia has also yielded a new peptide alkaloid in discarine E (37).[99] The synthesis of the cyclo-[-Gly-Thz(R)- and (S)-Gln-Thz-Val-Leu-Pro-] isomers of dolastin 3 has been accomplished. Their properties rule out an all-L configuration for this cell-growth-inhibiting cyclic peptide.[100]

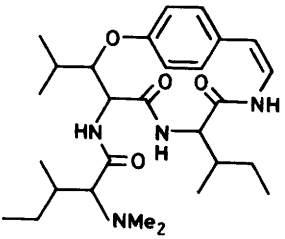
The total synthesis of ulicyclamide constitutes the first construction of a macrocycle containing both thiazole and dihydrooxazole rings. The final stages involve treatment of the compound (38) with trifluoroacetic acid followed by neutralisation under conditions of high dilution at 95°C. The cyclic monomer yield was 20%.[101] The second report of a macrocycle of this type followed shortly afterwards with the synthesis of ascidiacyclamide by a cyclodimerisation of (39) with diphenylphosphoryl azide (DPPA) in 27% yield, clearly establishing its stereochemistry.[102] Following this, two separate syntheses of patellamide B were reported

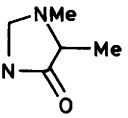
Htd - Abu - Thr - D - aThr - D - Leu - Gly - D - Gln - Gly - *N*-Me - aThr - Arg

(35) Abu = 2,3-dehydro-α-aminobutyric acid

Htd = (*R*)-3-hydroxytetradecanoic acid

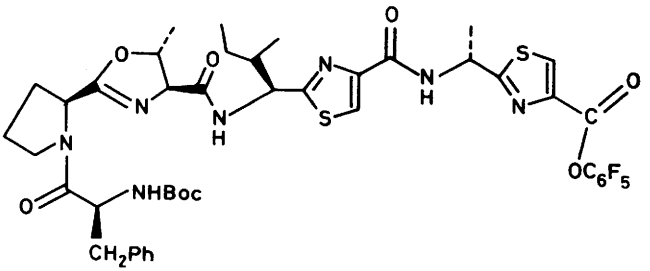




(36) a; $R^1 = -CHMeCH_2Me$, $R^2 =$ 

b; $R^1 = -CH_2Ph$, $R^2 = -CHMe(CH_2Me)$

(37)



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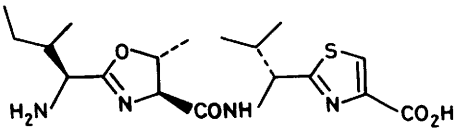

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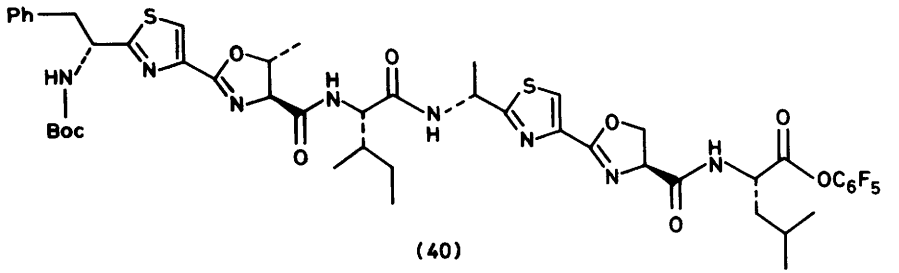

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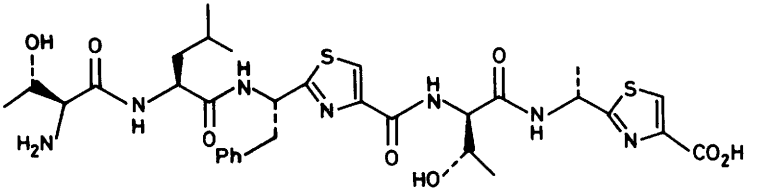

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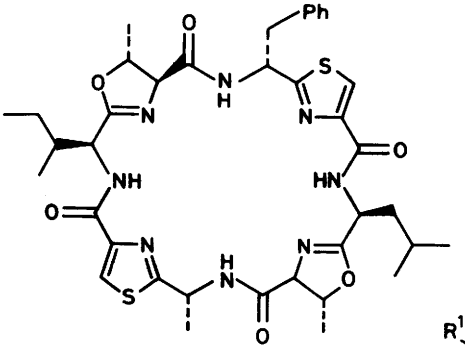

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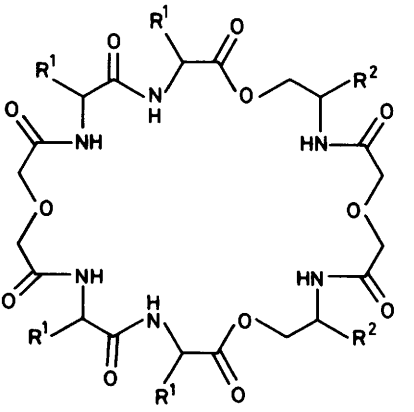


(43) R^1 = $-CH_2Ph$, R^2 = $-CH_2CHMe_2$

R^1 = R^2 = $-CH_2CHMe_2$

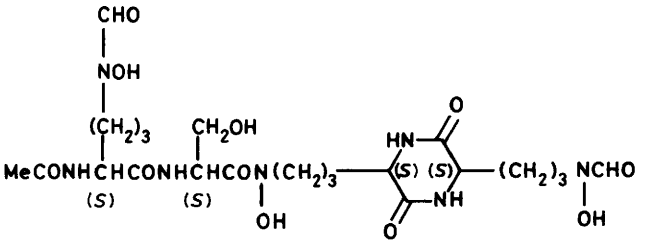
by the same groups. One involved a pentafluorophenyl ester cyclisation of (40) after N-deprotection in 50% yield [103] and the other a DPPA cyclisation of (41) in 12.5% yield and subsequent treatment with thionyl chloride to generate oxazoline rings from the two aThr residues; patellamide C was prepared also by an equivalent route.[104] None of the three products was identical with the natural materials (although showing potent cytotoxicity[104]) and as a result the structure of patellamide B has been revised to (42), which no longer contains directly linked thiazole and oxazoline rings. Analogous revisions have been made to the structures of patellamides A and C.[103,104] The revised structure of patellamide A has also now been confirmed by synthesis using the DPPA/$SOCl_2$ route.[105]

Two 30-membered macrocycles (43) containing residues of diglycolic acid, Phe, and Leu have been prepared. The synthesis builds on an earlier preparation of a 24-membered macrocycle containing these components whose ring size was thought to be too small to bind the primary ammonium cation.[106]

3 Modified Linear Peptides

3.1 Enzyme Inhibitors. - A new naturally occurring ACE inhibitor, foroxymithine (44), has been isolated from *Streptomyces nitrosporeus*,[107] while *Streptomyces tonabeensis* has yielded strepin P-I (isovaleryl-Tyr-Val-arginal), which inhibits trypsin as well as thiol proteinases. Synthetic material showed full activity.[108] Thiolstatin D, acetyl-Phe-arginal, from *Bacillus cereus* has also proved to be an inhibitor, albeit weak, of trypsin, thrombin, kallikrein, and plasmin.[109] The oxidation of captapril by glutathione disulphide via thiol-disulphide interchange has been studied in aqueous solution by ^{1}H-n.m.r. The results suggest that captapril has a greater tendency to reduce disulphide bonds at physiological pH than do the thiol groups in amino acids.[110]

The search for new and improved synthetic ACE inhibitors continues to be prosecuted with vigour. Replacement of the proline in enalapril by pyroglutamic acid, giving (45), halves the activity although the duration of action is unaffected.[111] Acyl tripeptide analogues of enalapril, e.g. (46), have been prepared to see if an extended peptide chain will give additional binding, but none was more potent than the parent.[112] Of a number of N-carbobenzoxy-γ-D-glutamyl analogues examined, the most potent



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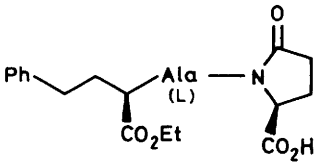

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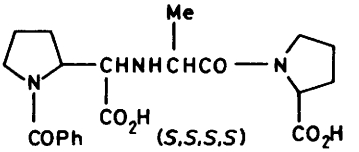

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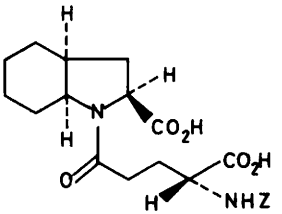

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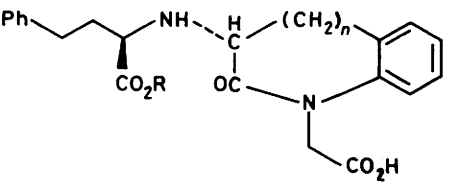
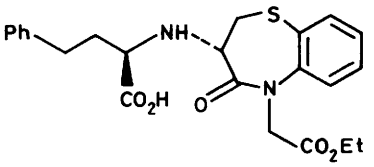


(48) n = 6–8, R = H



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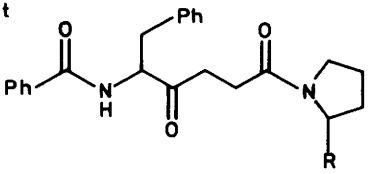
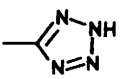


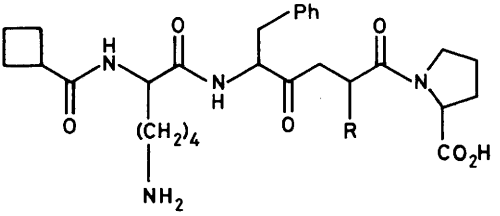
(50) R = —PO_3H_2, —CONHOH, or 

compound in *in vitro* tests was the indoline-2(S)-carboxylic acid (47), ACE IC_{50} 2.7×10^{-9}M.[113] In a series of analogues (48; R=H) differing only in lactam ring size, the 7-membered ring compound proved the most potent ACE inhibitor. These diacids have only marginal biological activity on oral administration, but the monoesters (48; R = Et) are much more potent.[114] Of a number of chiral 1,5-benzothiazines tested, the most potent was (49). However, replacement of the benzylic methylene by sulphur in these compounds resulted in less effective ACE inhibitors, particularly on oral administration.[115]

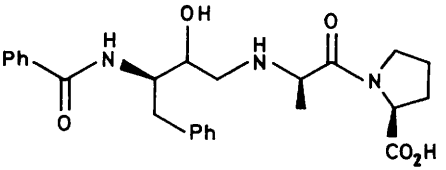
Of three compounds (50) involving replacing a proline carboxyl with other acidic groupings, the hydroxamic acid proved the most potent, although it was not as active as the parent.[116] Two pentapeptide analogues (51) of 5(S)-benzamido-4-oxo-6-phenylhexanoyl-L-proline are at least 10 times as active as their parent, but neither caused significant lowering of blood pressure in renal hypertensive rats. Experiments with a tritiated derivative showed that the desmethyl compound is excreted in greater than 90% as the unchanged form.[117] A new amino alcohol modification designed to mimic the transition state of amide-bond cleavage by proteolytic enzymes has been incorporated into the scissile bond position of the known ACE substrate N-benzoyl-Phe-Ala-Pro. The R-configuration of the alcohol in this modified tripeptide (52) proved to have 400-fold greater inhibitory potency than the corresponding S-isomer. Replacement of Ala by Arg or Lys did not diminish potency, but introduction of a methyl group to give a tertiary alcohol was extremely deleterious to activity.[118]

An analogue of the bradykinin potentiating peptide (Bpp) Glp-Lys-Phe-Ala-Pro with the Phe-Ala peptide bond inverted is a moderate inhibitor of ACE *in vitro* and more potent than Bpp as a hypertensive in normotensive rats without exhibiting the bradykinin potentiating action of the natural peptide.[119] N^{α}-(Diphenoxyphosphoroyl)-, N^{α}-[bis(4-nitrophenoxy)-phosphoryl]-, and N^{α}-[(2-phenylethyl)phenoxyphosphoryl]-Ala-Pro are moderately potent ACE inhibitors but at physiological pH and temperature *in vitro* become powerful ACE inhibitors by loss of phenol or 4-nitrophenol. The half-times for hydrolysis are 22 days, 3.5 hours, and 21 days respectively.[120]

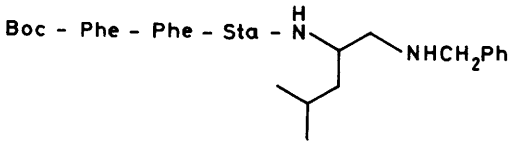
Details of the preparation of short-chain peptidic aldehyde renin inhibitors reported last year (Vol. 17, Ch. 4, ref. 95) have



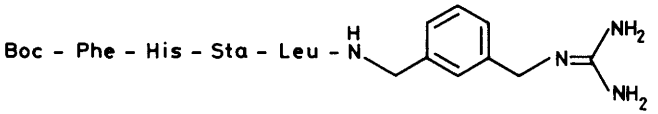
(51) R = H or Me



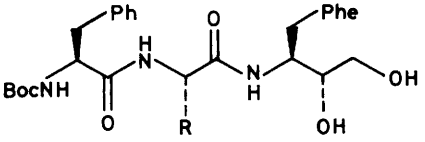
(52)



(53)

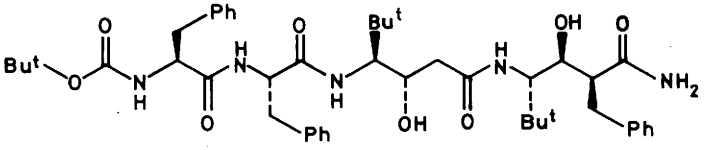


(54)



(55) a; R = Bu^i

b; R = CH_2 

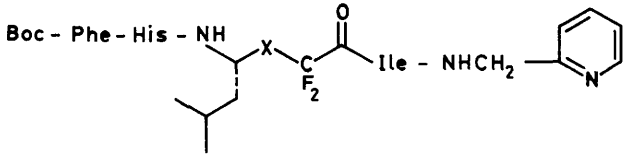


(56)

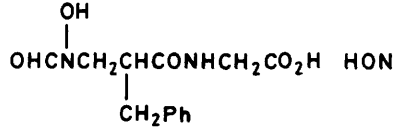
appeared.[121] A uniquely potent renin inhibitor (53), more than 200-fold more potent than the peptidal lead structure Boc-Phe-Phe-Sta-Leu-Phe-NH_2 (Sta = statine), has a $\underline{K}_i$ versus human renin of 2.6 x 10^{-11}M. It binds strongly to a plasma component, but can be regenerated by precipitation of the plasma protein with acetone. The compound (54) is also a potent inhibitor, but does not bind very much to plasma.[122] Incorporation of the novel side-chain analogue of statine (3$\underline{S}$,4$\underline{S}$)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid (Achpa) also gives very potent inhibitors, the most effective being Iva-His-Pro-Phe-His-Achpa-Leu-Phe-NH_2, $\underline{K}_i$ versus human renin 1.6 x 10^{-10}M.[123] In contrast to the results obtained with 4-amino-3-hydroxy-5-phenylpentanoic acid and statine itself, analogues containing 4-amino-3-hydroxy-3,6-dimethylheptanoic acid (Me^3Sta) or 4-amino-3-hydroxy-3-methyl-5-phenylpentanoic acid are more potent as their 3$\underline{R}$,4$\underline{S}$-isomers rather than the 3$\underline{S}$,4$\underline{S}$-forms. Difference n.m.r. spectroscopy results suggest that the C-3 methylated analogues must inhibit enzymes by a different mechanism to the corresponding statine peptides.[124]

On the basis that a diol may be bioisosteric with a formyl group, dipeptide glycols have been investigated as renin inhibitors. Compounds (55a) and (55b) are more potent than pepstatin; the epimer of (55a) at the asymmetric carbon bearing the hydroxyl group is inactive. Compound (55b) has the higher preference for inhibiting human renin over pepsin (385:1). Both compounds are species specific in that they do not inhibit rat renin.[125] The potency of the renin inhibitor (56) compares favourably with other peptides whose structures more closely resemble that of angiotensinogen,[126] while difluorostatine and difluorostatone peptides of the type (57) have also been investigated. (57a) is an order of magnitude less active than its parent, but (57b) shows high renin specificity, being 3-4 orders of magnitude less effective against pepsin and cathepsin D.[127] Z-Phe-His-leucinol has been found to be a highly potent renin inhibitor, and specific to human renin. Replacement of the benzene ring of the Phe with naphthalene does not reduce activity, but substitution with anthracene or phenanthrene is detrimental to activity. If statine or statinol ethyl esters or 2($\underline{S}$)-methylbutamides replace leucinol, highly active derivatives are produced.[128]

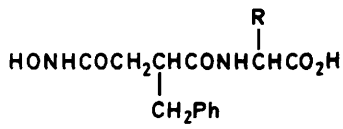
The dipeptide derivatives (58) and (59) have been designed as inhibitors for proteinases which degrade enkephalin. All three compounds inhibit three different metallapeptidases known to be



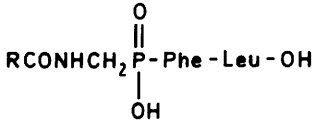
(57) a; X = CHOH
b; X = CO



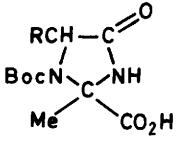
(58)



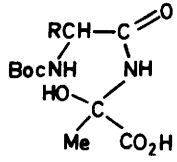
(59) R = H or Me



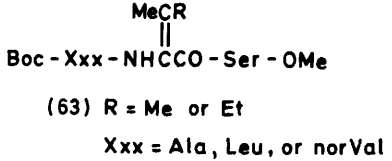
(60)



(61)



(62)



(63) R = Me or Et
Xxx = Ala, Leu, or norVal

a; Ac - Aib - Ala - Ala - Aib - Aib - Gln - Aib - Aib - Aib - Ser - Leu - Aib - Pro - Val - Aib - Ile - Gln - Gln - Trpol

b; Ac - Aib - Gly - Aib - Leu - Aib - Gln - Aib - Aib - Aib - Ala - Aib - Aib - Pro - Leu - Aib - Iva - Glu - Valol

(Ala) (Ala) (Aib)

c; Ac - Aib - Ala - Aib - Ala - Aib - Ala - Gln - Aib - Val - Aib - Gly - Leu - Aib - Pro - Val - Aib - Aib - Gln - Gln - Pheol

d; Ac - Aib - Ala - Aib - Ala - Aib - Ala - Gln - Aib - Aib - Aib - Gly - Leu - Aib - Pro - Val - Aib - Aib - Gln - Gln - Pheol

5 (Aib) (Leu) 10 15 (Iva) 20

(64)

involved, but only the alanine derivative (designated kelatorphan) proved able also to strongly inhibit aminopeptidase and dipeptidyl-aminopeptidase.[129] Enkephalin analogues with the Gly^3-Phe^4 amide bond replaced by a phosphonamidate group (60) are good enkephalinase inhibitors when the N-acyl group is small. Similar analogues derived from Leu^5- and D-Ala^2,D-Leu^5-enkephalin also showed potency as ACE inhibitors, but were inactive in GPI and MVD assays.[130] The dipeptide Phe-Ala is known to be a good inhibitor of enkephalinase. N-Phosphoryl derivatives of Phe-Ala, Phe-Ala-NH_2, and Phe-Gly-NH_2 have now been prepared and their analgesic activity evaluated in mice. Intracerebroventricular administration of the Phe-Ala-NH_2 derivative produced a 100-fold greater analgesic effect than Phe-Ala itself, and led to potentiation and prolongation of the analgesic effect of exogenously administered Met-enkephalin.[131]

Boc-Pab-Ala_3Lys(Z)-OPic and Boc-Pab-Ala_2-Val-Lys(Z)-OPic (where Pic = 4-picolyl and Pab = 2-amino-4-(3-pyridyl)butyric acid) have been synthesised and found to be only weak inhibitors of human leucocytic elastase, none being as effective as elastatinal.[132] It has been concluded from calculations on eight structurally diverse ACE inhibitors that there is a common low-energy conformation,[133] and the concept of transition-state analogue enzyme inhibitors has been discussed using pepstatin as the example.[134]

3.2 Dehydropeptides. - The condensation of amides of Boc-amino acids with pyruvic acid leads to Boc-dipeptides with C-terminal dehydroalanine. The formation of (61) as a side product supports the hypothesis that the primary product of condensation is the α-hydroxyalanine (62).[135] The removal of the N-protecting group from a series of twelve N-Z- or N-TFA-dipeptides containing C-terminal dehydro-Ala or (Z)-dehydro-Phe has been examined. Fission of the trifluoroacetyl group by ammonia caused no problems, but Pd/C-catalysed hydrogenolysis of N-Z-dipeptides containing dehydro-Ala caused side reactions; catalytic transfer hydrogenation proved a cleaner reaction.[136] The C-terminal dehydrotripeptide sequences of the antrimycins (cirratiomycins) (63) have been made in a stepwise fashion, introducing the dehydroamino acid as its N-carboxyanhydride derivative.[137] Hydrogenation of N-benzoyl-dehydro-Valyl--L- and D-Phe-OMe at 1 atm/5%Pd/C in n-propanol gives the protected Val-Phe dipeptide in nearly quantitative yield, but as expected diastereoselectivity is low. A previous report stated that these compounds were too hindered for hydrogenation under these

conditions.[138] The crystal structures of two dioxopiperazines containing dehydro-amino acids have earlier been mentioned (refs. 28 and 29).

3.3 Peptides Containing α,α-Dialkylamino Acids.- Three new peptaibophols have been sequenced. Trichorzianine A IIIc (64a) is one of the major components of an antifungal complex from Trichoderma horzianum, and the N-terminal part of the peptide is known to be ordered in a helix, the first turn of which is of the 3_{10} type.[139] Trichotoxin A40 (64b; exchanges due to natural microheterogeneity are shown) was sequenced by g.c.-m.s. of three N-acetylated dodecapeptides and two N-prolylhexapeptides obtained after selective trifluoroacetolysis.[140] Gliodeliquescin A (64c) from Gliocladium deliquescens represents the first peptaibophol to be isolated from this fungal genus.[141] Two peptides obtained from suzukacillin A by selective trifluoroacetolysis have been shown by synthesis to be Pro-Val-Aib-Iva(Aib)-Gln-Gln-Pheol, thereby proving the sequence 14-20 of the antibiotic.[142] Full details (a preliminary sequence was published in 1976) of a revised sequence for suzukacillin A (64d) have also been reported.[143]

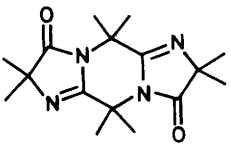
A fourth total synthesis of alamethicin has been accomplished; the product showed perfectly homogeneous conductance states of single pores on measurements in lipid bilayer membranes, which has not hitherto been reported for any synthetic or purified natural preparation.[144] The purification and structure determination by m.s. of peptaibophols has been discussed [141,145] and the relationship of paracelsin to other peptaibophols and the biotechnological applications of such mycotoxins reviewed.[146]

Preparation of the tricyclic bis-imidazolone (65), first obtained in 1973, from a linear homopentapeptide of Aib containing deuteromethyl groups in specific positions by treatment with thionyl chloride followed by alkali indicates that the reaction takes a different route to that observed for cyclisation from homo-tri- and -tetra-peptides.[147] The chemotactic peptide analogues OHC-Met-Leu or Aib-Phe-OMe both aggregate in $CDCl_3$ at concentrations $\geq$ 2mM, but this does not occur in DMSO-d_6.[148] Other chemotactic peptide analogues containing as the central residue Aib or 1-aminocyclopentane- or hexanecarboxylic acids (Acc^5 or Acc^6) have been examined. The Acc^6 compound proved 78 times more active than the parent peptide in inducing lysozyme release in rabbit neutro-

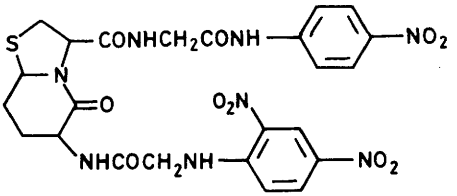
phils; the Aib and Acc^5 peptides were less active than the parent.[149] The taste of a series of L-aspartyl-α-aminocycloalkane carboxylic acid methyl esters is sweet when the rings contain from three to five carbon atoms, bitter for cyclohexane or heptane, but the cyclooctane compound is tasteless. Also tasteless is L-Asp--Deg-OMe (where Deg = diethylglycine), but L-Asp-Aib-OMe is sweet.[150] The reaction of Boc-$Leu_{3\ or\ 4}$-Aib-OH with H-Leu_3-OBzl and DCC initially gives the 4,4-dimethyl-5(4H)-oxazolone, which only slowly undergoes aminolysis. The oxazolone reacts faster with 1-hydroxysuccinimide than H-Leu_3-OBzl. By contrast, 1-hydroxybenzotriazole does not react with the oxazolone, but strongly catalyses its aminolysis through a biphilic pathway.[151] Other structural studies in peptides containing α,α-disubstituted amino acids are collated in Table 1.

Table 1 Application of physical methods

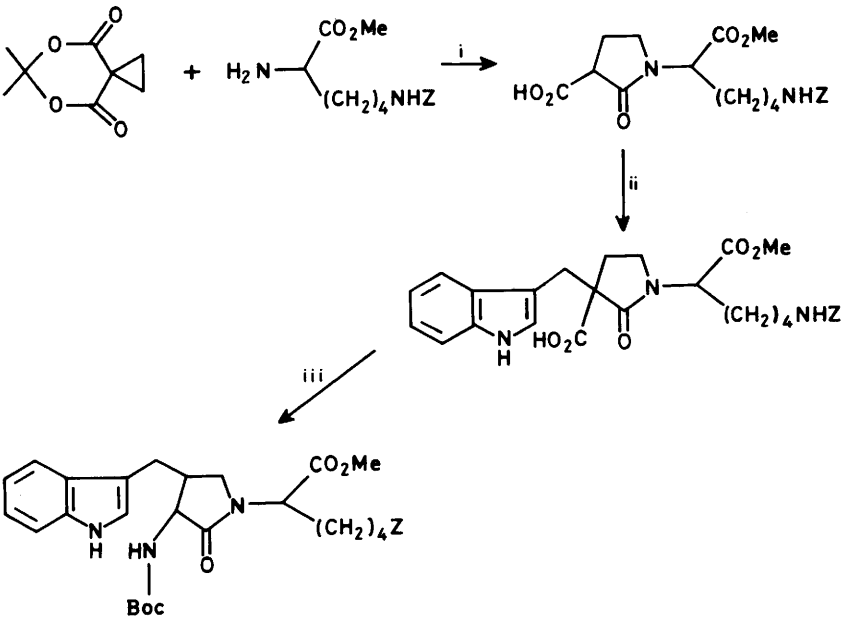
Peptide	Technique Used	Ref.
Boc-Aib-Acc^6-NHMe	X-Ray, ^{1}H-n.m.r.	152
Boc-Aib-Acc^6-OMe	X-ray	152
Boc-Gly-($^1\psi^2$CSNH)-S-Ala-Aib-OMe	X-ray	153
Boc-Gly-S-Ala($^2\psi^3$CSNH)-Aib-OMe	X-ray	153
Boc-Pro-Aib-Ala-Aib-Ala-OH	X-ray	154
Boc-Aib-Ala-Ile-Val-OBzl	Solubility Studies	155
Boc-Gly-Ala-Aib-Ala-Leu-Ile-Leu-OBzl	"	155
Bo -Val-Ala-Gly-Ala-Aib-Ala-Leu-Ile-Leu-OBzl	"	155
Boc-$(Val\text{-}Aib)_3$-Val-OMe	X-ray	156
Boc-$(Leu_{3\ or\ 4}\text{-}Aib)_{2\ or\ 3}$-OBzl		157
Boc-$(Leu_3\text{-}Ala)_{2\ or\ 3}$-OBzl		157
Boc-Aib-$Leu_{3\ to\ 6}$-OBzl		157
Boc-Aib-Leu_9-OBzl		157
Boc-Leu_{3-6}-Aib-OBzl		157
Boc-Leu_9-Aib-OBzl		157
Boc-$Leu_{3\ or\ 4}$-Aib-$Leu_{3\ or\ 4}$-OBzl		157
Boc-$(Ala\text{-}Aib)_2$-Ala-Glu(OBzl)-$(Ala\text{-}Aib)_2$-Ala-OMe	X-ray	158
Boc-Leu-Aib-Pro-Val-Aib_2-Glu(OBzl)-Gln-Pheol	X-ray	158



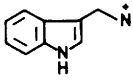
(65)



(65a)



(66)

Reagents : i, NEt_3 ; ii,  ; iii, diphenylphosphoryl azide, NEt_3

Scheme 7

3.4 Amide-Bond Analogues.- Of fourteen partial retro-inverso analogues of the N-terminal tetrapeptide of dermorphin, the most potent was H-Tyr-D-Ala-Phe-NHCH$_2$NHR (where R = OH, OBzl, OCHMePh(D), or adamantyloxycarbonyl), whose activity was comparable with the parent. All the compounds showed some activity.[159] However, in longer dermorphin analogues with the sequence H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH$_2$ and retro-inverso peptide bonds at Gly^4-Tyr^5, Phe^3-Gly^4, and Phe^3-Gly^4-Tyr^5 only low opioid activity was observed. The hydrogen bonds at the modified positions seem of more crucial importance to bioactivity in the heptapeptide.[160] Analogues of the C-terminal tetrapeptide of gastrin in which one of the peptide bonds is replaced by -CH$_2$NH- have been prepared. The Trp-Leu modified compound stimulated acid secretion identically with the parent, but the other two analogues had no agonist activity. Both were antagonists, the Leu^2-Asp^3 modification being the most potent.[161] Of a series of N-(L-asparty1)-1,1-diaminoethane derivatives the sweetest tasting was N-(L-asparty1)-N'-[(2,2,5,5-tetramethylcyclopentyl)carbonyl]-(R)-1,1-diaminoethane, which was 800-1000 times as sweet as sucrose. Surprisingly, the (S)-isomer was nearly as sweet. Compounds of this type are extremely stable towards hydrolysis and cannot form dioxopiperazines.[162] Double-bond isosteres of the dipeptides Pro-Gly, Pro-Leu, Pro-Phe, Ala-Gly, and Ala-Ala have been prepared.[163]

3.5 Peptides Containing Backbone Rings.- A bicyclic dipeptide with a fixed conformation simulating that of the two central amino acid residues in a type II' β-turn has been synthesised from L-glutamic acid and L-cysteine. A derivative (65a) showed a c.d. spectrum in MeOH closely similar to that of Dnp-Gly-D-Ala-Pro-Gly-pNa (pNa = p-nitroanilide), which is known to adopt the type II' β-turn.[164] A Trp-Lys derivative (66) has been synthesised by a novel approach (Scheme 7) that has potential generality for a variety of γ-lactam-constrained dipeptides.[165]

3.6 Peptides Containing D-Residues.- In the crystal phenylacetyl--D-Ala-D-Ala-OH adopts a partially folded conformation quite similar to that found earlier for the acetyl dipeptide. In solution i.r. indicates an extended conformation with possibly weak hydrogen-bonded C_5 rings. Theoretical studies suggest a highly flexible molecule since 55 minima within 3 kcal/mol were detected.[166] In the solid state both N-pivaloyl-D-Phe-Pro-NHEt and N-pivaloyl-D-Phe-L-thiazolidine-4-(N'-ethyl) carboxamide exhibit a type II' β-bend; the compounds are in fact isomorphous. The

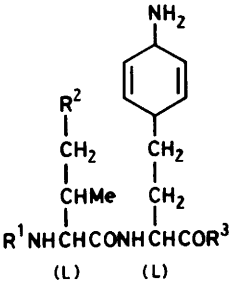
β-turn seen is formally analogous to the D-Phe-Pro bend observed in the solution conformation of gramicidin S.[167] Structure-taste studies have been made on a tripeptide series L-Asp-D-Ala-Xxx-OMe. When Xxx is Aib, α,α-diethylglycine, or α-aminocycloalkanecarboxylic acids (Acc's) containing from three-to six-membered rings, the compounds are sweet; when Xxx is an Acc with a seven-or eight-membered ring the compounds are bitter (*cf.* ref. 50).[168] The terminal amino groups of Ala-His-Ser, Ala-His-D-Ser, and D-Ala-His-Ser have been converted into isocyano functions and these derivatives polymerised with catalytic amounts of $Ni(II)Cl_2$ to give models of the charge relay system of chymotrypsin. On the basis of c.d. spectra, the first two of these compounds adopt helical conformations.[169] The molecular structure in the crystal of Boc-Pro-D-Pro-Pro-D-Pro-OH is characterised by two conformationally quasi-equivalent dipeptide halves with an overall left-handed screw thread. This structure is nearly identical to that predicted by minimisation of the conformational energy in terms of the torsional angles.[170]

The structure of [Val^1]-gramicidin A incorporated into sodium dodecyl-d_{25} sulphate micelles has been studied by 2D ^1H-n.m.r. The conformation indicated is an N-terminal to N-terminal (head to head) dimer formed by two right-handed, single-stranded β-helices with 6.3 residues per turn. This structure differs from that of Unry by the handedness of the helices but agrees with conclusions on N-pyromellityldesformyl, O-pyromellityl, and succinylgramicidin derivatives published earlier.[171] However, it has been reported that the conformation of synthetic gramicidin A with ^{19}F labels at both termini incorporated into small unilammellar vesicles of dimyristoylphosphatidylcholine agrees with that proposed by Urry, using ^{19}F n.m.r. measurements. Measurement of the accessibility of the labels to either aqueous or lipophilic paramagnetic probes shows that the N-terminus is located in the membrane interior and the C-terminus is at the membrane surface.[172] Des(formylvalyl)-gramicidin A has been obtained by treatment of gramicidin A with 4M HCl in dioxane followed by one cycle of the Edman degradation. Analogues with different amino acids in place of the N-terminal valine have been prepared from the des(formylvalyl) compound. Diphenyl phosphorazidate proved more efficient than dicyclohexyl-carbodi-imide in coupling N-formyl amino acids, the reaction proceeding with < 0.1% racemisation.[173]

3.7 Peptides Containing Other Non-protein Residues.- Six related di- and tri-peptide antibiotics (67) have been isolated from Streptomyces venezualeae; they all contain amiclenomycin, which has a 4-amino-2,5-cyclohexadiene ring in its side chain. Cleavage with Pronase gives free amiclenomycin, which may be converted (water, 100°C, 90 min) to p-aminohomophenylalanine. These antibiotics inhibit the growth of Gram-negative bacteria by blocking biotin biosynthesis.[174] The novel antibiotics alahopcin (from Streptomyces albulus) and nourseimycin (from S. noursei) have proved to be identical in structure. This dipeptide derivative (68) exists in equilibrium with two cyclic hemiacetal tautomers in aqueous solution; those depicted in Scheme 8 are thought on spectral grounds to be the most likely structures.[175] Another new antibiotic (69) has been obtained from an unidentified streptomycete. The stereochemistry of the novel cyclopentenonylglycine residue has not yet been determined.[176]

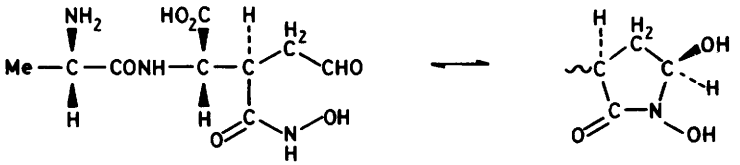
Vanoxonin (70), a new inhibitor of thymidylate synthetase, is produced (as its vanadium complex) by Saccharopolyspora hirsuta. The structure has been confirmed by synthesis.[177] The complete sequence of edeine D (71) has now been published. It only differs from edeine A in lacking a p-hydroxyl group on the aromatic ring.[178] A new basic peptide antibiotic containing 3-methylarginine and homoproline, lavendomycin (72), has been isolated from Streptomyces lavendulae. It is active against Gram-positive bacteria. The 3-MeArg side-chain configuration has not yet been elucidated.[179] A similarly basic antibiotic chitinovarin D (73) from a Flavobacterium species also has unusual structural features.[180]

The total synthesis of althiomycin (73a) has been achieved starting from D-cysteine (Scheme 9). The active ester shown (74) proved more effective than a p-nitrophenyl ester for the first coupling. Addition of the hydroxymethyl group to (75) using formalin gave an equimolar mixture of (76) and its anhydro derivative, but with paraformaldehyde only (76) was formed. The antibacterial activity of the synthetic product (73) was identical to that of the natural compound.[181] The first synthesis of a linear peptide alkaloid, hexaacetylcelenamide C (77), has also been accomplished. The key steps in the synthesis are two condensation reactions with α-dialkylphosphoryl amino acid derivatives to obtain the dehydroamino acid (78) and the dehydropeptide (79) derivatives respectively (Schemes 10 and 11).[182] New total syntheses of the

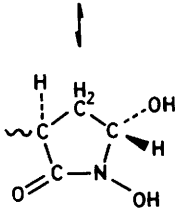


R^1	R^2	R^3	
Me	Me	OH	
H	Me	OH	
Me	H	OH	
Me	Me	Gln	(L)
H	Me	Gln	(L)
H	H	Gln	(L)

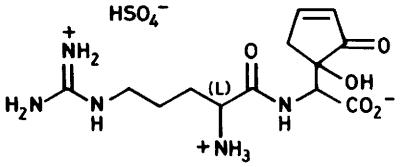
(67)



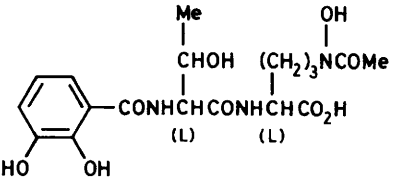
(68)



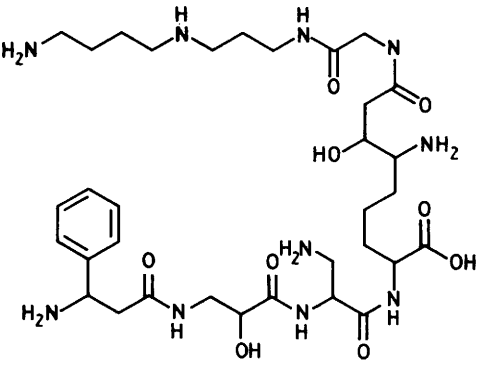
Scheme 8



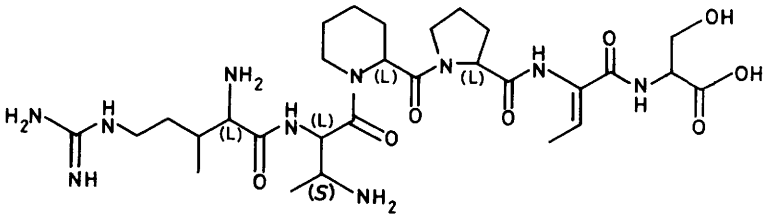
(69)



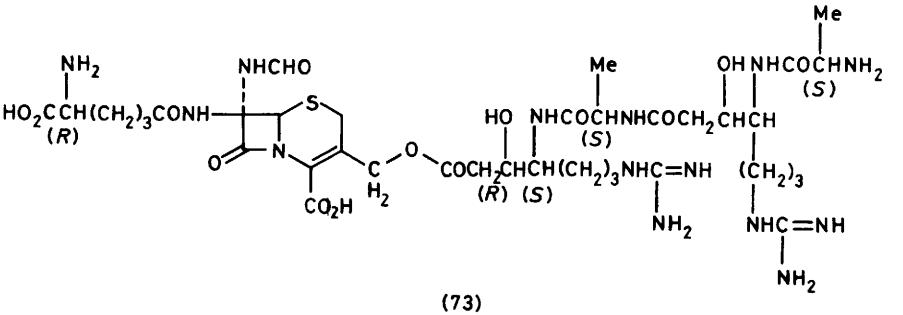
(70)



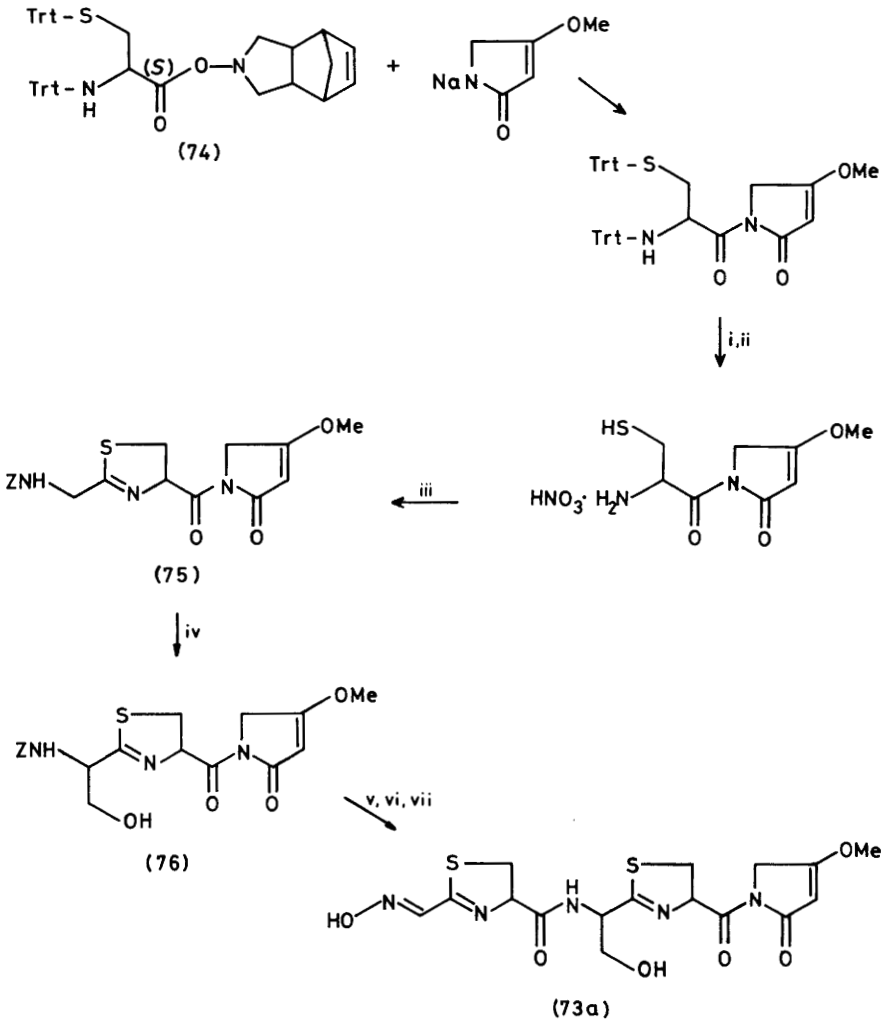
(71)



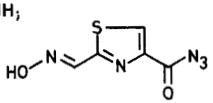
(72)



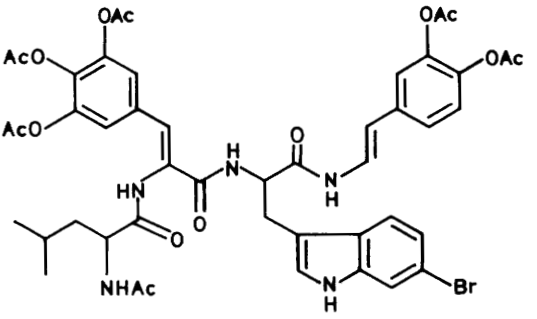
(73)



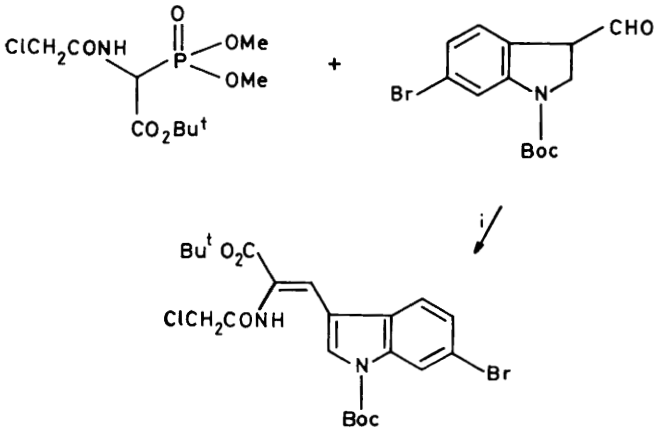
Reagents: i, $AgNO_3$, pyridine; ii, H_2S; iii, $ZNHCH_2C(OEt)=NH$;
iv, HCHO – DMSO; v, HF – anisole; vi, K_2CO_3; vii,



Scheme 9



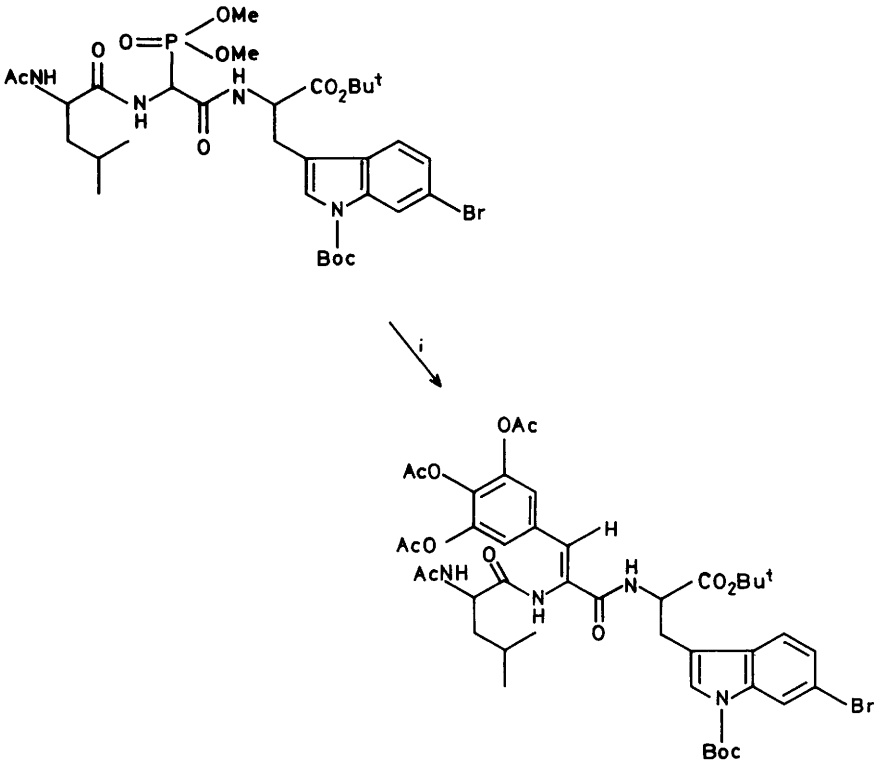
(77)



(78)

Reagent: i, $KOBu^t - CH_2Cl_2$, $-30\,°C$

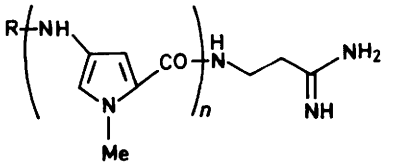
Scheme 10



(79)

Reagent: i, $LiNiPr_2$ - THF - 3,4,5 - triacetoxybenzaldehyde

Scheme 11



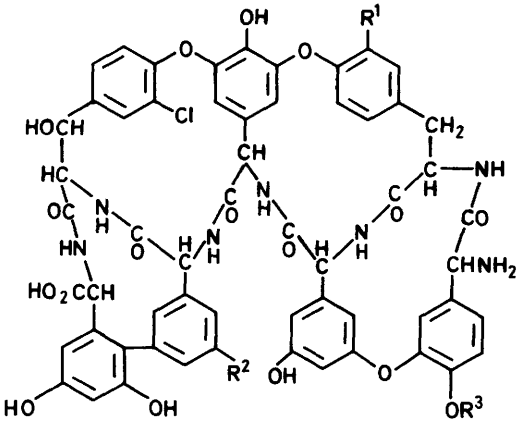
(80) a; R = $H_2NC(=NH)-$, $n = 2$
b; R = CHO, $n = 3$

antiviral antibiotics netropsin (80a) and distamycin (80b), first prepared in the 1960's, have been developed. The synthetic procedures are general, and it is proposed to apply these methods to other lexitropsins (oligopeptides designed to recognise specific DNA sequences in the minor groove).[183]

Synthesis of the sequence H-(γ-Glu-Cys)$_2$-D-Cys-γ-Glu-Gly-OH proposed in 1983 for the metallothioneine-like peptide cadystatin A (found in a fission yeast) gave material that was not identical with the natural compound. Compounds with D-Cys in other positions in the sequence were also prepared and found not identical. It has now been established that D-Cys is not present; the original assignment arose because racemisation occurred during the oxidation of cadystatin. The structure has therefore been revised to H-(γ-Glu-Cys)$_3$-Gly-OH, and cadystatin B to H-(γ-Glu-Cys)$_2$-Gly-OH; both sequences have been confirmed by synthesis.[184] A series of N^{ε}-acetylated derivatives of the [des-Trp^1, Cha^3, Lys^7] analogue (where Cha = cyclohexylalanine) of the *Saccharomyces cerevisiae* mating pheromone have been prepared. The biological activities of the acetyl, butyryl, capryl, and lauryl derivatives are similar to the unacylated parent, but the stearyl derivative is inactive.[185]

Both diastereoisomers of the dipeptide N-acetylbis-(1-pyrenylalanine) methyl ester have been studied by fluorescence measurements as model compounds of the randomly coiled peptide chain. The diastereoisomeric differences and solvent influences could be correlated with the populations of an extended conformation, which is unable to form an excimer, and with a folded conformation which does allow a transition to the excimer geometry within the lifetime of an excited pyrene moiety.[186] The crystal structure of Tyr-D-NleS-Gly-Phe-NleS (where NleS = α-aminopentanesulphonic acid), a biologically active enkephalin analogue, shows a II' β-bend conformation with an H-bond between Tyr-CO and Phe-NH for both molecules in the asymmetric unit.[187] Peptides containing N,N-dimethylamino acids, which are hard to obtain by conventional methods of peptide synthesis, have been prepared by using 3-cyano-4,6-dimethylpyridinethiol esters. In this way N,N-dimethylenkephalin was prepared without epimerisation.[188]

In the elongation of peptide chains containing a terminal N-carboxymethyl amino acid (CmAA), the coupling efficiency is



	R^1	R^2	R^3
A	Cl	Cl	H
B	Cl	H	H
C	Cl	Cl	Gal
D	H	Cl	H
E	H	H	H
F	Cl	Cl	Gal - Gal
G	H	Cl	Gal - Gal

(81)

generally poor. The 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide/HOBt method gave the best results for coupling C-terminal CmAA's, while coupling to the imino group is best done by the acid chloride method.[189] The coupling of Z-amino acid 1-succinimidyl esters with CmAA diesters has been accelerated by carrying out the reactions under 10 kbar pressure.[190]

4 Conjugate Peptides

4.1 Glycopeptide Antibiotics.- A complex of novel glycopeptide antibiotics A41030 produced by Streptomyces virginiae have been assigned structures (81). The major component A and three of the six minor factors are in fact aglycones; components D and G have two equivalent butyl groups attached to the peptide nucleus at an undetermined location. The complex is active against Gram-positive bacteria and shows growth promotion and feed efficiency improvement in poultry, swine, and ruminants.[191] A series of glycopeptide antibiotics related to the vancomycin-ristocetin family have been successfully analysed by FAB-m.s. Compounds of up to 2,100 daltons exhibit intense molecular and fragment ions from which information concerning carbohydrate composition and sequence is readily obtained. These methods have been applied to aridicins A, B, and C, three novel members of the vancomycin class.[192] Vancomycin is unstable in solution, an asparagine residue rearranging to isoaspartate, giving the inactive compound CDP-I. Peptide analogues of peptidoglycan such as Ac-D-Ala-D-Ala and diAc-L-Lys-D-Ala-D-Ala bind to vancomycin and have been found to stabilise the antibiotic against this degradation. Protection is effective even under prolonged heating at 80°C or steam sterilisation.[193]

Mono- and di-dechlorinated derivatives of vancomycin have been prepared by catalytic dehalogenation with 10% Pd/C. Heating the products to 80°C at pH 4.2 leads to CDP-I-type rearrangement products, and the dechlorinated products do not bind di- and tripeptides as effectively as the parent antibiotic.[194] The structure elucidation of the vancomycin group of antibiotics, including ristocetin, has been reviewed.[195] The conformation of ristocetin A in aqueous solution has been shown to be very similar to that previously found in DMSO, as are the conformations of its complexes with Ac-D-Ala-D-Ala and DiAc-Lys-D-Ala-D-Ala; unusual behaviour of some amide protons was again observed.[196] A series of

ristocetin analogues with modifications (OH, C=O, C=NOH, $NHCOCH_3$) at the C'-amino group have been prepared. They all possess antibacterial activity and bind to diAc-Lys-D-Ala-D-Ala. As they lack a positively charged amino group they are unable to form a salt bridge, indicating that the electrostatic interaction between the 1'-amino group and the carboxylate anion of ristocetin is not essential for complex formation.[197]

^{1}H-N.m.r. and u.v. difference spectroscopy studies of the binding energies of teicoplanin and degradation products of it lacking one, two, and three of the attached sugar moieties with N-Ac-D-Ala-D-Ala indicate that the complex formed is very similar in structure to that formed by ristocetin A and that the absence of the sugars has little effect on binding.[198] The copper(I)-bleomycin complex has been studied in detail by ^{1}H- and ^{13}C-n.m.r. and found to have a geometry distinct from Fe(II)-bleomycin. The degradation of DNA by this complex does not depend upon contaminating Fe(II) and does not result in the formation of thymine propenal.[199]

4.2 Other Glycopeptides. - The glycodipeptide β-D-Gal-p-(1→3)-α-GalpNAc-(1→O)-Ser-Thr-OH has been synthesised using an EEDQ mediated coupling of the O-glyco-amino acid,[200] and N-[2-^{3}H]acetyl-D-muramyl-Ala-D-iGln-Ala-2-(1',2'-dipalmitoyl-5N-glycero-3'-phosphoryl) ethylamide (MTP-cephalin), of specific activity 22 Ci $mmol^{-1}$, has been prepared for metabolic studies of its biological response-modifying activities.[201] The glycotripeptide Ala-(O-β-D-galactopyranosyl-Ser)-Ala has also been elaborated from a suitably protected O-glyco-serine.[202] Some hydroxy-bearing acyl analogues of FK-156 have been made and assayed for their ability to induce delayed-type hypersensitivity and antibody production. Three compounds in which the lactic acid group was replaced by $HOCH_2CO$-, PhCHOHCO- (D)- and $HOCH_2(CHOH)_4CO$- ($R_1S_1R_1R$) respectively were significantly superior to FK-156.[203]

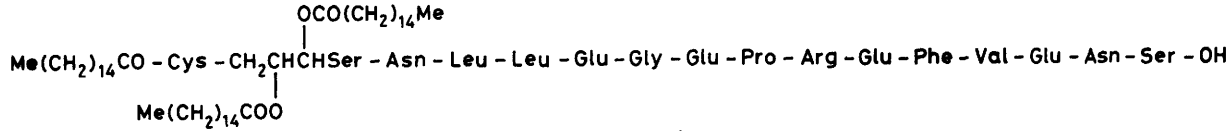
A glycotripeptide β-Glc-NAc(1→4)Glc(NAc)-Asn-Phe-Thr of the linkage region of the factor B of the human complement system and a glycopentapeptide β-Glc-NAc(1→4)Glc(NAc)-Asn-Ala-Thr-Ala-Ser of linkage region of the transmembrane neuraminidase of an influenza virus strain have been prepared using similar methodologies. In each case the fully acetylated disaccharide was converted to an amino derivative which was coupled to allyl Boc-Asp using EEDQ.

The allyl group was removed by a Pd(0)-catalysed transfer to morpholine before building up the peptide chain.[204] Two triglycosylated pentapeptides, Leu- and Ser-Ser*-Thr*-Thr*-Glu (where * represents 2-acetamido-2-deoxy-α-D-galactopyranosyl residues), which constitute the antigenic amino-terminal portions of human glycopherin A^N and A^{MC} respectively, have been synthesised in a stepwise fashion from the C-terminus using 2-azido-2-deoxy-α-D-galactopyranosyl-L-Ser or -Thr.[205]

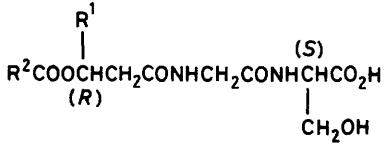
Using the recently developed method of allyl ester protection of carboxy functions (cf. ref. 204), the glycopentapeptide H-(Glc-NAcβ1)-Asn-Glu-Thr-Ile-Val-OH representing the partial sequence A^{80} - A^{84} of human fibroblast interferon has been prepared. C-Deprotection in this case was effected by Rh(I)-catalysed isomerisation of the allyl group followed by hydrolysis of the propenyl ester.[206] Treatment of peptidoglycan monomer from Brevibacterium divoricatum with aqueous ammonia leads to cleavage of the C-3 ether link in the N-acetylmuramyl residue to give the D-lactoyl pentapeptide and a saturated disaccharide. The latter has been identified by ^{13}C-n.m.r. as chitobiosamine.[207]

The glycohexapeptide O-(α-D-Glc)-(1→6)-O-β-Glc-(1→6)1-N-(Gly_3-Ser-Leu-glutam-5-oyl)-α-D-Glc-NH_2 has been synthesised as a model of a derivative possibly present in the glomerular basement membrane of rats, the protected L-glutamyl trisaccharide being coupled with a pentapeptide derivative using O,O-diethyl cyanophosphonate. The glutamyl trisaccharide was also converted into the acyl azide, which was condensed with bovine serum albumin to form a neoglycoprotein.[208] Using ^{1}H-^{1}H correlated n.m.r. spectroscopy and one and 1D-n.O.e. measurements, the preferred solution conformation of the disaccharide unit in the antifreeze glycopeptide (Galβ(1→3)Gal-NAcα1-Thr-Ala-Ala)$_n$ from the blood of the antarctic fish Trematomas borchgrevinki has been examined. The disaccharide, which is a rigid unit, exists with a restricted rotation around the GalNAc-α1-Thr linkage, the backbone peptide being in an extended helical conformation.[209]

4.3 α-Amino Conjugates. - The photo-affinity label N-(4-azido-2-nitrophenyl)-glycyl-$(Pro\text{-}Pro\text{-}Gly)_5$ for the binding site of prolyl 4-hydroxylase has been prepared. It proved to be a good substrate, and is capable of light-induced inactivation of prolyl 4-hydroxylase activity.[210] N^{α}-(5-Bromovaleryl)-N^{α}-deacetyl-α-melanotropin has been synthesised. On reaction with sodium thiosulphate it

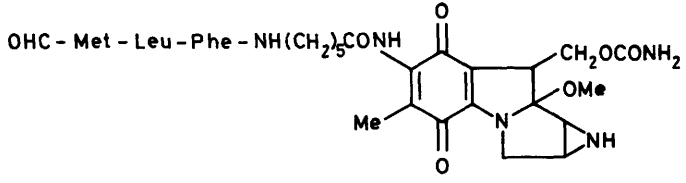


(82)

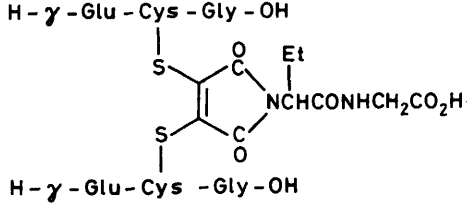


	R^1	R^2
A	$Me_2CH(CH_2)_{10}$—	$Me_2CH(CH_2)_7CH{=}CH(CH_2)_2$—
B	$Me_2CH(CH_2)_{11}$—	$Me_2CH(CH_2)_7CH{=}CH(CH_2)_2$—
C	$Me_2CH(CH_2)_{10}$—	$Me_2CH(CH_2)_{11}$—
D	$Me_2CH(CH_2)_{11}$—	$Me_2CH(CH_2)_{11}$—

(83)



(84)



(85)

gives the 5-sulphothio derivative which has been used to prepare tobacco mosaic virus-α-melanotropin disulphide conjugates. The work is aimed at an artificial antibody to study the localisation and cellular dynamics of neuropeptide receptors.[211]

A conjugate (82) of antigen with the synthetic lipopeptide which constitutes the N-terminus of the lipoprotein from the outer membrane of E. coli has been made. In contrast to other conjugates it required only a single administration without multiple boosting, and was highly efficient both in vitro and in vivo in producing specific antibodies.[212] One-electron reduction using pulse radiolysis techniques of a series of complexes $[(NH_3)_5Os^{III}$-isonicotinyl-peptide-$Co^{III}(NH_3)_5](BF_4)_5$, where the peptide is Pro_{1-4}, Phe_2, or Gly_2, gives the corresponding $[(NH_3)_5Os^{II}$-isonicotinyl-peptide-$Co^{III}(NH_3)_5]^{4+}$ compounds. The reduced Gly and Phe dipeptide derivatives undergo intramolecular electron transfer 5-10 times as fast as that of Pro. The rates for the Pro peptides are more rapid than the rate of trans to cis isomerisation and therefore it takes place while the proline is in its predominant trans configuration.[213] The structures of WB-3559 A, B, C, and D (83), new fibrinolytic agents isolated from a Flavobacterium species, have been recently elucidated. The total synthesis of component D has confirmed its structure.[214]

4.4 Other Conjugates.- An N-formyl peptide has been conjugated to mitomycin C, a highly reactive cytotoxic agent, at the N-7 position to give (84) as a targeting chemotherapeutic drug. Mitomycin C was treated with NaH and the p-nitrophenyl ester of the peptidic component then added, the reaction mixture finally being quenched with dry ice.[215] Three potent inhibitors of mammalian liver glyoxylase II, the S-(o-, m-, and p-nitrocarbobenzoxy)-glutathiones, have been prepared. They are almost inactive as inhibitors of glyoxylase I, which is in marked contrast to other glyoxylase II inhibitors.[216] Several N-dichloromaleoyldipeptides have been synthesised and conjugated with glutathione to give derivatives such as (85).[217] A photoreactive [D-Ala^2, p-N_3-Phe^4, Met^5]-enkephalin has been made, and competitive receptor binding showed it to be 4-fold more potent than [D-Ala^2, Met^5]-enkephalin in competing for binding to the enkephalin binding site. This suggests it may be suitable for the in situ photoaffinity labelling of the enkephalin receptor.[218]

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5
β-Lactam Antibiotic Chemistry

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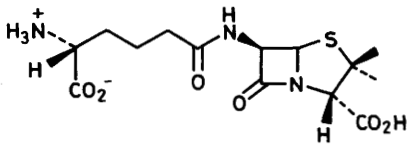
1 Introduction

While the general level of activity has remained high, an area which has received much attention this year has been the synthesis of chiral azetidin-2-one derivatives from chiral starting materials, often utilising diastereoselective reactions; such intermediates usually have been intended as precursors for monobactam or carbapenem syntheses. Reviews on naturally occurring β-lactams[1], recent developments in β-lactam antibiotic chemistry[2] and the utility of 4-acetoxyazetidin-2-one in the synthesis of potential antibacterials[3] have been published, while Knowles has presented an account of his elegant studies on the mechanisms of chemical inhibition of β-lactamases.[4]

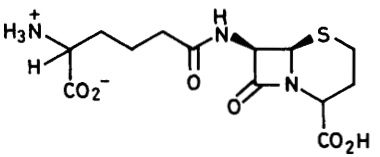
2 New Natural Products

Penam **(1)** and cepham **(2)** have been isolated from a Streptomyces species found in a soil sample;[5] although these bicyclic systems (with epimeric side chains) have been prepared from synthetic tripeptide precursors and purified enzyme systems,[6] they have not been isolated from intact organisms. Cephabacins M_{1-6}, which are cephamycins of the general type **(3)**, have been isolated from Xanthomonas lactamgena bacteria;[7] the R groups were principally polypeptide structures. Chitinovorin D **(4)**, where R is a large, strongly basic residue, was isolated from a Flavobacterium and possessed the characteristic chitinovorin 7α-formamido group.[8] Pluracidomycin A_2 **(5)**, a new carbapenem bearing a sulphinic acid C-2 side chain, was obtained along with other minor members of this family from a Streptomyces pluracidomycetus.[9] Chlorocardicin **(6)**, an aromatic-chlorinated derivative of nocardicin A, has been isolated from a Streptomyces;[10] while formadicins A-D **(7)** from a Flexibacter aligoliquefaciens[11] possess a more highly modified nocardicin-type structure, notably by the 3α-formamido group and the glucuronic acid residue.

It is of interest to note that azetidine **(8)** has been isolated from an Actinomycete and that it is active against plant pathogens although ineffective against bacteria.[12]



(1)



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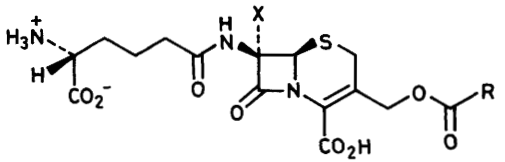
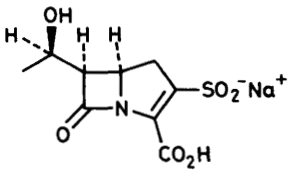


(3) X = OMe
(4) X = NHCHO



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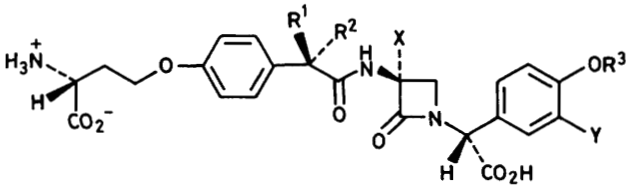
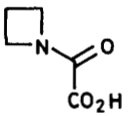


(6) $R^1, R^2 = =N-OH$, $R^3 = X = H$, $Y = Cl$

(7) A; $R^1 = OH$, $R^2 = H$, $R^3 =$ D-glucuronide, $X = NHCHO$, $Y = H$
B; $R^1 = NHCHO$, $R^2 = H$, $R^3 =$ D-glucuronide, $X = H$, $Y = H$
C; $R^1 = OH$, $R^2 = H$, $R^3 = H$, $X = NHCHO$, $Y = H$
D; $R^1 = NHCHO$, $R^2 = H$, $R^3 = H$, $X = H$, $Y = H$

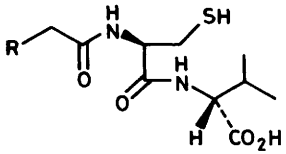


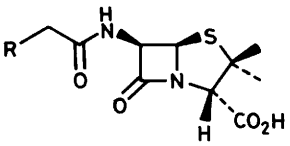
(8)

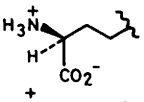
3 Biosynthesis

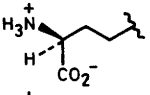
After the large number of important developments reported last year on enzymic aspects of penam and cephem biosynthesis, work presented this year in the main has been directed at more detailed examination and confirmation of key assumptions. Baldwin has shown by incubation of isopenicillin N (IPN) synthetase with δ-(L-α-amino-δ-adipoyl)-L-cysteinyl-D-valine, (LLD-ACV) **(9)**, labelled either with ^{34}S or ^{2}H at valine C-2 and failing to isolate any doubly labelled product, that the sulphur atom in isopenicillin N **(10)** is the same as that in the tripeptide precursor[13]; although generally assumed to be the case, this had never been rigorously demonstrated. Exposure of LLD-ACV thia-isostere **(11)** to IPN synthetase from Cephalosporium acremonium gave rise to the penicillin **(12)** in which the L side-chain stereochemistry was conserved[14]; the previously observed conversion to the penicillin N analogue was suggested as resulting from isomerisation of the side chain by IPN epimerase inside the C.acremonium mycelia. In contrast to evidence suggesting that modified aminoadipoyl analogues of LLD-ACV required a residue with a six-atom chain terminating in a carboxylic acid group, experiments with pure IPN synthetase have shown **(13)** and **(14)** respectively to be direct precursors of penicillin G **(15)** and penicillin V **(16)**, although with very low efficiency in relation to LLD-ACV.[15] Determination of k_m and v_{max} data suggested that the terminal carboxyl group may be of major importance in the catalytic event rather than in simple binding to the enzyme. Incubation of LLD-ACV **(19)** enriched so as to show ^{15}N-^{13}C nmr coupling at N-4 and C-5 of isopenicillin N **(10)** and thus at N-1 and C-4 of any monocyclic azetidin-2-one intermediate present during biosynthesis only gave results consistent with structure **(10)**, and so left clear identification of an intact monocyclic β-lactam intermediate in penicillin biosynthesis still to be demonstrated.[16]

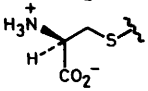
Incubation of D,L-(3R,4R)-[4-^{2}H,^{3}H] or D,L-(3R,4S)-[4-^{2}H,^{3}H] valine **(17)** with C. acremonium proceeded via **(10)**, correspondingly labelled at the 2β-methyl group, to cephalosporin C **(18)** in which the label, located at C-2, was racemic[17]; such results are consistent with activation of the 2β-methyl group by formation of a radical or carbocation prior to ring expansion to the cephem. These results along with some already published on the fate of the 2β-methyl group in penicillin N have also been published together.[18] In contrast to results reported for cephalosporin C biosynthesis by C. acremonium, it has been shown in Streptomyces clavuligerus that the formation of deacetoxycephalosporin C **(19)** and subsequent hydroxylation at C-3' are effected by two distinct and separable enzymes;[19] this represents the first major difference observed between the two organisms in cephalosporin C biosynthesis.

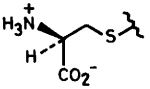




(9) R = 

(10) R = 

(11) R = 

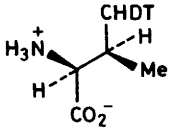
(12) R = 

(13) R = Ph

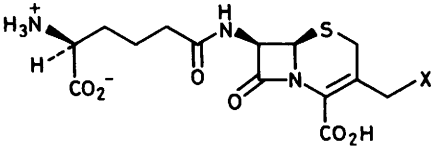
(14) R = PhO

(15) R = Ph

(16) R = PhO

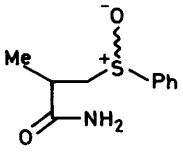


(17) 4*R* or 4*S*



(18) X = OAc

(19) X = H



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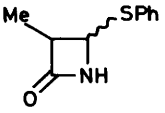

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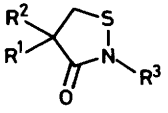

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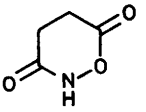
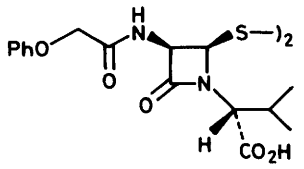


(23)

A number of reports have appeared on in vitro reactions intended to mimic either the formation of an azetidin-2-one intermediate from LLD-ACV or the subsequent ring closure of this enzyme-bound monocyclic species to the bicyclic penam nucleus. Pummerer reaction of model systems e.g. **(20)** with trimethylsilyl triflate and triethylamine yielded **(21)** in a cis:trans ratio of 2.7:1;[20] azetidin-2-ones were also the products from treatment of isothiazolines **(22)** with phenyllithium[21] and from thermolysis or photolysis of tetrahydrooxazinedione **(23)**.[22] Disulphide **(24)**, a model for a postulated monocyclic biosynthetic intermediate, was oxidised in vitro with iron(II) sulphate, ascorbic acid and EDTA in the presence of oxygen, to give a low yield of penicillin V **(16)** as well as two isomeric compounds, one of which was cepham **(25)**; these results were presented in support of a radical (or iron-carbon) intermediate in the enzymic process.[23]

Two reports on clavulanic acid **(26)** biosynthesis have shown intact incorporation of radiolabelled three carbon precursors corresponding to the C-(5,6,7) unit; in one case this was introduced as glycerate[24] and in the other the precursor was ß-hydroxypropionate[25]. Since in the latter study incubation of α-[2-^{3}H, 2-^{14}C] doubly labelled compound gave clavulanic acid with the same $^{3}H/^{14}C$ ratio it seems more likely that glycerate is reduced to ß-hydroxypropionate prior to incorporation into the biosynthetic pathway rather than the reverse oxidative process. Labelling studies on the origin of the remaining five carbon atoms, which had previously been introduced as the δ-hydroxynorvaline unit **(27)**, have shown ornithine **(28)** to be much more efficiently incorporated, suggesting that the latter is the true precursor of the C_5 unit;[26] in clavulanic acid biosynthesis **(29)** could therefore be the analogue of the LLD-ACV tripeptide in penicillin systems.

The acylase involved in exchange of pantotheinyl side chains in e.g. **(30)** during biosynthesis of the OA6129 carbapenems has been characterised,[27] while feeding radiolabelled ß-alanine to the biosynthetic system led to incorporation of this as the corresponding element in the C-2 side chain.[28] It was considered likely that the side chain was built up successively from pantoate, ß-alanine and cysteine and, after decarboxylation, was incorporated into the ß-lactam biosynthetic pathway. This group of carbapenems could be phosphorylated at the primary hydroxyl of the pantotheinyl side chain by Brevibacterium ammoniagenes, which resulted in some modification in antibacterial activity and dehydropeptidase stability.[29]



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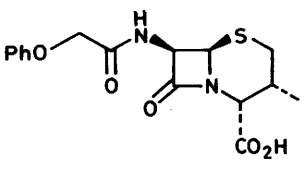

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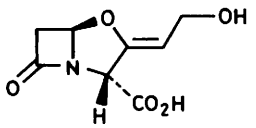

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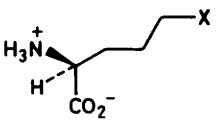
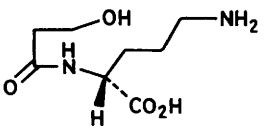


(27) X = OH
(28) X = NH_2



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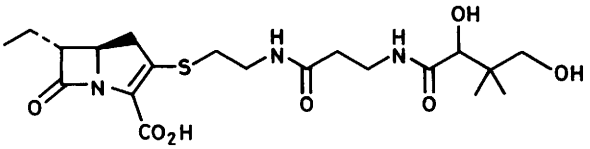

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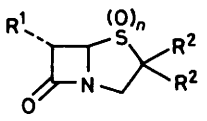
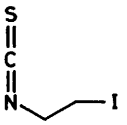
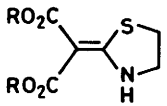


(31) a; $R^1 = R^2 = H$, $n = 0$
b; $R^1 = Me$, $R^2 = H$, $n = 0$
c; $R^1 = H$, $R^2 = Me$, $n = 1$
d; $R^1 = H$, $R^2 = Me$, $n = 2$



(32)



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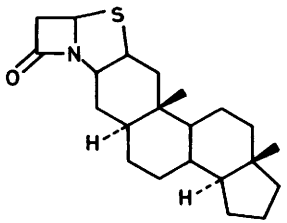


(34)

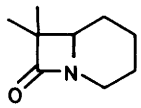
4 Penicillins and Cephalosporins

Three syntheses of the parent ring system of the penicillin nucleus, 1-aza-7-oxo-4-thiabicyclo[3.2.0]heptane (penam) **(31a)**, have been reported. A short synthesis starting from ethyl propiolate has been published, the final β-lactam-forming step by the Mukaiyama-Ohno method proceeding only in 8% yield.[30] By a similar β-lactam-forming reaction (±)-6-methylpenam **(31b)** was obtained in 70% yield[31]. A longer synthesis involved formation of the thiazolidine ring by treatment of a vic-iodoisothiocyanate **(32)** with malonate anion to give **(33)**: decarboxylation followed by reduction and cyclisation gave penam **(31a)**. Use of other vic-iodoisothiocyanates gave more complex polycyclic penams, including the penam-steroid hybrid **(34)**.[32]

Penam oxides **(31c)** and **(31d)**, synthesised from 4-(t-butylthio)-azetidin-2-one, showed no antibiotic or β-lactamase inhibitory activity,[33] probably due to the lack of a C-3 carboxyl group. Bicyclic β-lactams have been produced by intramolecular radical cyclisation reactions: carbacepham **(35)** was formed by thermolysis with tri-n-butylstannane of the 4-(phenylthio)azetidin-2-one **(36)**, presumably via radical **(37)**. A bicyclic [5.2.0]nonane could be made by a similar reaction, whereas a carbapenam could not[34]. Tricyclic 2,3-benzocepham **(38)** was synthesised in 42% yield by reaction of the 4-(t-butylthio)-azetidin-2-one **(39)** with tri-n-butylstannane;[34] the corresponding 2,3-benzopenam, however, could only be formed in very low yield (1%) under these conditions.[35]

The ready availability of 6-aminopenicillanic acid (6-APA) and 7-aminocephalosporanic acid (7-ACA) has long made these the starting materials for the many syntheses of variants of the penam/penem and cepham/cephem nuclei. A simple, efficient, stereospecific route to the nocardicin or monobactam precursor 3-aminoazetidin-2-one **(40a)** from 6-APA has been reported. Conversion to the N-1 unsubstituted compound by standard methods gave **(40b)** from which the acetoxy group could be readily displaced by borohydride in aqueous isopropanol. Hydrogenolysis gave **(40a)** in 100% yield. Reduction of the readily available 4-acetoxyazetidin-2-one with potassium borohydride gave the parent ring system of the β-lactam series, 2-oxoazetidine.[36] Reaction of the benzhydryl esters of 6-APA, 7-ACA or 7-aminodeacetylcephalosporanic acid with trimethyl orthoacetate or trimethylorthobenzoate gave the corresponding 6(7)-iminoethers **(41)** and **(42)** respectively.[37] Oxidation of sulphides **(41)** and **(42)** with mCPBA or H_2O_2 gave the more hindered 1β-sulphoxides; peroxo intermediate **(43)** was postulated to account for this observed stereochemistry, oxygen thus being transferred to sulphur from the more hindered β-face of the molecule.[38]

Condensation of the 6-ketopenicillanate **(44)** with the lithium salt of



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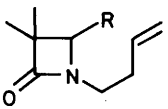
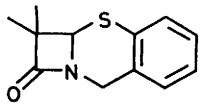


(36) R = SPh
(37) R = •



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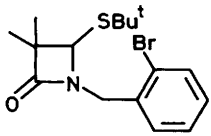
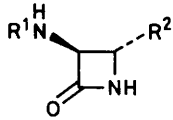
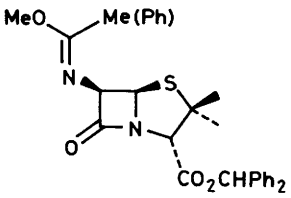


(39)



(40) a ; $R^1 = R^2 = H$
b ; $R^1 = PhCH_2OCO$
$R^2 = OAc$



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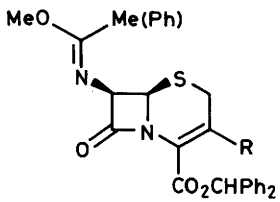
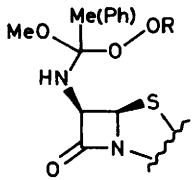
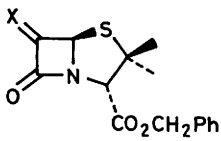


(42) R = Me or CH_2OAc

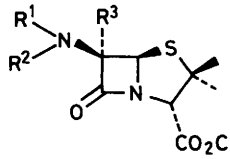


(43)

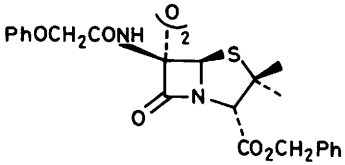


(44) X = O

(45) X =



(46) $R^1 = H, R^2 = Cl_3CCH_2OCO, R^3 = NHCHO$
(47) $R^1 = CF_3SO_2, R^2 = R^3 = H$
(48) $R^1 = PhCH_2CO, R^2 = H, R^3 = S(O)Me$
(49) $R^1 = PhOCH_2CO, R^2 = H, R^3 = SMe$



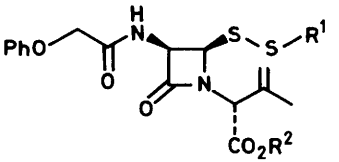
(50)

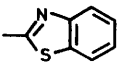
(methoxymethyl)trimethylsilane at -100 °C gave an alcohol which on acetylation followed by fluoride-induced elimination produced a mixture of (E) and (Z)-6-methoxymethylenepenicillanates **(45)**; the free acid of the (Z)-isomer showed β-lactamase inhibitory activity.[39]

Conversion of 6-APA esters to their 6-formamido derivatives **(46)** was accomplished by treatment of monotriflamide **(47)** with 2,2,2-trichloroethoxycarbonyl chloride followed by bis(trimethylsilyl)formamide (BSF). Use of MeOH in place of BSF gave the 6α-methoxy species; 7α-formamido derivatives of 7-ACA were synthesised by the same route.[40] 6α-Formamido and 6α-methoxy penicillin G derivatives could also be obtained in good yield by reaction of sulphoxide **(48)** with N,O-bistrimethylsilylformamide and MeOH respectively.[41] The bispenicillin ether **(50)**, synthesised in three steps from 6α-methylthio penicillin V derivative **(49)** (Scheme 1) had little biological activity.[42]

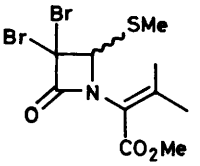
Thermolysis of penicillin sulphoxides has long been known to give unisolable sulphenic acids, which can be trapped by reaction with various thiols to give disulphides **(51)**. Most easily formed are the disulphides obtained by thermolysis of sulphoxide in the presence of heterocyclic thiols, such as 5-mercapto-2,3-benzothiadiazole.[43] These disulphides e.g. **(51)** and the bis(secopenicillanate)disulphides can be usefully converted to 2β-(chloromethyl) penams by treatment with $CuCl_2$.[44] In contrast, aryl thiols react with sulphenic acids much more slowly than heterocyclic thiols (significant decomposition to β-lactam-cleaved products also occurs), and the disulphides obtained e.g. **(52)** cannot be converted to 2β-(chloromethyl)penams under the same conditions as above.[45] Thermolysis of C-6 unsubstituted penicillin sulphoxides does not lead to disulphides, possibly because of lack of any stabilisation of the intermediate sulphenic acid by the C-6 amide substituent. A further incentive for sulphenic acid **(52)** formation is thought to be relief of strain on the β-face of the penicillin molecule by opening of the thiazolidine ring: this relief would not be as significant in thermolysis of C-6-unsubstituted sulphoxides.[45] Alkyl thiols react at a similarly slow rate as aryl thiols: use of a high boiling thiol such as ethyl thioglycolate shortens the reaction time and gives mainly the 1,2-seco-penicillanate plus decomposition products.[46] Thermolysis of penicillin sulphoxides in trimethylorthoacetate leads to no β-lactam-containing products, a mixture of heterocyclic and acyclic species being obtained.[47]

An alternative thiazolidine ring-opening reaction, the Nayler Reaction, was shown to give racemisation at C-4 of the resulting 4-(alkylthio)azetidin-2-one **(53)**.[48] A proposed anion-induced non-synchronous [3+2] cycloreversion mechanism for the racemisation in the case of 6,6-dibromopenicillanate **(54)** involved intermediacy of thioaldehydic species **(55)** which would recyclise to racemic

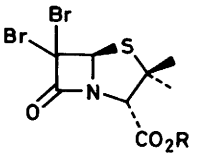


(51) R = 

(52) R = Ph

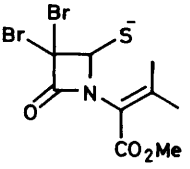


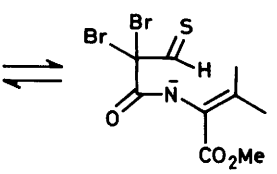
(53)



(54) R = Me

(56) R = $CHPh_2$





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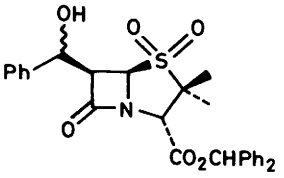

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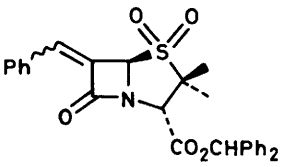

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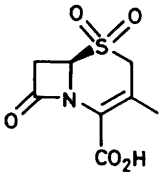

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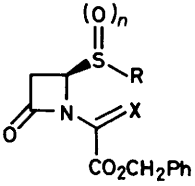


(60) a; R = OH, X = CMe_2, $n = 1$

b; R =  , X = CMe_2, $n = 2$

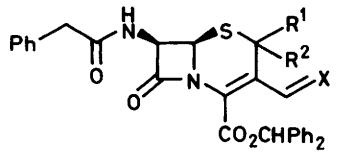
c; R =  , X = O, $n = 2$

β-lactam **(53)**.

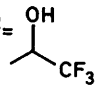
Benzhydryl 6,6-dibromopenicillanate **(56)** was converted to the 6-(hydroxybenzyl)- and 6-(E/Z)-(benzylidene)penicillanate β-lactamase inhibitors **(57)** and **(58)** respectively by conventional methods.[49] Other 6,6-dihalopenicillanates were produced from 6-diazopenicillanate pivaloyloxymethyl ester by reaction with N-halosuccinimide/potassium halide or haloiodides.[50] The stereoselectivity observed suggests that the reaction proceeds in a non-concerted fashion, involving displacement of nitrogen from diazonium ion as the final step. 6α-Monobromopenicillanates have appeared in two reports: incorporation of a deuterium or tritium label into the 2β-methyl group of the sulphoxide benzyl ester could be accomplished by thermolysis in the presence of deuterium oxide or tritium oxide.[51] Debromination of 6-bromopenicillanates could be brought about in yields of 44-72% by reaction with Zn/I_2 in dioxan followed by hydrolysis of the organozinc intermediates to the 6-dihydropenicillanates; both steps of the reaction were promoted by ultrasonic agitation of the reaction mixture.[52] An improved synthesis of penicillanic acid S,S-dioxide (sulbactam) from 6-APA S,S-dioxide has been reported.[53] Conversion of sulbactam itself to deacetylcephalosporanic acid sulphone **(59)** has been reported: thus sulbactam benzyl ester was ring-opened to the sulphinic acid **(60a)** by Stoodley's method (DBN,CH_2Cl_2,0°C) and was alkylated with chloroacetone to give ketone **(60b)**. Ozonolysis to oxalimide **(60c)** followed by phosphite-mediated reductive cyclisation and deprotection gave the free acid **(59)** which had no biological activity.[54]

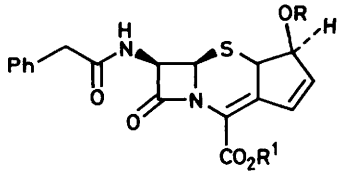
A number of ampicillin side-chain elaborations have been reported,[55,56,57] while in the cephem series efficient procedures for acylation of the 7β-amino group and C-3' substitution have been described,[58] as has a method for epimerisation of 7α-amino cephems to the biologically active 7β series.[59]

The C-3 methyl group of 3'-deacetoxycephalosporanates is converted to a bromomethyl group by photolysis in the presence of NBS.[60] The 3-(bromomethyl)-cephalosporanates produced may be converted to 3-(acyloxymethyl)cephalosporanates by treatment with tetraalkylammonium carboxylate salts[61] or to 3-(heterocyclyl-thiomethyl)cephalosporanates with thiols.[62] The triphenylphosphorane **(61a)** derived from 3-(bromomethyl)cephalosporanates could be made to undergo Wittig reactions with various aldehydes, although side reactions also occurred. For instance trifluoroacetaldehyde gave the Wittig product **(61b)** as well as side products whilst glyoxal gave tricyclic cephem **(62)**. Acetate **(63)** derived by acetylation of tricycle **(62)** showed low activity against Gram-positive and Gram-negative organisms.[63] Tricyclic sulphoxide **(64)**, synthesised from the sulphoxide of **(61a)** and acrylaldehyde, could be aromatised and desulphurised to nocardicin precursor **(65)**[64]. C-3 Alkyl cephems **(66)** have been made by reaction of 3-chloro-,



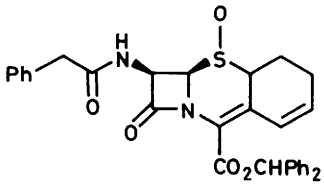
(61) a; X = PPh_3, $R^1 = R^2 = H$

b; X = $CHCF_3$, $R^1 = H$, R^2 = 



(62) $R^1 = CHPh_2$, $R^2 = H$

(63) $R^1 = H$, $R^2 = Ac$



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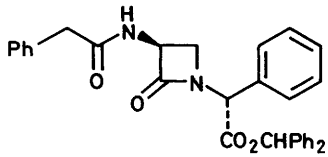

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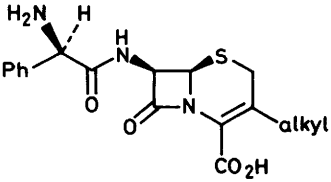

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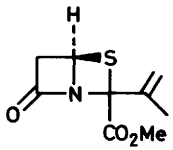

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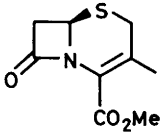

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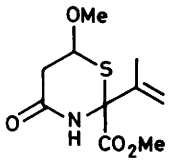

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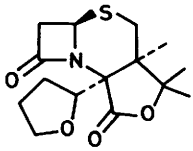

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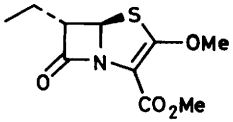

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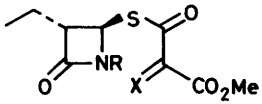
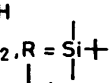
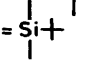


(72) X = O, R = H

(73) X = $CHNMe_2$, R = 

(74) X = H,H, R = 

3-(phenylthio)-, 3-vinyl- and 3-(iodomethyl)-cephems with lithium organocuprates: increasing the C-3 alkyl chain length increases Gram-positive but decreases Gram-negative activity with respect to the 3-methyl cephem.[65]

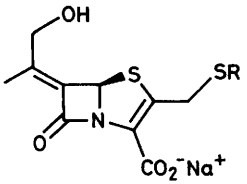
In an attempt to produce the bicyclo[2.2.0]hexane **(67)**, photolysis of cephem **(68)** gave instead the 1,3-thialactam **(69)** in MeOH and the cepham **(70)** in THF-acetone by radical reactions.[66]

5 Penem Chemistry

A synthesis of penem **(71)** was effected by the ready ring closure of reactive tricarbonyl compound **(72)**, obtained from photooxidation of enamine **(73)**, which in turn was synthesised from malonate **(74)**.[67] The same approach has been used in carbapenem and carbacephem synthesis and appears to present a general alternative to the NH-carbenoid insertion route to bicyclic systems. Penem variants **(75)** bearing the C-6 side chain characteristic of asparenomycins and which were reported to possess good biological activity have been synthesised using a combination of synthetic strategies that have been applied in the synthesis of the two types of parent compound.[68] A number of papers have appeared describing the synthesis by the phosphite-mediated cyclisation route of penems of the general type **(76)**[69,70,71].

6 Carbapenem and Carbapenam Chemistry

Reaction of thienamycin-*S*,*S*-dioxide derivative **(77)** with DBU and methyl iodide in DMF resulted in a Smiles-type rearrangement and isolation of **(78)** in 14% yield, but attempts to induce a similar rearrangement by the sulphide **(79)** were unsuccessful.[72] **(78)** was claimed as the first C-2 nitrogen-substituted carbapenem to be reported; however, later in the same year 2-azido compound **(80)** was described: it was obtained by azide substitution on the corresponding enol tosylate, underwent characteristic azide 1,3-dipolar cycloaddition reactions with alkynes and alkenes and could be reduced to a highly unstable enamine. The free acids from these derivatives were less biologically active than thienamycin **(81)**[73]. Several C-9 methoxythienamycin derivatives have been prepared, e.g. **(82)**, but all showed inferior activity to the parent compound;[74] these syntheses utilised C-3 *p*-methoxybenzyl esters which were ultimately cleaved with $AlCl_3$ in anisole. Both 1α- and 1β-methylthienamycins have been synthesised by routine methods and the latter has been shown to be more antibacterially active than



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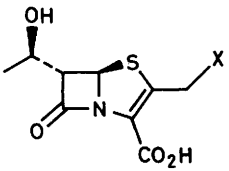
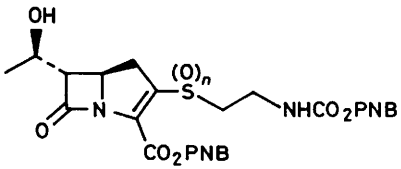


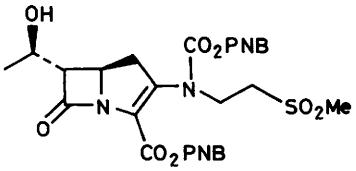
(76)



PNB = $CH_2 \cdot C_6H_4 \cdot NO_2 - p$

(77) $n = 2$

(79) $n = 0$



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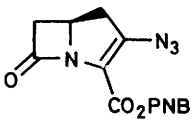

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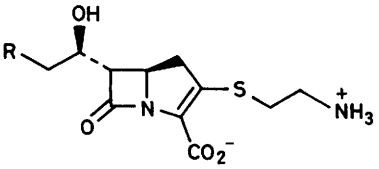
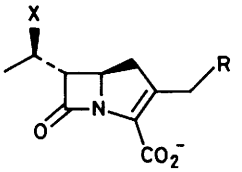


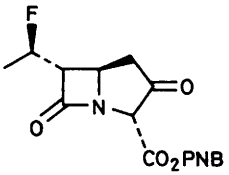
(81) R = H

(82) R = OMe



(83) X = OH, R = $(CH_2)_2\overset{+}{N}H_3$

(84) X = F, R = $S(CH_2)_2\overset{+}{N}H_3$



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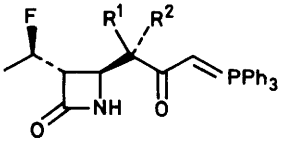
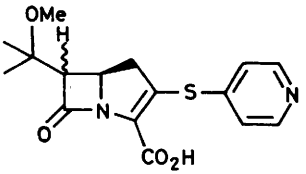


(86) R^1 = H, R^2 = Me, or

R^1 = Me, R^2 = H



(87)

thienamycin and to possess high resistance to renal dehydropeptidase-1 enzyme.[75] The 1-methyl, 1-methoxy and 1,1-dimethyl derivatives have also been synthesised via reaction of 4-acetoxyazetidinones with appropriate trimethylsilyl enolates.[76] Dethiathienamycin **(83)**, which showed approximately half of the activity of thienamycin, was synthesised from a penicillin-derived 4-acetoxyazetidinone intermediate utilising its reaction with tetraallyltin and BF_3-etherate to introduce an allyl group at C-4.[77]

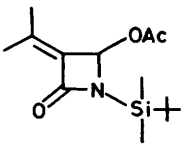
Fluorinated carbapenems e.g. **(84)** were synthesised via the Wittig cyclisation procedure, but they showed poor chemical stability;[78] attempts to utilise nucleophilic addition (EtOH, Me_3SiSMe, $PhCH_2NH_2$ or $\bar{C}H_2NO_2$) to **(85)** in each case resulted in opening of the five-membered ring by a retro-Dieckmann reaction.[79] Intermediates **(86)** were reacted with aldehydes and ketones and the resulting derivatives were cyclised to fluorocarbapenems bearing unsaturated C-2 side chains.[80]

Both 6α- and 6β-carpetimycin methyl ether derivatives **(87)** have been synthesised but these were less active than the corresponding alcohols.[81] Allylic bromination of acetonylideneazetidinone **(88)** gave a 1:1 mixture of isomers which after separation and substitution of bromide by hydroxyl were ultimately converted into asparenomycin C precursor **(89)**[82]; a variety of asparenomycins have been prepared via base-catalysed elimination of CO_2 from previously reported cyclic carbonate intermediates.[83,84]

Nitroalkene **(90)** synthesised from phenylthionitromethane and the appropriate azetidinone aldehyde was converted after N-deprotection into carbapenam **(91)** by reaction with base followed by ozonolysis of the nitronate anion; this approach was also applied to a carbacepham and an oxapenam.[85] Exo-methylene carbapenam **(92)** was obtained in 60% yield by an intermolecular base-promoted Michael addition - cyclisation sequence involving a 4-iodomethylazetidinone derivative and an allene.[86]

7 Azetidinones

This section forms the largest single part of the review and will include all syntheses of the β-lactam ring not described elsewhere and also novel chemistry of azetidin-2-ones. Syntheses of the β-lactam ring may be classified according to which bond(s) is (are) formed in the ring-forming step.



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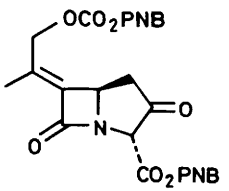

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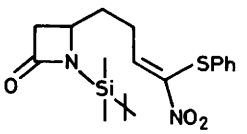

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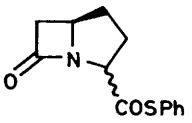

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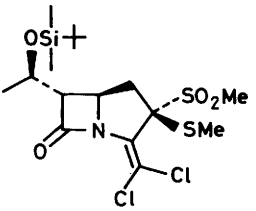

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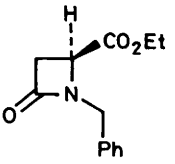

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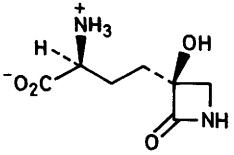

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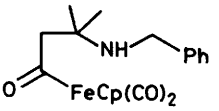

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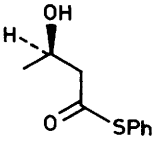

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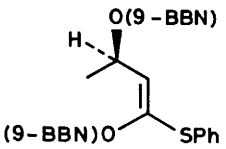

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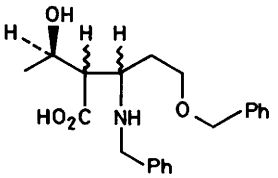

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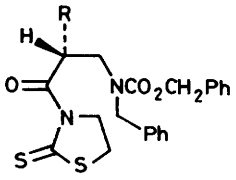
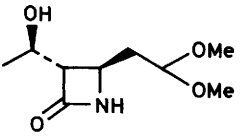


(99) a; R = H

b; R = 



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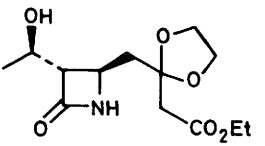


(101)

Synthesis

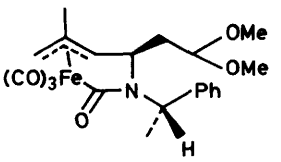
1-2 bond-forming reactions

Formation of the 1-2 bond is accomplished by attack of nitrogen (ultimately appearing as N-1 in the azetidinone) on a suitably activated carbonyl group: methods for effecting this reaction abound and a greater challenge to chemists is obtaining the 'correct' relative or, preferably, absolute stereochemistry of substituents on the azetidinone ring.

Only one example of a direct aspartic acid derivative being used to form the β-lactam ring has been reported; thus N-benzyl diethylaspartate was cyclised to **(93)** by treatment with ethylmagnesium bromide;[87] a similar 1-2 cyclisation ultimately gave the (±)-trans-3-acetyl-4-carboxylic acid.[88] Other racemic syntheses included that of tabtoxine β-lactam **(94)**[89] by refinement of a previously reported route.[90] Reaction of the acyl iron complex **(95)** with bromine and triethylamine gave a 90% yield of 4,4-dimethylazetidin-2-one.[91]

The 6(R)-hydroxyethyl side chain of carbapenems in the thienamycin series can be visualised as being derived from 3(R)-hydroxybutyrate **(96)**, the C-1 and C-2 atoms of the butyrate ultimately becoming, respectively, C-2 and C-3 of the β-lactam ring. Thus conversion of **(96)** to the vinyloxyborane **(97)** and condensation with the appropriate imine gave the β-amino acid derivative **(98)**; cyclisation by the Mukaiyama-Ohno method gave a mixture of azetidinones, the major component of which had the required 3(S),4(R) absolute stereochemistry.[92] An example of a directed aldol reaction was the tin-mediated condensation of the enolate derived from acylthiazolidine-2-thione **(99a)** with, for example, acetaldehyde, to give β-hydroxy carbonyl compound **(99b)** and its diastereoisomer; species **(99b)**, having all the required atoms and relative stereochemistry for a β-lactam with a 3α-(R)-hydroxyethyl moiety, was favoured in a 9:1 ratio. Cyclisation of **(99b)** was accomplished after N-deprotection by treatment with N-methyl-2-chloropyridinium iodide and triethylamine in dichloromethane.[93]

1,3-Dipolar cycloaddition of methyl crotonate to 3,3-dimethoxypropionitrile-N-oxide gave an isoxazolone having 3,4-trans relative stereochemistry. Conversion to azetidinone **(100)** and further elaboration by dehydration and ozonolysis to an azetidin-2,3-dione followed by treatment with hydroxylamine hydrochloride gave an oxime; reduction (H_2,Rh/Al_2O_3) completed a novel route to 3-amino-substituted azetidinones in which the cis-3,4 isomer was produced in a 3:1 ratio to the trans isomer.[94] A chirally N-substituted nitrone underwent 1,3-dipolar cycloaddition with benzyl crotonate to yield separable diastereoisomeric isoxazolidines; use of previously reported methodology gave



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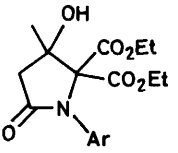

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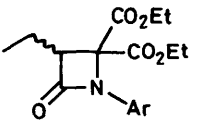

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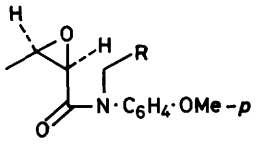
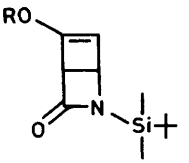
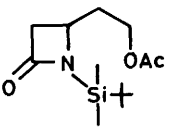


(105) a; R = ≡—SPh
b; R = COPh



(106) a; R = Ac
b; R = Si



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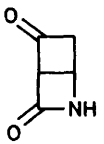

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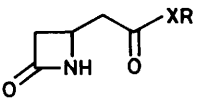
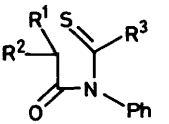


(109) X = O or S



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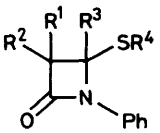
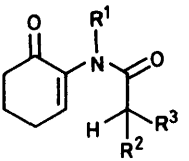


(111) a; R^4 = H
b; R^4 = Ac



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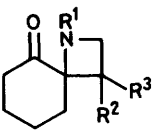

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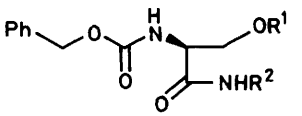
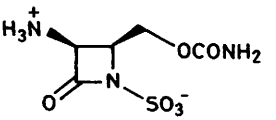


(114) a; R^1 = SO_2Me, R^2 = OCH_2Ph
b; R^1 = SO_2Im, R^2 = C_6H_4·OMe-*p*



(115)

chiral azetidinone **(101)**.[95]

2-3 bond-forming reactions

In the only example of this reaction, lactam **(102)** was oxidised (ceric ammonium nitrate (CAN)) to a β-lactam which was readily converted into the chiral carbapenem precursor **(100)**.[96]

3-4 bond-forming reactions

Pyrrolidones **(103)** derived from β-ketoamides gave β-lactams **(104)** in 54-80% yields on treatment with I_2/NaOEt.[97] Syntheses of racemic esters of the carbapenems PS-5 and PS-6 were reported, involving similar intramolecular displacement of bromide by anions derived from malonates as the 3-4 bond-forming step.[98] An extension of this synthesis started from L-threonine: conversion to an α-bromo-β-hydroxy amide followed by treatment with lithium hexamethyldisilazide (LHMDS) gave a trans β-lactam[99] which was converted to a carbapenem by standard reactions. Alternatively, conversion of L-threonine to epoxides **(105)** led to a carbapenem precursor[100] and a penem precursor[101]. The last few references have seen the emergence of aromatic groups, especially p-methoxyphenyl, for the protection of β-lactam nitrogen; deprotection is usually accomplished with potassium persulphate or CAN in CH_3CN.

Photolysis of 4-oxygenated 2-pyridones led to azabicyclo[2.2.0]hexanes. Thus 4-acetoxy-2-pyridone isomerised to **(106a)**, which upon hydrogenation, reduction and acetylation gave azetidinone **(107)**.[102] Alternatively, acid hydrolysis of **(106b)** gave 2-aza-3,5-dioxobicyclo[2.2.0]hexane **(108)**, which on treatment with alcohols or thiols formed esters **(109)**[102,103]. Photolysis of N-phenyl thioamides **(110)** gave thiols **(111a)**, by a [1,6] H-abstraction reaction, which were trapped by acyl halides to give azetidinones **(111b)**[104]; enamines **(112)** were photolysed to give spiro-β-lactams **(113)** in 45-73% yields.[105]

4-1 bond-forming reactions

All but one of the reactions in this section involved displacement of a good leaving group at C-4 by N-1. Thus, displacement of mesylate[106] or imidazolosulphonate[107] from serine derivatives **(114a)** and **(114b)** gave the corresponding 4-unsubstituted azetidinones. Similarly sulfazecin **(115)** was

produced from an N-sulphonated species derived from ascorbic acid,[108] and tin-mediated directed aldol reactions were used to form β-hydroxy amides, which were cyclised by the Mitsunobu Reaction.[109,110,111] Bromination of O-acyl vinylacetohydroxamate **(116)** under basic conditions led to 4-(bromomethyl)azetidin-2-one.[112] Finally, a radical 4-1 bond formation was reported, which possibly has some relationship to azetidinone ring formation in the biosynthesis of isopenicillin N (q.v.): photolysis of the tetrahydro-1,2-oxazine-3,6-diones **(23)** led to N-O bond cleavage, decarboxylation and radical coupling to give azetidinones.[22]

Reactions in which two bonds are formed

This section will include formal [2+2] or [3+1] additions which may be either concerted or stepwise reactions under the experimental conditions used.

[3+1] additions: 2-3 and 1-2 bond formation

Several examples of this type of reaction have been reported, involving reaction of a metal carbonyl complex with a nitrogen-containing molecule, one of the carbon monoxide ligands ultimately becoming the β-lactam carbonyl group. 3-Methylene β-lactams are formed by reacting 2-bromoallylamines with a catalytic amount of palladium (II) acetate and triphenylphosphine in an atmosphere of carbon monoxide.[113] This reaction has so far been applied mainly to the synthesis of 4-unsubstitued azetidinones, most usefully that of (±)-3-aminono-cardicinic acid (3-ANA) **(117)**.[114] The β-lactam components of cephem, oxacepham and clavam derivatives could be synthesised by photolytic reaction of chromium and molybdenum carbene complexes with imines. Thus photolysis of methoxymethylmethylenepentacarbonylchromium **(118)** with a thiazine and an oxazine gave cephem **(119)** and oxacepham **(120a)** respectively. Similarly, an oxazoline reacted with methoxymethylmethylenepentacarbonylmolybdenum to form clavam **(120b)** in low (14%) yield.[115]

[2+2] Additions

2-3 and 4-1 bond-forming reactions

The addition of various aryloxy-, alkyloxy-, aryl-and alkyl- sulphonyl-

isocyanates to alkenes and cyclic vinyl ethers has been investigated,[116] as has the high-pressure [2+2] cycloaddition of tosyl isocyanate to glycals.[117] The more commonly used chlorosulphonylisocyanate (CSI) reacted with allenyl sulphides to give, regiospecifically, 3-alkylidene-4-thioazetidin-2-ones.[118]

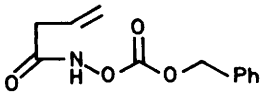
1-2 and 3-4 bond-forming reactions

A very large number of these reactions were reported in 1985, ranging from simple ketene-imine condensations[119,120] to more complex examples in which a very high degree of enantioselectivity was obtained. The stereoselectivity of the reaction has been studied, as has the [2+2] v [4+2] periselectivity observed when ketenes condense with a cinnamaldimine.[121] Variation of the ketene/imine precursors has also been studied; thus, when carbodi-imides were used in place of imines, double condensation occurred giving the novel tricyclic bisazetidinones **(121)**.[122] Bisazetidinones could also be obtained by using 2,3-azabutadienes instead of a simple imine: condensation of the lithium enolate of ethyl 2-methylpropionate with 2,3-diaza-1,4-diphenylbuta-1,3-diene gave a mixture of 1-aza- and 1-azetidinyl- azetidinones.[123] Using a mesionic oxazolone **(122)** as a ketene equivalent gave good yields (60-80%) of 3-acylamino-3-phenyl azetidinones.[124] Using more 'conventional' ketene equivalents, such as α-substituted acetic acids, phenyldichlorophosphate has again proved to be an efficient activating agent for the condensation with imines,[125,126,127] as have, to a lesser extent, PPh_3-Br_2 and Me_2S-Br_2.[128]

Few stereospecific syntheses of azetidinones from achiral precursors have appeared, although (±)-trans azetidinones **(123)**[129] and **(124)**[130] were obtained in a 3:1 ratio over their cis isomers by enolate-imine and silylenol ether-imine condensations respectively.

Many enantioselective syntheses from chiral precursors have been reported, in which the chiral auxiliary is present in either the imine or the ketene equivalent. On treatment with organolithium reagents N-(cyanomethyl)amines are converted to formaldehyde imines which condense with lithium enolates to give 4-unsubstituted azetidinones; thus a chirally N-substituted (cyanomethyl)amine reacted with ethyl (1-aza-2,5-bis(dimethylsila)-cyclopentyl)acetate **(125)** in the presence of 2 equivalents of LDA to give an enantiomerically pure 3-ANA analogue.[131] cis-Azetidinones were obtained in 90% enantiomeric excess from chiral imines **(126)** and appropriate ketene equivalents.[132]

The majority of enantioselective syntheses in which the chiral auxiliary is



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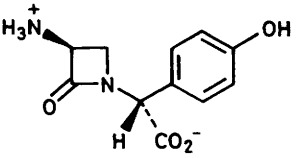

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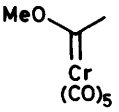
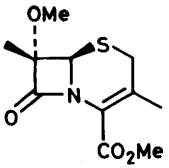


(118)



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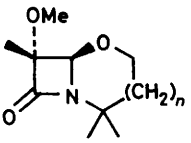
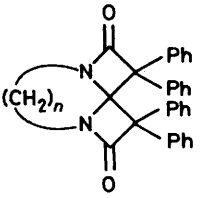
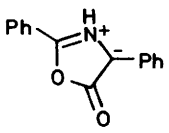


(120) a; $n = 1$
b; $n = 0$



(121) $n = 5, 6,$ or 11



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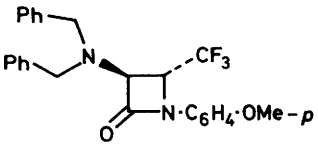

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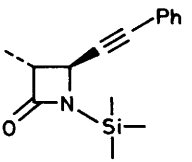

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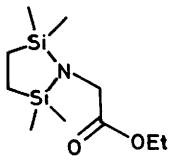

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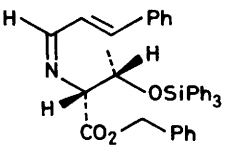
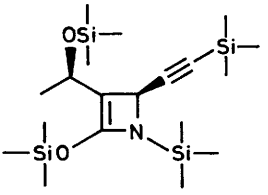


(126)



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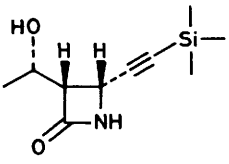

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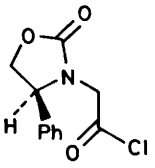
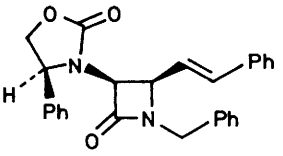


(129)



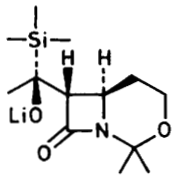
(130)

present in the ketene equivalent involve a 3(R)-hydroxybutyrate or its enantiomer. Reaction of N-trimethylsilyl-1-propynaldimine with methyl 3(R)-hydroxy butyrate gave a cis-azetidinone which could be converted to the isomeric trans-β-lactam by N-silylation, MnO_2 oxidation, reduction to the (R)-alcohol and desilylation; O-silylation and acidic hydrolysis in the presence of a mercury (II) salt to the 4-acetyl β-lactam followed by Baeyer-Villiger oxidation gave a chiral 4-acetoxyazetidinone.[133] In another case, the cis-trans conversion was effected via the silyl enol ether **(127)**;[134] using the other enantiomer of the ketene equivalent, cis-azetidinone **(128)** was, predictably, obtained: epimerisation of the alcohol by the Mitsunobu process was followed by conversion to a 4-acetoxyazetidinone, the acetoxy group of which could be displaced stereospecifically by silyl enol ethers, leading to trans-1-(alkyl) carbapenem precursors.[135] Similarly high enantioselectivity could be obtained by condensing 3-(S)-hydroxybutyrates with cinnamaldimines[136,137,138] and benzaldimines.[139,140]

Finally, glycine derivative **(129)** could be used as an alternative chiral ketene equivalent, giving 92-97% asymmetric induction in the product cis-azetidinone **(130)**; cleavage of the side chain followed by further elaboration led to a monobactam.[141]

Novel Azetidinone Chemistry

New methods for introducing a 1-(hydroxyethyl) side chain at C-3 of the azetidinone ring of carbapenem precursors have appeared: condensation of an enolate with 3,3-dimethyl-3-silabutan-2-one gave trans-β-lactam **(131)**, which rearranged with stereochemical retention to the O-silylated alcohol on treatment with potassium t-butoxide.[142] Similarly, the enolate from **(132)** and acetaldehyde gave all possible isomers of the expected products, which were separated by HPLC and used as 1-methylcarbapenem precursors.[143] Oxidation of diol **(133)** with $NaIO_4$ gave corresponding 3-acetylazetidinone, which was reduced to the R-alcohol by K-Selectride.[144] Introduction of the 1-hydroxy-1-methylethyl side chain by condensing an enolate with acetone gave carpetimycin precursors; thus the titanium enolate of methoxyethoxymethyl (MEM) protected ether **(134)** reacted with acetone to give mainly the 3,4-cis-azetidinone, the reaction being directed by chelation of the acetone molecule and enolate to the titanium atom.[145] Other methods which have been used to obtain the required cis-carpetimycin stereochemistry are kinetic protonation of enolate **(135)**[146] and abstraction by radical **(136)**.[147] In each case reaction with tributyl- or triphenylstannane occurs from the least hindered side of the molecule to give mainly the cis product.



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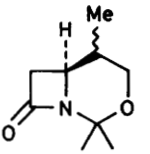

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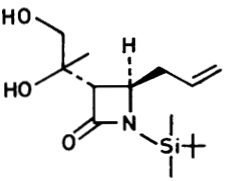

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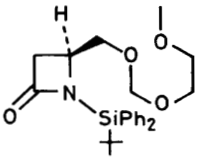

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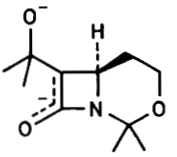

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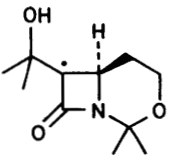

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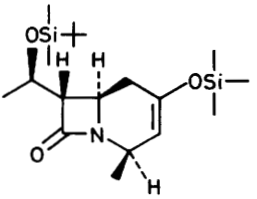

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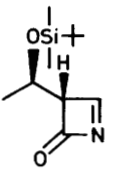
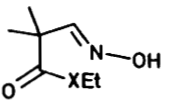
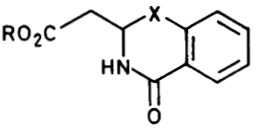


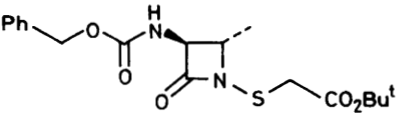
(138)



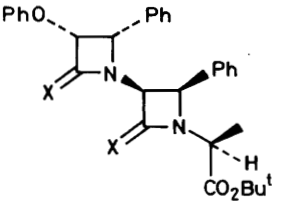
(139) X = O or S



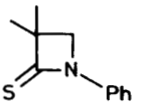
(140) a; X = O
b; X = S



(141)



(142) a; X = O
b; X = H,H



(143)

Successful displacement of the acetoxy group from 4-acetoxyazetidinones by carbon nucleophiles has been accomplished using organocuprates,[148] allyl stannanes,[149] allylsilanes,[150] α-silylthioesters[151] and silylenol ethers.[152] As well as the expected displacement product, reaction of silylenol ethers with N-unsubstituted 4-acetoxyazetidinones in the presence of $ZnCl_2$ also gave low yields of 2-carbacephem **(137)**, possibly by Diels-Alder reaction of the intermediate imine **(138)** with the silylenol ether.[152] Attempted displacement of the acetoxy group of 3,3-dimethyl-1,4-diacetoxyazetidin-2-one using heteronucleophiles RX^- led instead to ring opening to oximes **(139)**.[153] Salicylates and 2-mercaptobenzoates caused ring opening of 4-acetoxyazetidin- 2-one, giving benzoxazin-4-ones **(140a)** and benzthiazin-4-ones **(140b)** along with low yields of the displacement products.[154] β-Lactam ring opening by ethylmercaptide ion was used by Baldwin to produce β-aminoalanine derivatives.[155] In contrast, displacement of acetoxy by other heteroatom nucelophiles, e.g. azide ion and thioglycolate ion, led to 4-heteromonobactams, certain derivatives of which were biologically active.[156]

Attempted displacement reactions of iodine from 4-iodomethylazetidin-2-one with alkynylcuprates led instead to N-alkynylated products.[157]

Thiamazin **(141)** was made in excellent yield by reaction of the 1-unsubstituted azetidinone with S-phthalimido-t-butylthioglycolate.[158] Electrolysis in methanol of simple N-alkyl or N-benzyl azetidinones gave mixtures of 4-methoxy- and N-(methoxyalkyl)-or N-(α-methoxybenzyl)azetidinones.[159,160] The N-benzyl derivatives could be converted to the free N-H compounds by hydrolysis with aqueous p-toluenesulphonic acid. Bis(azetidin-2-one) **(142a)** was reduced to bis(azetidine) **(142b)**[161] using chlorodihydroalane; azetidin-2-thione **(143)**, obtained in low yield from the oxo compound using P_2S_5 or Lawesson's Reagent, was reduced to the azetidine with Raney nickel.[162]

8 Major Structural Variants

This section will describe β-lactam compounds which have not been discussed in the preceding sections and compounds in which the β-lactam ring has been replaced by a structural element intended to retain some of its steric or electronic characteristics.

The Δ^1-1-azapenem **(144)** was synthesised from a 4-isothiocyanatoazetidinone precursor but only displayed weak activity, and attempts to isomerise it to the Δ^2 compound were unsuccessful.[163] Azide **(145)** underwent ready cyclisation to a

triazoline which lost nitrogen upon heating to yield the tricyclic 2-azacephem **(146)**; neither the free acid from this nor the similarly synthesised 7-β-phenoxyacetamido compound was biologically active.[164] Azadiene **(147)** underwent photoisomerisation to **(148)**, which upon ozonolysis and photolysis yielded **(149)**; attempts to cleave the ethoxycarbonyl group and to N-sulphonate were unsuccessful.[165] A related 2,4-diene underwent photoisomerisation to **(150)**.[166]

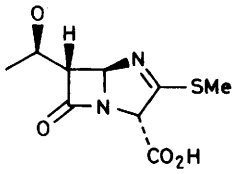
Clavalanine **(151)** was synthesised via coupling of racemic 4-acetoxyazetidin-2-one and a chiral fragment derived from xylose; although an antibacterial, **(151)** apparently does not act by inhibiting cell-wall biosynthesis.[167] Oxapenam **(152)** utilised a novel intramolecular Michael reaction in the formation of the bicyclic framework.[85]

Chiral carbacephem **(153)** was synthesised via an enantioselective ketene-imine cycloaddition to form **(154)**; the methoxyaryl group was of key importance since Birch reduction to a 1,4-cyclohexadiene followed by ozonolysis yielded a β-keto ester, which after transesterification was cyclised onto the β-lactam nitrogen by the carbenoid method.[168] A formal [2+4] cycloaddition between an azetidinone and a diene gave rise to carbacephem **(137)**,[152] and the synthesis of carbacepham **(155)** has been described.[85]

Carbenoids derived from **(156)** cyclised by N-H insertion to yield 4-acyl-1,2-diazetidinones;[169] alternatively photolysis and Wolff rearrangement of compounds **(157)** gave rise to a series of diazetidinones including bicyclic compound **(158)**.[170]

The three γ-lactam carbapenam analogues **(159)**, **(160)** and **(161)** were synthesised and found to be inactive against bacteria and β-lactamases; it was suggested that the relatively large distance between the lactam carbonyl and the carboxylic acid group in each case (ca. 4.1Å) contributed significantly to their inactivity.[171]

Analogues in which a cyclobutanone ring replaces the β-lactam have received the attention of a number of groups. 'Oxapenam' **(162)**, an analogue of a known β-lactam antibacterial was synthesised using the addition of dichloroketene to benzyl 2,3-dihydrofuran-4-carboxylate to create the bicyclic framework; although inactive as an antibacterial, **(162)** displayed weak carboxypeptidase inhibition, while both **(162)** and **(163)** were β-lactamase inhibitors.[172] Penam analogue **(164)**[173] and thienamycin analogues **(165)**[174] were synthesised by synthetic approaches similar to that for **(162)**; whereas biological data were not presented for 'penam' **(164)**, β-lactamase inhibitory and antibacterial activities were displayed by benzhydryl esters of **(165)**, R' = $CHPh_2$, although the free acids, R'=H, were inactive.



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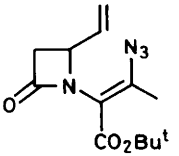

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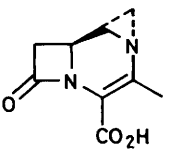

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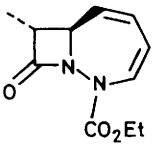

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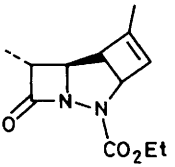

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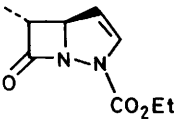

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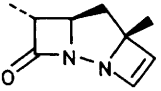

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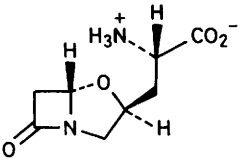

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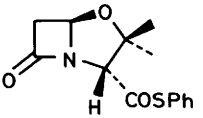

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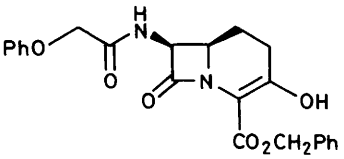

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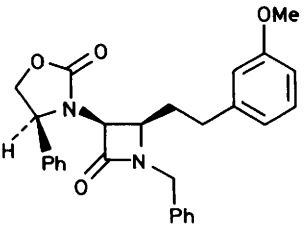

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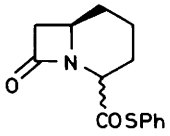

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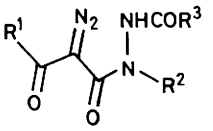

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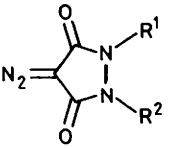

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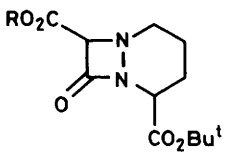

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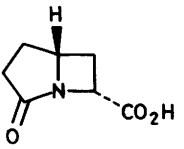

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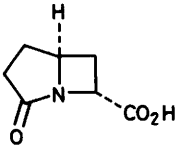

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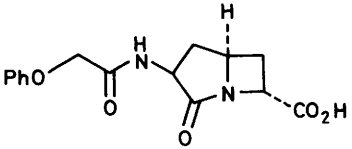

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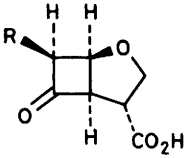
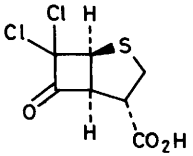


(162) R = $PhCH_2CONH$
(163) R = Cl



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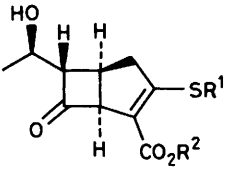

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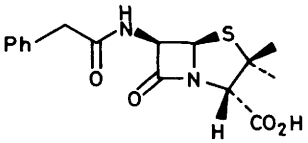

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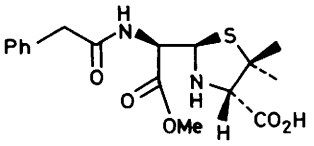


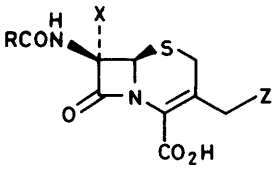
(167)

9 Mechanistic Studies Relating to Biological Activity

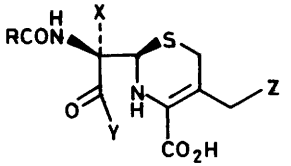
Two studies, intended to give an insight into the behaviour of enzyme-bound penicilloate intermediates, used nmr and uv spectroscopy to examine the behaviour of penicillin G **(166)**[175] and methyl penicilloate **(167)**[176] under alkaline aqueous conditions and reached similar conclusions. Ester **(167)** may undergo simple hydrolysis followed by unimolecular opening of the thiazolidine ring at the C-5,S bond to form an iminium ion which can cyclise with epimerisation at C-5; the ester hydrolysis step competes with proton abstraction at C-6, which leads to opening of the thiazolidine by elimination at C-5. Since penicillin G can only form penicilloic acid upon β-lactam hydrolysis, only the first mechanism was then observed, although the second could be induced by the presence of Hg(II) ions.

Studies on cephalosporins **(168)** and cephamycins **(169)** have concentrated principally upon the nature of the leaving group, Z, and the relationship between β-lactam lysis and its elimination. Ammonolysis in liquid ammonia at -50° of cephamycins, where Z = $OCONH_2$, and observation by ^{13}C nmr provided clear evidence for intermediates **(170)**, which subsequently underwent elimination to species of the type **(171)**.[177] Kinetic data from the hydrolysis of cephalosporins with PCl β-lactamase from S. aureus suggested that intermediates **(172)** were ca. 10^3 times more stable to acyl-enzyme cleavage than their precursors **(173)**; Michael addition of nucleophiles from the enzyme to **(172)** does not appear to be a significant factor in this stabilisation.[178] The general structural and electronic factors which may influence β-lactam cleavage and loss of Z have also been discussed.[179] Degradation of cephalosporins **(174)** in solution and study of kinetic data suggest that these may undergo self-aminolysis by attack of the amino group from one molecule upon the β-lactam of a second, resulting in dimer formation.[180]

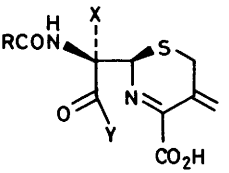
The degradation of sulbactam **(175)** under alkaline methanolic or aqueous conditions has been studied, and in the former case β-aminoacrylate derivative **(176a)** was isolated, while it was shown that in both cases intermediates **(176)** underwent further decomposition with dilute acid to the corresponding formyl acetates.[181] Triflamide sulphone **(177)**, although an inhbitor of β-lactamases, does not appear to be active by the same mechanism as **(175)**, and it was suggested that, instead, changes associated with the substrate-enzyme complex might be the basis for the biological activity.[182] Under weakly basic aqueous conditions clavulanic acid may decompose to four pyrazine derivatives; this is in accord with the previously reported formation of one of these and identification of 1-amino-4-hydroxybutan-2-one as an intermediate in its formation.[183]



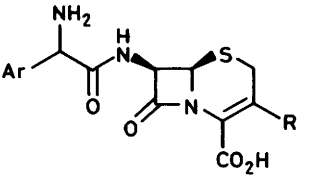
(168) X = H
(169) X = OMe



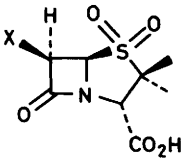
(170) X = OMe , Y = NH_2
(173) X = H , Y = enzyme



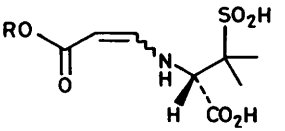
(171) X = OMe , Y = NH_2
(172) X = H , Y = enzyme



(174)



(175) X = H
(177) X = CF_3SO_2NH



(176) a; R = Me
b; R = H

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6
Metal Complexes of Amino Acids and Peptides

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

1 Introduction

Reviews dealing with metal-amino acid and related complexes include a critical survey of formation constants of complexes of histidine, phenylalanine, tyrosine, L-dopa and tryptophan;[1] complex formation between palladium(II) and amino acids, peptides and related ligands;[2] metal carbonyl complexes with amino acids, peptides, porphyrins, nucleic acids and nucleosides;[3] the computation of energy interaction parameters from the absorption spectra of praseodymium and neodymium complexes with amino acids and other ligands;[4] reversed-phase high-performance ion-pair chromatography of cobalt(III) complexes;[5] and metal-amino acid chelates in trace-mineral nutrition.[6]

In the area of metal-peptide complexes a variety of reviews have appeared. Non-covalent interactions in palladium(II)-peptide complexes have been the subject of a dissertation[7] and palladium(II) and platinum(II) complexes with amino acids and peptides discussed.[8] A further review has dealt with lanthanide complexes of peptides and proteins[9] with an emphasis on the application of the trivalent lanthanide ions as n.m.r. probes for peptides and proteins.

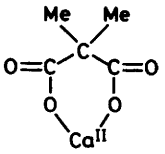
A recent book[10] has reviewed the area of molybdenum enzymes.

2 Amino Acids

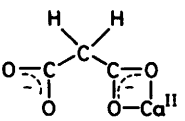
Synthesis, Spectroscopy and Structures. - A large number of papers dealing with synthesis, spectroscopic properties and structures of metal-amino acid complexes have been published in 1985 and a significantly high proportion of these describe structures determined by X-ray diffraction methods.

Metals of the s-Block and First-Row d-Block. - In order to improve our understanding of calcium binding of proteins such as collagen, a tissue calcification substrate which possesses a high

content of hydroxy-L-proline (HPro-OH), the crystal structure of the complex $Ca(Pro\text{-}OH)_2.5H_2O$ has been determined by X-ray diffraction methods.[11] The structure contains monomeric units of seven-coordinate $Ca(Pro\text{-}OH)_2(H_2O)_3$ linked together by two additional water molecules to give Ca-Ca distances of 6.21 Å. This structure is unusual by comparison with other calcium-amino acid complexes, which, with the exception of $Ca(Glu)_2.4H_2O$, tend to form dimers or chain-like polymers with Ca-Ca distances of 3.74-4.79 Å. The complex has distorted pentagonal-bipyramidal geometry in which each Pro-OH behaves as an N,O (carboxylate) bidentate ligand. The structures of the complexes $Ca_3(Memal)_3.4H_2O$ and $Mg(Memal).4H_2O$ (Memal = methylmalonate) have been investigated as models for binding of (and discrimination between) Ca^{II} and Mg^{II} by γ-carboxyglutamate and β-carboxyaspartate residues in proteins.[12] The calcium complex is polymeric and contains two inequivalent metal ions, one of which is seven-coordinate with three differently coordinated Memal ligands, one monodentate and one each showing the bidentate coordination modes in (1) and (2).



(1)

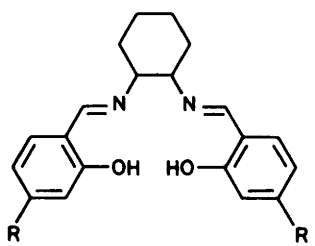


(2)

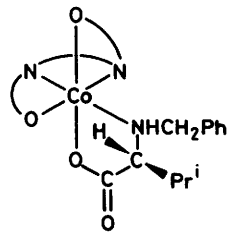
The mixed-ligand chromium(III) complex [Cr(A)L-His].H_2O (H_2A = L-alanine-N-monoacetic acid) has been synthesised and its structure determined by X-ray diffraction.[13] Of the six possible isomers, the one synthesised was found to have *mer*-octahedral geometry with the amino groups of each ligand *cis* to each other and the nitrogen atom of ligand A having R-chirality. Four of the six possible isomers of the complex Cr(Cys)(L- or D-His) (H_2Cys = L-cysteine) have been isolated and assigned structures on the basis of their high-speed liquid chromatograms.[14] The iron(II) complexes $FeL^1_2.nH_2O$ and $FeL^2.mH_2O$ (L^1 = monoanions of Gly, Ala, Ph-Gly, Phe, Leu, Ser, Glu, Trp, His, Met, S-Me-Cys, Gly-Gly, L^2 = dianions of Asp, Glu, Cys, n = 0-2, m = 0,2) have been synthesised.[15] Results of magnetic, reflectance and Mössbauer studies suggest that these complexes have extended structures

containing high-spin six-coordinate iron(II) bridged by carboxylate ligands. A series of trinuclear complexes $[Cr_{\underline{n}}Fe_{3-\underline{n}}(\mu_3\text{-}O)(O_2CCH_2NH_3)_6(H_2O)_3]X_7.\underline{x}H_2O$ ($\underline{n}$ = 0-3, X = NO_3) has been prepared and the crystal structure of one of its members ($\underline{n}$ = 1, X = ClO_4, $\underline{x}$ = 6) reported.[16] This complex contains a planar-triangular array of metal atoms, a central μ_3-O ion and glycine ligands which bridge across the metal centres via the carboxylate groups.

One of the most interesting papers on metal-amino acid complexes published for some time describes the design and synthesis of a complex for use in chiral recognition.[17] Reaction of $Co(OAc)_2$, N-benzyl-D-valine and the Schiff-base ligand (3) in the presence of air gave the cobalt(III) complex (4), for which a Δ,β_2-configuration was established. Copolymerisation of (4) with styrene and divinylbenzene (1:20:4) in THF gave a polymer-embedded complex from which the amino acid ligand could be removed by treatment with HCl in MeOH. The resulting diaquo complex (*trans* configuration) showed almost 100% chiral discrimination in favour of N-benzyl-D-valine when treated with a racemic mixture of the amino acid. Some cobalt(III) complexes containing the (3N,O) tetradentate ligand D,L-8-amino-2-methyl-3,6-diazaoctanoate and Gly or D,L-Ala have been prepared and separated into isomers by cation-exchange chromatography.[18] Electronic and ^1H n.m.r. spectroscopy have been used to assign the configurations of these isomers. The crystal and molecular structure of the complex Δ-*cis*-α-ethylenebis-S-prolinato(1,2-diaminoethane)cobalt(III) perchlorate dihydrate has been reported.[19] The complexes Δ- and Δ,Λ-$[Co(en)_2(AlaenH)](ClO_4)_2(NO_3)_2$ (5), Δ- and Δ,Λ-$[Co_2(en)_4(AlaenAla)](ClO_4)_6.2H_2O$ (6) and $[Co(en)_2(Ala\text{-}GlyOC_3H_7)]Cl_3$ (en = 1,2-diaminoethane) have been synthesised and the crystal structure of the first of these studied by X-ray diffraction.[20] No interaction was found between the NH_3^+ group and other ligands



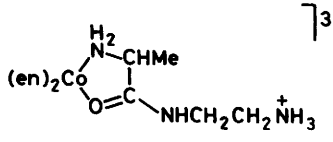
(3) R = $OCH_2CH(Ph)=CH_2$



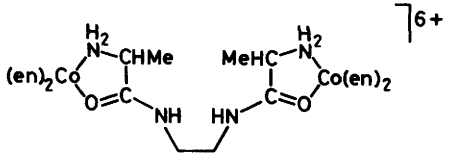
(4)

in the complex, and results of amide hydrolysis studies showed no evidence for a mechanism involving proton transfer from this group to the amide nitrogen. The complexes trans(CN)-, cis(N)-$Li[Co(CN)_2(L\text{-Ala})_2]$ and trans-$Li[Co(CN)_2(tmdda)]$ (tmdda ▪ tetramethylenediamine-N,N'-diacetate) have been prepared and characterised by electronic, n.m.r., Raman and c.d. spectroscopy.[21] Four cobalt(III) complexes [Co(en)L]Cl (L ▪ dianions of S-carboxymethyl-L-cysteine or L-homocysteine) which contain the derivatized amino acids as N,S,2O tetradentate ligands have been synthesised and separated into isomers which were characterised by ^{13}C n.m.r., electronic absorption and c.d. spectroscopy.[22] The complex CoA(L-Met) (H_2A = N,N'-ethylenebis(1,1,1-trifluoro-4-imino-2-pentanone)) has been prepared and separated into enantiomers by selective crystallisation.[23] The crystal structure of the $(-)_{435}$ isomer was determined and shown to have a mer-N_3O_3, Λ-cisβ_2 configuration with the methioninate ligand coordinated to the metal as an NH_2, CO_2^- bidentate ligand. Electronic, c.d. and ^1H n.m.r. spectra are reported for both enantiomers. The (iminodiacetato)(L-methioninato or S-methyl-L-cysteinato)cobalt(III) complexes have been prepared and separated into three geometric isomers, i.e. trans-N,N, trans-N,S and trans-N,O, by ion-exchange chromatography.[24]

A number of tosylated glycine (Ts-GlyH) and alanine (Ts-α or -β-AlaH) complexes, which display a range of ligand coordination modes and ionization states corresponding to the loss of one or two protons, have been reported. In the complexes $Co(Ts\text{-}Gly)_2(H_2O)_4$ and $Zn(Ts\text{-}\beta\text{-}Ala)_2(H_2O)_4$, for which crystal and molecular structures have been determined, the metals lie in distorted octahedral fields containing two trans carboxylato ligands and four water molecules.[25] A series of complexes (of which the aforementioned are members) of general formulae $M^{II}(Ts\text{-}L)_2(H_2O)_4$ and $Zn^{II}(Ts\text{-}L)_2(H_2O)_2$ (M = Co, Ni, Zn, L = Gly, α- or β-Ala) have been prepared and characterised. The crystal structure of the complex $[Cu(Ts\text{-}Gly)_2(2,2'\text{-bipy})].H_2O$ has been determined.[26] The structure consists of discrete dimeric units in which the metal ions are bridged by two oxygen atoms from the carboxylate groups of different amino acid ligands. The coordination geometry at each copper is distorted square pyramidal with a ligand arrangement as shown in (7). Results of magnetic susceptibility and e.s.r. measurements are consistent with weak magnetic exchange interactions between the metals. Crystal structures are also reported



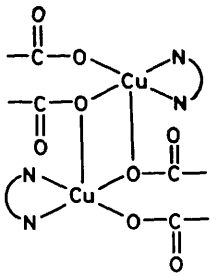
(5)



(6)

for $Cu(Ts\text{-}\beta\text{-}Ala)_2(ImH)_2$ (ImH ▫ imidazole) and for the amide deprotonated complexes $Cu(Ts\text{-}\alpha\text{-}AlaH_{-1})(H_2O)_2.H_2O$ and $(PipH_2)_2[Cu(Ts\text{-}\alpha\text{-}AlaH_{-1})_2]$ ($PipH_2$ = piperidinium).[27] The first of these is a square-planar complex in which the metal lies in a centrosymmetric field of two carboxylate oxygen atoms (one from each amino acid) and two imidazole nitrogen atoms. The second complex contains polymeric carboxylato-bridged $[Cu(Ts\text{-}\alpha\text{-}AlaH_{-1})(H_2O)_2]_n$ units; each copper lies in a distorted square-pyramidal ligand field in which the deprotonated sulphonamide nitrogen, one carboxylate oxygen and two waters are basal, with the second carboxylate oxygen of an adjacent amino acid molecule occupying the apical position. The 2:1 complex is square planar with the ligands (2N,2O) coordinated centrosymmetrically about the metal ion. The complexes $Cu(Ts\text{-}\alpha\text{-}Ala)_2$, $Cu(Ts\text{-}\beta\text{-}Ala)_2$, $A_2[Cu(Ts\text{-}\alpha\text{-}AlaH_{-1})_2]$ (A ▫ K, morpholinium) and $Cu(Ts\text{-}Ala)_2B_2$ (B ▫ MeImH, PipH, morpholine for Ts-β-Ala; B = ImH, MeImH for Ts-α-Ala) have also been isolated and their magnetic and spectroscopic (e.s.r., i.r., electronic) properties reported.

The addition of chiral metal complexes to the mobile phase has been used in the h.p.l.c. resolution of D,L-amino acids and their derivatives. The mechanism of such chiral recognition is thought to involve ligand exchange with the formation of diastereoisomeric ternary complexes. To gain further insight into the mechanistic details, the complex bis(L-phenylalaninamidato)copper(II) (8) has been prepared, its crystal structure determined and its enantiomeric selectivity towards D,L-dansyl-amino acids measured.[28] The immediate coordination environment of the copper is distorted square planar ($Cu\text{-}NH_2$ distances



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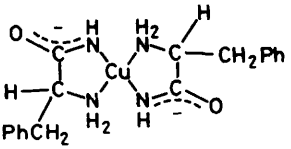
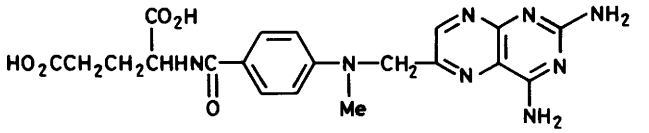


(8)

2.00-2.11 Å, Cu-NH^- distances 1.93-1.94 Å), although a long interaction (2.732 Å) between the metal and a carbonyl oxygen from a second molecule gives a very elongated square-pyramidal structure. High separation factors (α up to 2.75) were observed in the resolution studies. Crystal structures and spectroscopic (e.s.r., i.r. and visible) properties are reported for the complexes $Cu(Glu)L^1$ and $[Cu(Glu)L^2(H_2O)].3H_2O$ (Glu = L-glutamate anion, L^1 = imidazole, 2,2'-bipyridyl, L^2 = 1,10-phenanthroline).[29] Polymeric structures with bridging glutamate ligands are observed for the imidazole (square planar) and bipyridyl (square pyramidal) complexes, while the phenanthroline complex consists of mononuclear units with square-pyramidal geometry around the metal ion and glutamate acting as a simple bidentate ligand. The crystal structure of $Cu(L\text{-Lys})_2Cl_2.2H_2O$ has been determined by X-ray analysis.[30] The complex is a tetragonally elongated octahedron containing trans-N,O-chelated lysine ligands in the equatorial plane and two very long Cu-Cl bonds. The e.s.r. spectra of a number of copper(II) complexes with N-protected amino acids (Ac-Gly, Ac-Met, Ac-Ala, Ac-Val, Ac-Glu) have been studied as a function of temperature in the solid state and found to be characteristic of dimeric carboxylato-bridged species in which the metals are coupled by antiferromagnetic exchange.[31] In coordinating solvents these complexes are monomeric.

A number of copper(I) complexes of the type [Cu(terpy)L].HCl (terpy = 2,2',2"-terpyridine, L = $^-$S coordinated cysteine, penicillamine, N-acetylcysteine and N-acetylpenicillamine anions) have been prepared.[32] These are readily oxidised to copper complexes which also contain metal-thiolate bonds as indicated by the $\pi(S)\rightarrow Cu^{II}$ charge-transfer bands at ~420 nm in their electronic spectra. Complexes of general formulae $CuX_2\{O_2C(CH_2)_mNH_3\}.nH_2O$ (n = 2-5, X = Cl, Br) and $CuX_{3/2}(O_2CCH_2NH_3)$ (X = Cl, Br) have been synthesised and characterised.[33] The temperature dependence

of magnetic susceptibilities as well as results of i.r. studies indicate that these complexes consist of binuclear subunits with carboxylate bridging ligands similar to those present in copper(II) acetate monohydrate. The thermal behaviour of 11 hydrated copper(II) carboxylate complexes (including some of amino acids) has been studied and the factors influencing the thermodynamic curves are discussed.[34] A number of copper(II)-glycinate complexes containing Cl^-, Br^-, NO_3^-, SO_4^{2-}, ClO_4^- or H_2O as co-ligands have been reported.[35] The complex K[Cu(Gly)L] (H_2L ▫ amethopterin ▫ methotrexate) (9) has been prepared and its i.r. spectrum reported.[36]



(9)

Some mixed-ligand complexes containing glycinate and either uracil (Ur) or thiouracil (Tur) anions as co-ligands have been prepared and characterised by elemental analysis, conductance, spectroscopy (i.r. and electronic) and magnetic measurements.[37] These are $Na_2[Cu(Gly)Ur(H_2O)].H_2O$, $[Cu_2(OH)_2(Gly)Tur].2H_2O$, $Na[Ni(Gly)Ur(OH)].H_2O$, $[Ni(Gly)Tur(H_2O)_2].H_2O$, $Na[Co(Gly)_2(Ur)_2].2H_2O$, $[Co(Gly)Tur].H_2O$, $Na_2[Zn(Gly)Ur(OH)_2H_2O].H_2O$ and $Zn(Gly)Tur(H_2O)$. In all of these complexes the glycinate ligand is bidentate whereas the uracil anion is bidentate (N,O donor) in some cases and monodentate (N donor) in others. The thiouracil anion on the other hand may be bidentate (N,O or N,S) or tridentate (N,O,S). Crystal structures are reported for four zinc(II) complexes of cysteine (H_2-Cys), cysteine ethyl ester (H-CysOEt) and L- or D,L-pencillamine (HA).[38] The complexes $Na_2[Zn(Cys)_2].6H_2O$ and $Zn(CysOEt)_2$, which both contain (N,S^-) coordinated ligands, were isolated from alkaline (pH 11) and neutral solutions respectively. The complex $ZnBr_2A_2$ which contains monodentate carboxylato-bonded penicillamine ligands was isolated from solution of pH 2. In the complex $ZnCl_4(L\text{-Pro})_2$, for which crystal and molecular structures are reported, the metal lies in a tetrahedral field containing two chloride and two monodentate carboxylato-bonded amino acid ligands.[39]

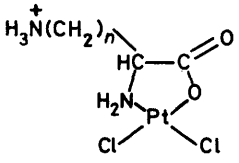
Some mixed-ligand complexes containing vitamin U (HL^1 = $^+NH_3CH(CH_2CH_2SMe_2{}^+Cl^-)CO_2^-$) and other amino acid ligands have

been prepared and characterised.[40] These complexes have formulae $ML^1L^2.\underline{n}H_2O$ (M ▫ Co, HL^2 = Gly, Asp, Glu; M = Cu, Ni, HL^2 = Gly; M ▫ Zn, HL^2 = Gly, Ala, Val; $\underline{n}$ = 0-3) and $ML^1_{3-\underline{x}}L^2_{\underline{x}}.\underline{n}H_2O$ (M ▫ Cr, HL^2 = Gly, Asp, Glu; M ▫ Fe, HL^2 ▫ Gly, Ala, Val; $\underline{n}$ = 1-3, $\underline{x}$ ▫ 1,2). Similar complexes but which in addition contain Cl^- ligands have been reported.[41] The N-phenylglycinate complexes $CrL_3.\underline{n}H_2O$ and $ML_2.\underline{n}H_2O$ (HL = $RC_6H_4NHCH_2CO_2H$, R = H, o-, m- and p-Me, m- and p-NO_2, p-Cl, M ▫ Co, Cu, Ni) have been prepared and characterised.[42] The complexes bis(4-methyloxazolidine-4'-carboxylato)copper(II) dihydrate and 3N,7N-(1,3,5,7-tetraazabicyclo[3.3.1]nonyl)diacetatonickel(II) have been prepared and the heterocyclic ligands isolated following treatment with BH_4^-.[43] The vanadyl complexes $VOL_2.\underline{n}H_2O$ (HL = Gly, α- and β-Ala, $\underline{n}$ ▫ 1; HL ▫ PhCo-Gly, $\underline{n}$ ▫ 0) were obtained by the reaction of $VO(OAc)_2.2H_2O$ and the amino acids in ethanol.[44] The reported cytostatic properties of titanocene dichloride, Cp_2TiCl_2, has led to a study of its interaction with L-cysteine (H_2-Cys), anthranilic and hippuric acids (HL).[45] The complexes $CpTi(Cl)Cys.2H_2O$, which contains thiolate- and carboxylate-chelated cysteinate dianion, and $Cp_2Ti(L)_2$ have been isolated and characterised.

Heavier d-Block, f-Block and p-Block Metals. - The majority of publications in this section deal with complexes of platinum(II) and most of these are either directly or indirectly related to the search for anticancer drugs. The complexes [Pt(bipy)L]X (X = Cl^-, HL ▫ L-His, L-Asn, L-Phe, L-Trp; X ▫ NO_3^-, HL ▫ L-Tyr), [Pt(bipy)L-Lys]Cl_2,[46] $[Pt(NH_3)_2Met]Cl$ and $Pt(NH_3)(H_2O)L^1$ (H_2L^1 = Cys),[47] for which syntheses, spectra and structures are reported, have been tested for antitumour activity. The complexes cis-$PtCl_2(His)_2$, cis-$[Pt(NH_3)_2(His)_2]Cl_2$ (three isomers) and $PtCl_2(NH_3)$His, which contain various modes of monodentate histidine, as well as the bidentate histidine complex cis-$Pt(NH_3)_2$His have been prepared and analysed by n.m.r. (^1H, ^{13}C) spectroscopy and by electrophoresis and ion-exchange chromatography.[48] Series of N-coordinated amino acid complexes of formulae cis-$PtCl_2(HL^1)Bu^tNH_2$ (HL^1 = neutral D- and L-Val, D- and L-Leu, D-Phe, D-Ser, L-Pro) and cis-$Pt(HL^2)(Bu^tNH_2)A_2$ (HL^2 ▫ neutral Gly, D- and L-Ala, D- and L-Val, D- and L-Leu, D- and L-Phe, D- and L-Ser, L-Thr and L-Pro, A ▫ guanosine; HL^2 = neutral L-Val, L-Ser, A = 9-methylguanine) have been isolated and studied by i.r., n.m.r. (^1H, ^{13}C) electronic

and c.d. spectroscopy.[49] The reactions of some of these complexes with calf thymus DNA have been investigated and attempts have been made to correlate antitumour activity with chemical properties.

Reaction of K_2PtCl_4 and 1,2-diaminopropionic acid (Dap) hydrochloride in aqueous solution gives the carboxylato-bonded complexes cis- and trans-$[PtCl_2(H\text{-}Dap)_2]Cl_2$,[50] both of which are converted to the N,O-chelate $PtCl_2(Dap)$ (10; n ▫ 1) on short heating. Prolonged heating gives the N,N-chelated isomer (11; n ▫ 1). The analogous 2,4-diaminobutyric acid (Dab) complex $PtCl_2(Dab)$ (10; n = 2) also isomerises to the N,N-chelate (11; n = 2) on heating, but unfavourable ring size prevents similar rearrangements of the L-ornithine (10; n ▫ 3) and L-lysine (10; n = 4) N,O-chelates.[51]



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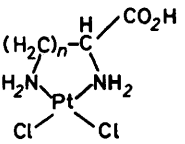


(11)

The complexes $[Pt(HA)Cl_2].3H_2O$ and $[Pt(HA)_2]Cl_2.3H_2O$ containing hydrazides of aspartic and glutamic acids (HA) have been synthesised.[52] In these complexes the ligands are thought to coordinate to the metal through the amino and hydrazide carbonyl groups with the carboxylic groups remaining uncoordinated.

Reaction of K_2PtCl_4 and S-alkylated L-cysteine (R-CysH, R ▫ Me, Et, $PhCH_2$, p-NO_2PhCH_2, Ph_2CH, Ph_3C, $(p\text{-}MeOPh)_2CH$) derivatives in neutral or acidic solutions led to the isolation of the complexes $Pt(R\text{-}CysH)Cl_2$, $[Pt(R\text{-}CysH)_2]Cl_2$ and $Pt(R\text{-}Cys)_2$, which were shown to consist of diastereoisomers (^1H, ^{13}C n.m.r.) and to contain N,S-coordinated cysteine or cysteinate ligands.[53] The $Pt(R\text{-}CysH)Cl_2$ complexes react with guanosine and inosine (L) to give $[Pt(R\text{-}CysH)L_2]Cl_2$ products.

A number of salts containing square-planar $[Pt(CN)_4]^{2-}$ and $[AuCl_4]^-$ anions and protonated amino acid cations have been reported.[54,55] The platinum(IV) complexes cis-$PtCl_4L_2$ (L = Gly-OEt, Gly-Gly-OEt, Gly-Leu), trans-$PtCl_4$(Met-OEt), cis-$PtBr_2Cl_2(Gly\text{-}Gly\text{-}OEt)_2$ and $PtBr_2(C_2O_4)(Gly\text{-}Gly\text{-}OEt)_2$ were obtained in high yield by the oxidative addition of halogens to

$PtCl_2L_2$, $PtCl_2$(Met-OEt) and $Pt(C_2O_4)$(Gly-Gly-OEt).[56] The ^{15}N n.m.r. signals of the coordinated amino groups in these complexes are shifted ~50 ppm upfield relative to those for the free ligand, and the Pt^{II} and Pt^{IV} complexes may readily be distinguished by ^{195}Pt n.m.r. spectroscopy.

A number of gold(I) thiolates [Au(SR)] containing S^--coordinated cysteinate, N-acetylcysteinate, cysteinate ethyl ester, C,C-dimethylcysteinate and other thiolate ligands have been synthesised.[57] Temperature-dependent n.m.r. (^1H, ^{13}C) spectra of these complexes suggest the presence of polymeric rings, while Mössbauer spectra are consistent with linear coordination of gold(I) by two sulphur donors. From these studies, the non-equivalence of thiolate ligands and of gold atoms respectively was implied. The spectra of these complexes are compared with those of 'Myocrisin', 'Solganol' and 'Auranotin', gold(I) thiolates used in the treatment of rheumatoid arthritis.

Reaction of MoO_3 with amino acids HA (HA = Gly, Ala, Val, Pro, Ser, Leu) and hydrogen peroxide in aqueous solution gave complexes $MoO(O_2)_2(HA)H_2O$, two of which (HA = Gly, Pro) were investigated by X-ray crystallography.[58] These complexes have distorted pentagonal-bipyramidal structures with the oxo and aquo ligands at apical sites and the symmetrically bidentate peroxo ligands and the carboxylate group of HA in the equatorial plane. The binuclear molybdenum(VI) complexes $Mo_2O_4(\mu\text{-}O)(\mu\text{-}A)(OH)_2$(HA ◘ Gly, L-Ala, D-Pro, L-Val, D,L-Val, L-Leu) were obtained as products of the reaction of Na_2MoO_4 and the amino acids in aqueous solution.[59] A number of complexes of general formula $[Ru(NH_3)_mL]X_3$ (L ◘ Gly, β-Ala, γ-Aba, His, imidazole, adenine, X = tosylate, methanesulphonate or triflate, m = 4,5) have been prepared and their structures investigated.[60]

Sixteen organotin(IV) complexes of general formulae R_2SnL_2 and $R_2(L)SnOSn(L)R_2$ (L = Ac-Leu, Ac-L-Phe, R = Me, Et, Bu^n, Oct^n) have been prepared by the reaction of the ligands and R_2SnO in 2:1 and 1:1 mole ratios respectively.[61] Results of spectroscopic (i.r., ^1H n.m.r. and Mössbauer) investigations show that the 2:1 complexes have distorted octahedral geometries; with chelating carboxylate ligands, the binuclear complexes have O-bridged five-coordinate trigonal-bipyramidal structures. The di- and triorganotin(IV) complexes Ph_2SnCys (H_2-Cys ◘ L-cysteine), R_2SnPen (R ◘ Me, Bu^n, Ph, H_2-Pen = D,L-penicillamine), $Me_2Sn(PhCO\text{-}Gly)_2$ and $R_3Sn(R^1\text{-}Gly)$ (R = Me, Bu^n, R^1 ◘ Ac, Dnp)

have been synthesised and tested against lymphocytic P-388 leukaemia.[62] The antitumour effects of these complexes are rationalised on the basis of possible structures in solution. The complex $Bi(H\text{-}Cys)_3.H_2O$, which contains S^--coordinated cysteinate ligands, has also been investigated for comparative purposes.

The crystal structure of the uranyl complex $[UO_2(Gly)_4](NO_3)_2$ has been determined by X-ray diffraction.[63] The coordination about the metal is hexagonal bipyramidal with four carboxylato-bonded glycine ligands, two of which are monodentate and the other two bidentate, in the equatorial plane. A number of rare-earth (Ln) complexes of the type $LnCl_3L_3.nH_2O$ (L = Gly, Ala, Val) have been prepared and characterised by elemental analysis, i.r. spectroscopy, thermal analysis and X-ray powder diffraction.[64]

Complexes in Solution. - A very large number of papers on the solution chemistry of metal-amino acid complexes has been published in 1985. These deal with structure determination, reaction kinetics and mechanisms and formation constants.

Structures in Solution. - The structures of the glycinate complexes $[Zn(Gly)(H_2O)_4]^+$ and $[Zn(Gly)_3]^-$ in aqueous solution at 20°C have been determined by X-ray diffraction and both complexes have been shown to have regular octahedral structures.[65]

Results of e.s.r. studies (g and hyperfine values were obtained from the spectra of ^{63}Cu and ^{65}Cu complexes) on a number of CuL_2 complexes (HL = Lys, Glu, Ala, Val, Glu, Asp, Pro, Asn) in aqueous solution show that they consist of cis-, trans-isomers which deviate from ideal square-planar geometry.[66] The complex $Cu(L\text{-}His)_2$ in aqueous solution has been analysed by ^1H and ^{13}C n.m.r. and shown to consist of a species (24%) in which both ligands are coordinated 'histamine-like' to the metal and another species (76%) in which one ligand is coordinated 'histamine-like' and the other ligand 'glycine-like'.[67] In acid solution the ligand 2-fluoromethyl-L-histidine shows bidentate N(amino), O-coordination in a number of binary and ternary copper (II) complexes.[68] In neutral solution however the ligand becomes tridentate, and evidence from c.d. spectroscopy supports apical rather than equatorial binding of the imidazole group to the metal in these complexes. Potentiometric, polarographic and spectrophotometric measurements show that copper(II) forms the

same complexes with N-dansylglycine in aqueous and methanolic solutions.[69] These complexes are mixed hydroxy-dansylglycinate species. Below pH 5 no complex formation was observed in either solvent. On raising the pH, however, monodentate coordination through the carboxylate group followed by N,O-bidentate coordination involving the deprotonated amide nitrogen and the carboxylate oxygen was observed. The effect of solvent on equilibria and polarographic parameters is discussed. Measurement of ^{13}C relaxation times of solutions containing L-proline and $Cu(ClO_4)_2$ (10^{-4}M) at pH 11 give results which suggest that the structure of the 2:1 complex in solution is similar to that in the solid state.[70] The absorption spectra of solution mixtures containing 1:1:1 ratios of Cu^{2+}, ATP^{4-} and glycine, histidine or histamine have been used to characterise the species present.[71] The copper(II) complexes of L-histidine and related ligands (3-Me-His, Gly-His, Gly-Gly-His) and their interaction with L-ascorbic acid at pH 7 have been investigated by paramagnetic ^1H n.m.r. line broadening.[72] The ternary complexes $CuL^1L^2.xH_2O$ (HL^1 = Gly-Gly, HL^2 = Gly, Ala, Ser, Thr, Val, x = 0,2) have been studied by i.r. spectroscopy and by polarography.[73]

Vibrational circular-dichroism spectra in the regions 3,400-2,500 cm^{-1} and 1,600-1,180 cm^{-1} have been reported for Δ- and Λ-Co(acac)$_2$L-Ala (acac = acetylacetonate) in d^6-DMSO and $CDCl_3$ solutions.[74] The appearance of a large negative v.c.d. band in the N-H stretching region of the spectrum of the Δ complex, which is not observed in the case of the Λ complex, is due to H-bonding between the L-alaninate and one of the acac ligands in the former complex, which is not present in the latter. Spectra (v.c.d.) have also been obtained for the C-H stretching regions of the complexes CuL_2 (L = anionic L-Ala, L-Ser, L-Val, L-Pro, L-Thr) and Λ-Co(L-Ala)$_3$ and compared with those of the free amino acids.[75]

Paramagnetic relaxation rates of the ^{13}C (glycine) and ^{31}P atoms in Mn^{II}/glycine/ATP^{4-} complexes at pH 7.4 as a function of temperature, magnetic field strength and ligand concentration have been investigated.[76] Whereas only one ternary complex, $[Mn(ATP)Gly]^{2-}$, was observed over a wide range of ligand concentrations, three binary complexes, $[Mn(ATP)]^{2-}$, $[Mn(ATP)_2]^{6-}$ and $[Mn(ATP)_3]^{10-}$, were observed in the same ATP^{4-} concentration range. Relaxation mechanisms and the structures and geometries of the

complexes are discussed. Equilibria between nickel(II) complexes containing pentadentate and hexadentate amino carboxylate ligands such as EDTA have been studied by ^{13}C n.m.r. spectroscopy.[77] These equilibria are very temperature sensitive near ambient conditions (the pentadentate form predominates at low temperatures) and this, combined with an ionic strength sensitivity, is believed to be responsible for contradictory literature reports regarding EDTA coordination. The stepwise coordination of L-histidine to cobalt(II) with increasing pH has been examined by ^{1}H n.m.r. spectroscopy, and successive steps involving $-CO_2^-$, α-NH_2 and imidazole N coordination have been identified.[78] Complexes of Mn^{II}, Co^{II} and Cu^{II} with amino acid (Gly, Ala, Pro) and cyclic peptide ligands in aqueous solution have been studied by ^{17}O and ^{14}N n.m.r.[79]

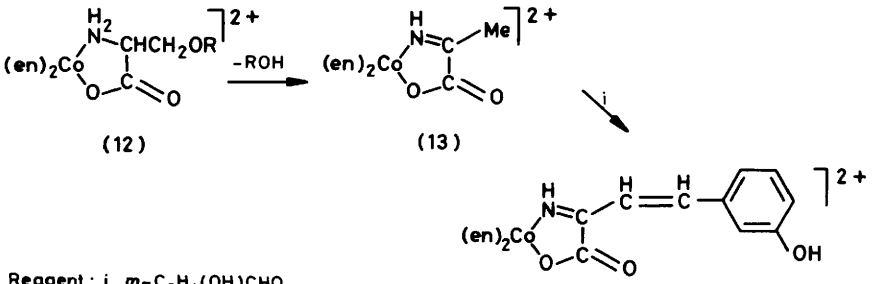
The interaction of Se^{IV} compounds with sulphur-containing amino acids and related compounds (Cys, Cys-OMe, penicillamine, glutathione, 2-mercaptoethanol, 2-mercaptoethylamine) produces u.v. absorptions at 255-258 nm and ^{77}Se n.m.r. signals at 580-730 ppm (relative to Me_2Se at 1.298), both of which indicate the presence of -S-Se-S- groups.[80] The technique of circularly polarised luminescence spectroscopy has been used to study complexes of α-amino acids with terbium(III)-EDTA.[81] For amino acids without side-chain complexing sites, NH_2, CO_2^--bidentate coordination was observed at pH > 8. Where ring size permits, chelation involving the carboxylate group and a side-chain complexing site occurred. In some cases oligomeric species formed. The interaction of Pd^{II} with thiaproline (thiazolidine-4-carboxylic acid) and its N-acetyl derivative both in the absence of and in the presence of glycine has been investigated by u.v., c.d. and ^{13}C n.m.r. spectroscopy.[82]

Reactions, Kinetics and Mechanisms. - In weakly alkaline solutions vanadium(II) and vanadium(III) form the intensely yellow coloured complexes $[V(Cys)_3]^{4-}$ and $[V(Cys)_2]^-$, both of which contain S,N-chelated cysteinate dianions.[83] The vanadium(II) complex is a powerful reductant and can reduce water to hydrogen under mild conditions. The kinetics of formation of these complexes and of the reduction of water have been studied and reaction mechanisms with possible intermediates are described. Similar complexes have been obtained with cysteamine and cysteine methyl ester. The involvement of cysteinyl residues in biological

redox processes is discussed.

Complex formation between chromium(III) and L-cysteine in the pH range 3.4-4.2 has been studied polarimetrically.[84] The results are similar to those obtained for glycine, and under the pH conditions used it appears that the initial complex formed contains N,O-chelated cysteinate. A mixture of Cr^{III}, nicotinic acid, glycine, glutamic acid and cysteine, which stimulates the rate of CO_2 production in a yeast bioassay system, was separated into inactive fractions by ion-exchange chromatography.[85] A Cr^{III}-bis(nicotinate) complex is proposed as the active ingredient in the original reaction. The effect of CN^- on the reaction of $[Fe(CN)_5NO]^{2-}$ with cysteine has been investigated.[86] The amino acids glycine and methionine react with $[Fe(CN)_5NO]^{2-}$ to give complexes with high molar absorption coefficients.[87] The implications of this for the analysis of these amino acids is discussed. Complex formation between Fe^{II}, Fe^{III} and glycine in aqueous solution (pH = 0-10.5, T ◻ 308K) and the catalytic activity of the resulting complexes in cysteine oxidation by O_2 have been studied.[88] A linear free-energy relationship between stepwise rate constants and stability constants in $FeL(OH)(CN)_x$ (L = aminocarboxylates such as EDTA, x = 0-3) complexes is reported.[89]

Base-catalysed elimination reactions of O-acetyl and O-sulphonylserine ligands in the complexes $[Co(en)_2(R\text{-}Ser)]^{2+}$ (12; R = CH_3CO, SO_3^-) give the chelated 2-iminopropanoate (13) product (Scheme 1).[90] This reaction, for which mechanisms are discussed, occurs 10^7 times faster than the corresponding reaction for the free ligands. Elimination of methanethiol is similarly facilitated in the S-methyl-cysteinate (Me-Cys) complex p-N,O-[Co(tren)(Me-Cys)]$^{2+}$. Complex (13) reacts rapidly with aldehydes in alkaline solutions (via carbanionic species), and the product (14) of such a reaction with 3-hydroxybenzaldehyde has been isolated (Scheme 1).



Reagent: i, *m*-$C_6H_4(OH)CHO$

Scheme 1

Base-catalysed hydrolyses of the amide groups in complexes (5), (6) and in $[Co(en)_2(\underline{S}\text{-Ala-Gly-OPr}^i)]^{3+}$ have been studied and the data interpreted in terms of OH^- attack on the acyl carbon.[20] The rates of racemization of L-alanine in the complexes $[CoL(\underline{L}\text{-Ala})_{\underline{n}}]^{\underline{m}+}$ (L = $(C_2O_4^{2-})_2$, $\underline{n}$ = 1, $\underline{m}$ = -2; L = $C_2O_4^{2-}$, $\underline{n}$ = 2, $\underline{m}$ = -1; L = O, $\underline{n}$ = 3, $\underline{m}$ = 0; L ▫ en, $\underline{n}$ ▫ 2, $\underline{m}$ ▫ +1; L ▫ tren, $\underline{n}$ ▫ 1, $\underline{m}$ = +2; L = $(NH_3)_5$, $\underline{n}$ = 1, $\underline{m}$ ▫ +3) have been found to increase with increasing charge.[91] Decarboxylation of the complex $\Lambda\text{-}\beta_1\text{-}[Co(\underline{R},\underline{R}\text{-picchxn})L](ClO_4)_2.2H_2O$ ($\underline{R},\underline{R}$-picchxn = $\underline{N},\underline{N}^1$-di(2-picolyl)-1$\underline{R}$,2$\underline{R}$-diaminocyclohexane, HL = 2-amino-2-methylpropanedioic acid) in aqueous HCl solution gave a mixture of $\underline{R}$- and $\underline{S}$-alaninato complexes in ratios 89.0 ± 0.5:11.0 ± 0.5, independent of reaction conditions.[92] The photolysis of aqueous solutions of $[Co(Gly)_2(Gly\text{-}OMe)]Cl_2$ has been investigated.[93]

The kinetics of ternary complex formation between Ni^{II}(EDDA) (EDDA ▫ ethylenediaminediacetate) and the ligands glycine, sarcosine and others (ethylenediamine, 2,2'-bipy and 1,10-phen) have been studied, and an isomerization mechanism involving a change in bonding (cis-α → cis-β) of the EDDA ligand which allows chelation of the amino acid ligands to take place at the observed rate is postulated.[94] The kinetics and mechanisms of oxidation of cysteine, cysteine methyl ester, penicillamine and glutathione, RSH, coordinated to Cu^{II}-Me_6tren (Me_6tren ▫ 2,2',2"-tris(dimethylamino)triethylamine) and Cu^{II}-tmpa (tmpa = tris-(2-pyridylmethyl)amine) in the pH range 5.6-11.1 have been investigated.[95] The Cu^{II}-tmpa($^-$SR) species appear to be stabilised by delocalisation of thiolate charge over the aromatic π systems. The mixed-valence complex $[Cu^{II}_6Cu^{I}_8\{(\underline{D}\text{-penicillamine})_{12}Cl\}]^{5-}$ has been examined for superoxide dismutase activity, and its photochemical decomposition has been investigated by electronic, c.d. and e.s.r. spectroscopy.[96] The superoxide dismutase activity of copper(II) complexes with ethylene-bridged amino acid ligands varies according to amino acid in the order Leu, Ala, 2-Me-Ala > Ile, Val > Gly.[97] The reduced activity of the Ile and Val complexes is a result of the methyl groups blocking coordination of superoxide, while the low activity of the Gly complex is thought to be due to its dimeric nature and the fact that the axial sites on copper are completely blocked. The reduction of the complexes $CuL^1_2(H_2O)_2$ and $Cu(2,2'\text{-bipy})L^2$ (L^1 = Gly, L-Ala, L-Ile, L-Pro, L-Ser, L-Orn, L-Lys, L^2 = Gly, L-Ala, L-Ile, L-Val, L-Ser) has been studied by cyclic volt-

ammetry.[98,99] In all cases intermediate Cu^{I} complexes, which dissociate to Cu^{O} at the mercury electrode, were observed. The photochemical behaviour of $Cu(Gly)_2$ and $Cu(Ala)_2$ in aqueous solution has been investigated.[100] Charge-transfer excitation of the former complex gives NH_3 and HCHO as ligand decomposition products. In oxygenated solutions H_2O_2 is also formed. The reaction of the macrocycle 1,4,8,11-tetraazacyclotetradecane with Cu^{II}-glycinate and other complexes has been investigated in an attempt to correlate reactivity and stability.[101]

The addition of chiral complexes to the mobile phases in h.p.l.c. has been used in the separation of enantiomers of free and derivatised amino acids. High separation factors were obtained for the dansyl derivatives (Dns) Dns-Glu, Dns-Asp, Dns-Thr, Dns-α-Aba, Dns-Val, Dns-Met, Dns-Phe and Dns-Trp when solutions of bis-(L-phenylalaninamidato)copper(II),[28] or of copper(II) acetate and the ligands $NH_2CH(R)CONH(CH_2)_nCONHCH(R)NH_2$ (R = $-CH_2Ph$, $-CHMe_2$, Me, n = 2,3),[102] were added to the mobile phase. The pH dependence of the separation in the latter case suggests that the complex $Cu(LH_{-2})$, containing amide deprotonated ligands, is the one responsible for the separation. A number of underivatised amino acids have been separated into enantiomers using the complexes Cu^{II}(L-Phe), Cu^{II}(Me-L-Phe) and Cu^{II}(Me_2-L-Phe) as chiral eluents.[103] The separation of enantiomers using copper(II) complexes of N,N-dialkyl-α-amino acids is described.[104]

The catalytic cycle of the enzyme sulfite oxidase is thought to involve cis-dioxomolybdenum(VI) and oxohydroxomolybdenum(V) complexes containing two or three S^--coordinated cysteinate ligands. This has prompted a recent investigation into the air oxidation of PPh_3 using the cysteinate ester complexes $Mo^{VI}O_2(Cys\text{-}OR)_2$ (HCys-OR = cysteine ester, R = Me, Et, Pr^i, Bzl) as catalysts in DMF solution.[105] The formation of PPh_3O was shown to require the presence of water, while e.s.r. and n.m.r. investigations showed that the complex $Mo^{V}(O)OH(Cys\text{-}OR)_2$ was involved in the catalytic cycle. The formation of $Mo^{V}_3O_3(Cys\text{-}OR)_4$ or $Mo^{V}_2O_4(Cys\text{-}OR)_2$, however, was shown to break the cycle. The catalytic effect was found to increase with increasing size of R. The complex $[Mo_2O_4(Cys)_2(H_2O)_2]^{2-}$ (Cys = cysteinate dianion) is reported to have nitrate reductase, xanthine oxidase and sulfite oxidase activities and, as in the enzymes, these activities are stimulated by spinach ferridoxin but inhibited by KCN, Na_2AsO_3 and NaN_3.[106] The complexes

$[Mo^{IV}Mo^{V}O_4(L)]^{3-}$ (L ▫ EDTA, (L-Cys)$_2$, or $(C_2O_4)_2(H_2O)_2$) were obtained from the corresponding $[Mo^{V}_2O_4(L)]^{2-}$ species by reduction with hydrated electrons (generated by pulse radiolysis) or with Zn^+ (generated by the reaction of hydrated electrons with Zn^{2+}).[107] In the presence of oxygen these complexes are rapidly redoxidised back to the Mo^V species.

The reaction of $[Pt(H_2O)_4]^{2+}$ with glycine gives $[Pt(H_2O)_3(O_2CCH_2NH_3)]^{2+}$ and $[Pt(H_2O)_2(O_2CCH_2NH_3)_2]^{2+}$ (cis- and trans-), the latter of which on standing undergoes chelation to $Pt(NH_2CH_2CO_2)_2$ (N,N-cis and -trans).[108] With methylimidodiacetic acid, $[Pt(H_2O)_4]^{2+}$ gives $[Pt(H_2O)_3\{O_2CCH_2NH(Me)CH_2CO_2H\}]^{2+}$, which on heating gives a complicated mixture of bridged species. The complex cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ reacts with glycine to give cis-$[Pt(NH_3)_2(H_2O)(O_2CCH_2NH_3)]^{2+}$ and this slowly chelates on standing. Analogous reactions occur with methyl imidodiacetic acid. The complex cis-$Pt(NH_3)_2(OH)_2$ reacts slowly with glycine to give $Pt(NH_3)_2(OH)(NH_2CH_2CO_2)$ and cis-$Pt(NH_3)_2(NH_2CH_2CO_2)_2$. These reactions were studied by ^1H, ^{13}C, ^{15}N and ^{195}Pt n.m.r. Reaction mixtures containing glycine, L-histidine and the complexes cis-$Pt(NH_3)_2L_2$ (L_2 ▫ Cl_2, cyclobutane-1,1-dicarboxylate, ethyl malonate) were analysed by electrophoresis, ^1H and ^{13}C n.m.r. spectroscopy.[109] The kinetics of the Ce^{IV} oxidation of amino acids AA (AA ▫ Gly, N-Ac-Gly and α-Ala) and their complexes $[Co(NH_3)_5AA]^{3+}$ and $[Co_2(NH_3)_6(\mu\text{-}OH)_2(\mu\text{-}AA)]^{4+}$ in 1.2M $HClO_4$ solution have been investigated and mechanisms involving radical formation are discussed.[110]

Formation Constants. - Using reported formation constants and the known variation of these constants as a function of metal ion and ligand, estimations are made of unreported formation constants for a wide range of complexes.[111] Such an approach has been used to establish a formation constant database for complexes of unprotonated (22), monoprotonated (14) and diprotonated (6) amino acids with the cations H^+, Na^+, Mg^{2+}, La^{3+}, Am^{3+}, UO_2^{2+}, Ni^{2+}, Cu^{2+}, $MeHg^+$ and Fe^{3+} at 25°C, ionic strength 0.1M. The equation $\log K = A/\gamma^2 + B$, which relates formation constants (K) of amino acid complexes to cation radii (γ) and to ligand (A) and cation (B) coefficients, has been derived.[112]

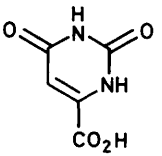
A number of papers report stabilisation effects which result from interligand interaction in metal complexes. A comparison of stability constants for 2:1 complexes of Co^{II}, Ni^{II},

Cu^{II} and Zn^{II} with the amino acids Gly, Ala, Phe, Tyr, Leu, Trp, nor-Val and nor-Leu (25°C, $\underline{I}$ = 0.05-0.1) provides concrete evidence for intramolecular hydrophobic and aromatic ring-stacking interactions in some cases.[113] The addition of Cu^{II} (10^{-3}M) to an aqueous solution of $\underline{L}$-Leu (2 x 10^{-3}M) at pH 7 considerably enhances an interaction between the isopropyl groups of two amino acid ligands, and the concentration of this 'metal bridged adduct' is increased on adding dioxane, its distribution rising from ~ 17% in H_2O to ~ 80% in 1:1 H_2O-dioxane. The biological relevance of these results is discussed. Evidence of stacking between the imidazole ring of histidine and the aromatic side chains of other amino acids has been obtained from a comparison of formation constants (25°C, $\underline{I}$ = 0.1M KNO_3) and of c.d. spectra (deviations from additivity, solvent dependence) of the mixed-ligand complexes Cu(A)($\underline{L}$-B) (A = $\underline{L}$-Tyr, $\underline{L}$-His, $\underline{D}$-His, en, B = Ala, Val, Ser, Thre, Asn, Glu, Glu, Arg, Lys, Phe, Tyr, Trp).[114]

Values of $\underline{K}$ for equilibrium (1)

$$Cu(N\text{-}N)\underline{L}\text{-Ala} + Cu(en)AA \overset{\underline{K}}{\rightleftharpoons} Cu(N\text{-}N)AA + Cu(en)\underline{L}\text{-Ala} \quad (1)$$

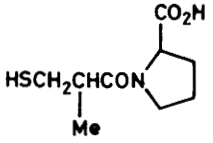
have been measured for seven diamines (N-N) and for 6 amino acids (AA) at 25°C, $\underline{I}$ = 0.1M KNO_3.[115] The results provide evidence for stacking interactions between aromatic rings of N-N and AA and this is further supported by c.d. spectra. Phosphorylation of $\underline{L}$-tyrosine has been found to regulate aromatic ring stacking in ternary complexes involving this amino acid. Formation constants for mixed-ligand complexes of Mg^{II} and Mn^{II} with orotic acid (15) and amino acid (Gly, Ala, Ser, Thr, Leu, Ile, Val, Phe, Trp, nor-Val, nor-Leu) ligands at 25°C, $\underline{I}$ = 0.15M NaCl, also provide evidence for π stacking interactions in some cases, and this has been confirmed by ^1H n.m.r. spectroscopy.[116] The stacking phenomenon has also been invoked to



(15)

explain trends in formation constants for complexes of Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} with cytidine,[117] or xanthosine,[118] and other (Gly, His, histamine, oxalate) ligands at 35°C, $\underline{I}$ = 0.1M KNO_3.

The biological toxicity of heavy-metal ions and their compounds has in recent years caused an upsurge of interest in the determination of formation constants for complexes of these and related ions with various ligands. This work has been carried out to pinpoint possible binding sites to biomolecules, to help design suitable ligands for clinical therapy of heavy-metal toxicity and to help eliminate side effects arising from the interaction of these ligands with essential metal ions. With the chelation therapy aspect in mind, formation constants have been measured for complexes of 'Captopril' (16) with Zn^{II}, Ca^{II} and Pb^{II} at 37°C in 0.15M NaCl solution.[119] This drug was



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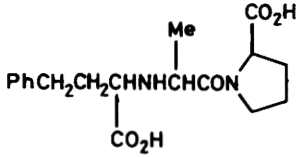
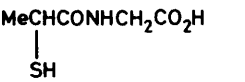


(17)

originally used as a hypotensive agent and subsequently in the treatment of chronic congestive heart failure, rheumatoid arthritis and migraine. It does, however, have serious side effects which are thought to arise from the interaction of the -SH group with metal ions (e.g. taste disfunction due to complexing with Zn^{2+}). The drug 'Enelaprilat' (17), which does not contain -SH groups, was therefore synthesised in the hope of producing a hypotensive agent with fewer side effects than 'Captopril'. The new drug, however, despite its lack of -SH groups and despite its higher potency in the treatment of hypertension was found to have adverse side effects related to Zn^{2+} and Cu^{2+} deficiency. Formation constants for complexes of 'Enelaprilat' with Cu^{2+} and Zn^{2+} at 37°C in 0.15M NaCl solution have therefore been determined, and from the results obtained it is estimated that mobilisation of these ions from human blood plasma by the drug is negligible.[120] Formation constants have also been obtained for complexes of Zn^{2+}, Ni^{2+}, Ca^{2+} and Pb^{2+} with $\underline{N}$-(2-mercaptopropionyl)glycine (18), a ligand



(18)

which had previously been studied as an antidote to mercury, alkyl- and aryl-mercury, lead cadmium and copper poisoning.[121] Here also the results were used to estimate the ability of the ligand to mobilise metal ions from plasma proteins and tissues. Complexes of Zn^{2+} and Ca^{2+} with α-penicillamine and other thiol and amino-carboxylate ligands have been similarly investigated.[122] The distribution of Cd^{2+} and Ni^{2+} amongst complexes formed with ~ 50 ligands under human blood plasma conditions has been the subject of a computer simulation study.[123] Formation constants are reported for cation (H^+, Ca^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}) binding to meso- and racemic $(CO_2^-)(CONH_2)NCH(Me)CH(Me)N(CONH_2)CO_2^-$ ligands, which are produced by hydrolysis of cyclic imide homologues of the anti-tumour drug razoxane.[124]

Equilibrium constants are reported for the formation of $[Co_2(2,2'\text{-bipy})_2L_2(\mu\text{-OH})(\mu\text{-}O_2)]^+$, $[Co_2(2,2'\text{-bipy})_2L_2(H_2O)_2\mu\text{-OH}]^+$ (HL = Ala, α-Aba, nor-Val, nor-Leu)[125] and $Co_2L^1{}_4(\mu\text{-}O_2)$ (HL^1 = L-Cys).[126] Formation constants have been measured for ternary complexes of Cu^{2+}, L-His and L-Asp-L-Ala-L-HisNHMe, a tripeptide which mimics the Cu^{2+} binding site of human serum albumin.[127] Five octadentate chelating agents each of which contains two amido groups and two terminal iminodiacetate groups have been synthesised, and formation constants for their complexes with Co^{2+}, Ni^{2+} and Cu^{2+} at 25°C, I ▫ 0.1M KNO_3, have been determined.[128]

A list of other complexes for which formation constants have been measured is given in the Table.

Complexes with Schiff-Base Ligands. - The synthesis, structures and properties of metal complexes with Schiff-base ligands derived from amino acids are covered in a number of papers. The nickel(II) complex (19) was obtained as a mixture of diastereoisomers by the reaction of NiX_2, R,S-Ala and (S)-aldehyde.[168] Deprotonation of (19) with BuLi followed by alkylation gave diastereoisomers (20), which were separated on SiO_2. Hydrolysis in 0.6M HCl gave enantiomerically pure R- and S-α-alkyl amino

(Text continues on page 281)

Table Formation constant measurements for metal-amino acid and related complexes

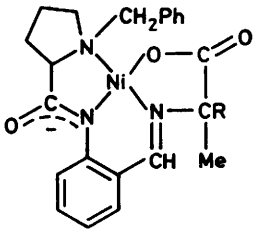
Cation	Ligand; complexes	Method; conditions; comments	Ref.
VO^{2+}	L-Cys, L-His, L-Phe; binary complexes	potentiometry; pH=2-11, I=0.15M KNO_3; thermodynamic parameters	129
Cr^{3+}	Met or Cys; 1:1, 2:1 and 3:1 binary complexes	potentiometry, spectrophotometry; T=20°C, I=0.1M $NaClO_4$	130
Fe^{2+}, Fe^{3+}	imidazole, benzimidazole, His; 1:1 binary complexes	calorimetry; T=288-318K, I=1.0M $NaClO_4$	131
H^+, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}	$NH_2(CH_2)_2NHCHCO_2H$; 1:1 and 2:1 complexes	potentiometry; T=25°C, I=0.5M KCl; Irving-Williams series obeyed, structures proposed	132
Co^{2+}, Ni^{2+}, Cu^{2+}	amethopterin (methotrexate) (9) and Ala, Phe or Gly; 1:1:1 ternary complexes	spectrophotometry; comparison with folic acid complexes	36
Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}	benzimidazole and Gly; binary and ternary complexes	potentiometry; T=30°C, I=0.1M $NaClO_4$; Irving-Williams series obeyed	133
H^+, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}	$RCH(ONH_2)CO_2H$ (R=H, Me, Et, Bu^n, Bu^t, Bzl); 1:1 complexes	potentiometry; T=25°C, I=0.5M; structures proposed	134
Co^{2+}, Cu^{2+}, Zn^{2+}, UO_2^{2+}	nitrilotriacetic acid, valine; binary and ternary complexes	paper electrophoresis (new method); T=35°C, I=0.1M	135, 136
Al^{3+}, Cr^{3+}, Ni^{2+}, Pb^{2+}, Th^{4+}	Gly; 1:1, 2:1, 3:1 complexes	paper electrophoresis; I=0.1M	137

Cation	Ligand; complexes	Method; conditions; comments	Ref.
Al^{3+}	succinic acid, Asp, Glu, His; binary and ternary (with OH^-) complexes	potentiometry; T=25°C, I=0.5M $NaClO_4$	138
Cu^{2+}	dipicolinic acid and Gly, Ala or Val; 1:1 binary and 1:1:1 ternary complexes	potentiometry; T=25°C, I=0.1M KNO_3	139
Cu^{2+}	bipy (A) and D,L-2,3-diaminopropionic acid, D,L-2,4-diaminobutyric acid or ornithine (B); Cu(A)B, Cu(A)BH	potentiometry; T=37°C, I=0.1M $NaClO_4$	140
Cu^{2+}	Gly	potentiometry (Cu-selective electrode), spectrophotometry; pH distribution of complexes	141
Cu^{2+}	thiodiglycolic acid and Gly, α-, β-Ala, Ser, en, malonic acid; ternary complexes	potentiometry, spectrophotometry; T=30°C	142
Cu^{2+}	$ROCH_2(CHOH)_4CO_2H$ (R=H or $-COCH_2NMe_2$) and Gly, Ala, Val, Trp, Ser, Lys or His; ternary 1:1:1 complexes	potentiometry, spectrophotometry; correlation of formation constants with pK_a values	143
Cu^{2+}	L-Asn, L-Gln, L-iso-Asn, L-aspartimide; 1:1, 2:1 binary complexes	potentiometry, spectrophotometry; T=25°C, I=0.2M	144
Cu^{2+}	Val or N-R^1R^2-Val and Pro, N-R^3-Pro, Pro(4-OH), N-R^3-Pro(4-OH) or a-Pro(4-OH) (R^1=H, R^2=Me, Bzl; R^1=Me, R^2=Me, Bzl; R^3=Bzl); binary and ternary complexes	potentiometry; T=25°C, I=1M KNO_3	145

Cation	Ligand; complexes	Method; conditions; comments	Ref.
Cu^{2+}	N-malonato-α-Aba	potentiometry; T=25°C, I=0.1M KNO_3	146
Cu^{2+}	Gly-NH_2, Gly-Gly-NH_2, N-Ac-L-His, Gly, L-His, histamine and other ligands; binary and ternary complexes	potentiometry	147
Ni^{2+}	bipy, phen, 2-(2^1-pyridyl)-benzimidazole or -imidazoline and diamines or Gly, α- or β-Ala, His, histamine, malonate	T=30°C, I=0.2M $NaClO_4$; dioxan-H_2O (1:1)	148
Zn^{2+}	ATP(H_4L) and Gly, Ala, Val, Leu, Ile, Phe, Ser, Thr, Met, Trp, Pro (HA); $[Zn(L)A]^{3-}$, $[Zn(L)A(OH)]^{4-}$	potentiometry; T=25°C, I=0.1M KNO_3	149
Zn^{2+}	L-His or 1,2-diaminopropane; binary complexes	polarography	150
Zn^{2+}, Cd^{2+}	$2,2^1$-bipy and amino acids; 1:1:1 ternary complexes	potentiometry; T=30°C, I=0.2M	151
Cd^{2+}	naturally occurring amino acids	differential pulse polarography, potentiometry; T=25°C, I=0.7M $NaClO_4$	152
Cd^{2+}	en and amino acids; 1:1:1, 1:2:1 and 1:1:2 complexes	polarography; T=25°C, I=1M KNO_3	153
Cd^{2+}, Pb^{2+}	Gly, Asp, mono- and diethanolamine; mixed-ligand complexes	polarography	154
Cd^{2+}, Pb^{2+}	D,L-norleucine; 1:1, 2:1 complexes	polarography; T=20,30°C, I=0.1M $NaClO_4$, pH=4.0,8.8; thermodynamic parameters	155

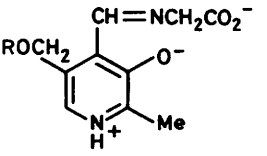
Cation	Ligand; complexes	Method; conditions; comments	Ref.
Pb^{2+}	oxalate and en, malonate or Gly; 1:1:1 complexes	polarography	156
Hg^{2+}	en and Gly, L-Lys, L-His, D,L-Ala, Glu, Cys or 2-aminoethanol; 1:1:1 ternary complexes	potentiometry; T=30,45°C, I=0.1M KNO_3, 10% EtOH; thermodynamic parameters	157
$MeHg^{+}$	selenomethionine	potentiometry	158
Me_3Sn^{+}	Gly, His, Cys	potentiometry; T=25°C, I=0.3M $NaClO_4$	159
La^{3+}, Pr^{3+}, Nd^{3+}, Gd^{3+}, Dy^{3+}	iminodiacetic or nitrilotriacetic acids and pyridine-2,6-dicarboxylic acid; 1:1:1 complexes	potentiometry; T=25°C	160
La^{3+}, Ce^{3+}, Pr^{3+}, Na^{3+}, Sm^{3+}	EDTA and Gly, Ala, Leu or Val; 1:1:1 ternary complexes	potentiometry; T=25°C, I=0.2M $NaClO_4$	161
Th^{4+}, Zr^{4+}	EDTA and Asp or Glu; 1:1:1 ternary complexes	potentiometry	162
H^{+}, all Ln^{3+} ions	$X[CH_2CH_2N(CH_2CO_2^{-})_2]_2$ (X=S-, PhN-, EtN-)	potentiometry; T=25°C, I=0.1M KNO_3	163
H^{+}, all Ln^{3+}	$(^{-}O_2CCH_2)_2N(CH_2)_2O(CH_2)_2$-$NH(CH_2CH_2CO_2^{-})$	potentiometry; T=25°C, I=0.1M KNO_3	164
Tb^{3+}	EDTA and 27 amino acids; 1:1:1 ternary complexes	circularly polarised luminescence spectroscopy	81
Ag^{+}	D,L-Met, D,L-ethionine, S-Me-Cys, S-Et-Cys, $NH_2CH_2CH_2SCH_2CO_2^{-}$, $NH_2CH_2CH_2SCH_2CH_2CO_2^{-}$; mononuclear (1:1,2:1) and binuclear complexes	potentiometry (pH,pM); T=25°C, I=0.5M KNO_3	165
Au^{+}	I^{-} and Gly; $[Au(Gly)_2]^{-}$, $[Au(I)Gly]^{-}$	light-scattering titration; T=20°C, I=0.5M $NaClO_4$	166
Th^{4+}, In^{3+}, Ga^{3+}, Sc^{3+}	Gly and carboxylic acids	T=20°C, I=0.1M $NaClO_4$	167

acids in high yields with recovery of the parent aldehyde. Copper(II) and nickel(II) complexes



(19) R = H

(20) R = Me, Bzl, or $CH_2{=}CHCH_2$

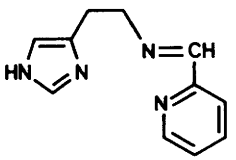


(21) R = PO_3H^-

(22) R = H

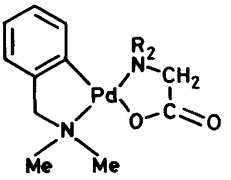
with Schiff-base ligands derived from S-o-[(N-benzylprolyl)-amino]acetophenone (S-bap) and various amino acids (Gly, R- and S-Ala, R- and S-Val, R- and S-adamant-1-yl-Ala and -Gly) have been synthesised and characterised, and the structures of Cu(S-bap-S-Val) and Ni(S-bap-S-Val) have been determined by X-ray crystallography.[169] Deuterium exchange on the -CH groups of the -Ala and -Val residues occurs with epimerisation, and the enantioselective effects, which are greater for the Ni^{II} than for the Cu^{II} complexes, are rationalised on the basis of X-ray structures, c.d. and ^1H n.m.r. spectroscopic data. The crystal structures of two pyridoxylideneglycinato, (21), (22), complexes are reported.[170] The complex $[Cu(21)H_2O].3H_2O$ is a one-dimensional polymer in which each metal is in a square-pyramidal ligand field containing phenolate O, imine N, carboxylate O and H_2O as basal ligands with a phosphate O from a neighbouring molecule occupying the axial site. In the complex $[Ni(22)_2].6H_2O$ the metal ion is octahedrally coordinated by two tridentate Schiff-base ligands.

The structures of the azido-bridged Cu^{II} complexes $[Cu_2L_2(N_3)_2](ClO_4)_2$ and $[Cu_2L_2(N_3)_3]Cl.2H_2O$, which contain the Schiff-base ligand L (23), have been determined by X-ray methods.[171]



(23)

Some Ni^{II} and Pd^{II} complexes with Schiff-base ligands derived from DMF and glycine or alanine have been prepared.[172] The complex (24) reacts with formaldehyde to give Schiff-base complex (25), which reacts further with formaldehyde (or acetic acid) to give serine (or threonine) after reductive elimination of the metal. The mechanism of the Akabori reaction is discussed and a series of Schiff-base complexes have been prepared by the reaction of amino acids with pyruvate using K_2PtCl_4 or K_2PdCl_4 as templates.



(24) R = H

(25) R_2 = CH_2

The crystal structure of the complex FeL.MeOH (H_3L = N-2-{(o-hydroxyphenyl)glycinato}ethylsalicylidenimine), a model for the iron-binding site in transferrins, has been determined.[173] This complex has pseudo-octahedral geometry in which two nitrogen atoms and the two phenolate oxygen atoms lie in the equatorial plane with the carboxylate oxygen and the MeOH group in the axial sites. In aqueous solution the MeOH is replaced by H_2O and the hydrolysis of the aquo complex has been studied by Mössbauer spectroscopy. New interpretations of the binding sites in the transferrins are presented. The synthesis and properties (adduct formation, redox potentials, photochromic behaviour of iodine derivatives) of a large number of iron(II) complexes with Schiff-base ligands containing electron-withdrawing substituents have been reported.[174]

Transamination and dephosphonylation of the Schiff bases of pyridoxal with aminoalkylphosphonic acids and with 2-amino-3-phosphonopropionic acid were studied in the absence of and in the presence of Al^{III}, Zn^{II} and Cu^{II}.[175] The kinetics of complex formation between Fe^{III} and pyridoxal-5-phosphate (pH = 1-2.5, T = 18-30°C, ionic strength = 0.5M NaCl) have been studied using stopped-flow techniques.[176] Reaction of Me_2SiCl_2 with various Schiff-base ligands in benzene gives the N-coordinated imine complexes $Me_2SiCl_2\{RCH{=}NCH(R')CO_2H\}_2$ (R=H,Me, R'=Ph, o-PhOH,

3- and 4-pyridyl), for which antibacterial activities are reported.[177]

Miscellaneous. - The application of perturbed angular correlation methods to the determination of oxidation states and ligand structures of simple molybdenum complexes ($Mo^{V/VI}$ with EDTA, tartaric acid or Cys) and of molybdoenzymes is discussed.[178] The ^{99}Tc radiopharmaceutical ^{99}TcN-Cys and others have been synthesised in high radiochemical purity from $[^{99}TcNCl_4]^-$, and their biological distributions in mice have been determined.[179] The mutagenic potentials of Cr^{III} complexes of Arg, Asp, Gly, Pro(4-OH) and Lys have been studied and compared with those of other Cr^{III} and Cr^{VI} compounds.[180] Formation constants for Cu^{II} complexes of Leu, Glu, Met, Trp and Ala have been measured as part of a programme to evaluate trace-metal requirements in total parental nutrition.[181] The addition of glycine and methionine to soils was found to cause increased water-soluble copper content, increased copper uptake by rye and increased polyphenol oxidase activity in plants.[182] Other papers describe: the catalytic decomposition of H_2O_2 by Mn^{II}-His complexes in aqueous solutions buffered by borax;[183] the use of the metallocarborane sandwiches $[(C_2B_9H_{11})_2M^{III}]^-$ (Me = Fe, Co, Ni) in the solvent extraction and analysis of amino acids and other organic bases;[184] the Fourier-transform i.r. detection of metal-amino acid complexes in h.p.l.c.;[185] the mechanism of quenching of riboflavin fluorescence by M^{II}-His and M^{II}-Ala (M = Cu, Ni) complexes;[186] the complexing of ^{239}Pu by amino acids and other organic and inorganic acids in blood;[187] quantitative relationships between the formation constants of lanthanide(III)-aminocarboxylate complexes and the number of f-electrons;[188] the preparation of serine by the hydroxymethylation of Cu^{II} and Ni^{II} glycine complexes with formaldehyde in alkaline solution;[189] a model based on the *in vivo* patterns of absorption, distribution, reaction and excretion of chelating agents, metal ions and their complexes for the selection of therapeutic chelating agents;[190] the preparation of complexes of Cu^{II} with glycinate and biologically active phosphate ligands;[191] and the mechanism of the catalytic electro-reduction of In^{III} in the presence of cysteine, thiourea and thiosemicarbazide.[192]

3 Peptides

A large number of papers dealing with syntheses, structures, solution studies and reactions of metal-peptide complexes have been published during 1985. Reviews covering this area are discussed in the Introduction. Particularly interesting developments are the use of platinum(II) and cobalt(III) complexes in peptide synthesis and the solution equilibrium studies of Pettit and coworkers on metal complexes of tetra- and penta-peptides. There is continuing interest in the use of peptide ligands to stabilise unusual high oxidation states of metal ions such as Cu(III), Ni(III) and Pd(IV).

Synthetic Aspects. - Beck[193] and his collaborators have described the preparation of sixteen Pt(II) peptide complexes such as cis-$[PtCl_2(Gly\text{-}Gly\text{-}OEt)_2]$ and trans-$[PtCl_2(Gly\text{-}Gly\text{-}Gly\text{-}OEt)_2]$ by condensing the corresponding platinum amino acid complexes, e.g. cis-$[PtCl_2(Gly\text{-}OH)_2]$, trans-$[PtCl_2(Gly\text{-}OH)_2]$, with the appropriate amino acid or peptide ester using $Me_2N(CH_2)_3NCNEt.HCl$ as the condensing reagent. The amino protecting Pt(II) was removed from the peptide esters by 1,2-(diphenylphosphino)ethane or by hydrogenolysis. Peptide formation and Pt(II) removal took place without racemisation.

Isied et al.[194] have developed the use of $[Co(NH_3)_5]^{3+}$ as a carboxyl-protecting group for peptide synthesis. A recent paper discusses $[Co(NH_3)_5]^{3+}$ as a protecting group for threonine.[195] The $[Co(NH_3)_5(C_4H_9NO_3)]Br_3$ complex has been characterised and its crystal structure determined. The threonine ligand is bonded to cobalt(III) via one carboxylate oxygen, and the absolute configuration of the complex was established. The use of the complex in peptide synthesis was established by coupling with N-protected L-Ala to give the dipeptide complex.

The tetrapeptides Z-Cys-Ala-Ala-Cys-OMe, Z-Cys-Val-Val-Cys-OMe and Z-Cys-Gly-Pro-Cys-OMe (Z ◘ benzyloxycarbonyl) having Cys-X-Y-Cys sequences have been synthesised by using an acetamidomethyl (Acm) protecting group.[196] The Hg(II) complexes of these peptides were prepared by reaction of the S(Acm)-protected peptides with $HgCl_2$ in DMF. The complexes $HgCl_2$(Z-Cys-Ala-Ala-Cys-OMe), $HgCl_2$(Z-Cys-Gly-Pro-Cys-OMe) and $HgCl_2$(Z-Cys-Ala-Cys-OMe) display Raman bands due

to Cl-Hg-S in the solid, whereas a single band due to S-Hg-S was found at 326 cm^{-1} for Hg(Z-Cys-Ala-Ala-Cys-OMe) and Hg(Z-Cys-Val-Val-Cys-OMe).

The Fe(II) complexes of Z-Cys-Thr-Val-Cys-OMe and Z-Cys-Pro-Leu-Cys-OMe have been prepared in solution as analogues of reduced rubredoxin from Clostridium pasteurianum. The complexes were characterised by visible, c.d. and m.c.d. techniques.[197] The Fe(II) complexes of Z-Cys-Ala-Ala-Cys-OMe, Z-Cys-Ala-Cys-OMe and Z-Ala-Cys-OMe were also prepared in solution for comparative purposes. The tetrapeptides and the tripeptides give macro-ring Fe(II) chelates similar to the Cys-X-Y-Cys sequences found in rubredoxin. The redox potentials of the Fe(II)/Fe(III) couple in these peptide complexes in Me_2SO solvent occur at ca. -0.5V versus S.C.E., which are substantially more positive than the -0.99V of $(Et_4N)_2[Fe(S_2\text{-}o\text{-}xyl)_2]$.

The dipeptides H-Gly-X-OH (X = Gly, Leu, Val) have been complexed with Cp_2TiCl_2 (Cp ◘ cyclopentadienyl) in the presence of base and the products characterised by i.r. and mass spectroscopy.[198] Mercury(I) and Fe(II) complexes of PEG-CO-Cys-Pro-Leu-Cys-OMe [PEG = poly(oxyethylene)] each having an invariant sequence of rubredoxin have been characterised by ^{13}C n.m.r. and c.d. measurements.[199] In aqueous solution the Fe(II) complex is much more resistant to hydrolysis compared with the Fe(II) complex of PEG-CO-Cys-OMe because of protection of the Fe(II) core by the hydrophobic side chains of the Pro and Leu residues.

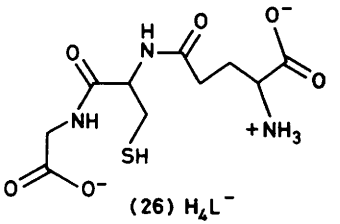
Cysteine-containing peptides have been incorporated as pendant groups on styrene-divinylbenzene copolymers.[200] The resulting resins had absorption capacities for Hg(II) ranging up to 10mg Hg/mmole Cys for H-Cys-Gly-Gly-polymer. The complexes $[Fe_4S_4(Z\text{-}Cys\text{-}Gly\text{-}Ala\text{-}Cys\text{-}OMe)_2]^{2-}$ and $[Fe_4S_4(Z\text{-}Cys\text{-}Ile\text{-}Ala\text{-}Cys\text{-}OMe)_2]^{2-}$ have been prepared from $[Fe_4S_4(S\text{-}t\text{-}Bu)_4]^{2-}$ as [4Fe-4S] ferredoxin models[201] having the invariant sequence for Cys-X-Y-Cys of bacterial ferredoxins. The chelation of Cys-X-Y-Cys to $Fe_4S_4^{2+}$ was confirmed by ^1H n.m.r. and c.d. spectra. A large positive shift of the redox potential of $[Fe_4S_4(Z\text{-}Cys\text{-}Gly\text{-}Ala\text{-}Cys\text{-}OMe)_2]^{2-}$ in CH_2Cl_2 was observed at low temperatures (-0.93V versus S.C.E. at 24°C, and -0.80V at -42°C), while no such temperature dependence occurred with the second complex. The presence of a Gly residue adjacent to the Cys thilato group was found to be advantageous for the formation of an

NH···S hydrogen bond, which is probably one of the important factors for the more positive redox potential of native ferredoxins.

Solid complexes containing Fe(II) and glutathione or Fe(III) with oxidised glutathione have been characterised.[202] Two stoichiometries 1:1 and 1:2 for iron(II)-glutathione complexes occurred. The 1:1 complexes contain high-spin iron(II) in five- and six-coordination, whereas the 1:2 complexes contain only distorted six-coordinate high-spin iron(II).

A number of polymeric iron(III) oxidised glutathione complexes were also prepared.

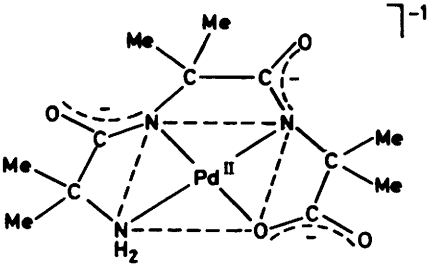
The glutathione (H_5L) complexes of chromium(III) $K_2[Cr(H_3L)(H_2L)].3H_2O$, $K_2[Cr(H_2L)(A)].\underline{n}H_2O$ (A ▫ the dianion of Cys, Glu or Asp) and $K_2[Cr(H_2L)(Gly)OH].2H_2O$ have been prepared.[203] All of the complexes exhibit an intense u.v. charge-transfer band characteristic of a Cr-S bond. The sulphydryl-to-chromium linkage undergoes an acid-catalysed hydrolysis. Glutathione is bound to Cr(III) by the terminal Gly(N,O) and the deprotonated sulphur of Cys. The glutamic acid residue of glutathione (26) does not appear to interact with chromium.



(26) H_4L^-

The two trimethyltin-dipeptide complexes PhCO-Leu-His-OMe($SnMe_3$) and PhCO-His-Cys-OMe($SnMe_3$) have been prepared and characterised as models for triorganotin-protein interactions.[204] The former complex is bonded to tin via the imidazole ring while the latter has a Sn-S bond.

Transient palladium(IV) intermediates have been invoked to account for certain palladium-catalysed reactions of organic compounds. Palladium(IV) has now been shown to be stabilised by the tripeptide of α-aminoisobutyric acid, $H\text{-}[NHC(CH_3)_2CO]_3\text{-}OH$.[205] The palladium(II) complex is shown in (27) in which the amino group, two deprotonated amide nitrogens and the carboxylato oxygen act as donors. The Pd(II) complex may be oxidised both chemically and electrochemically to the Pd(IV) complex (28) with



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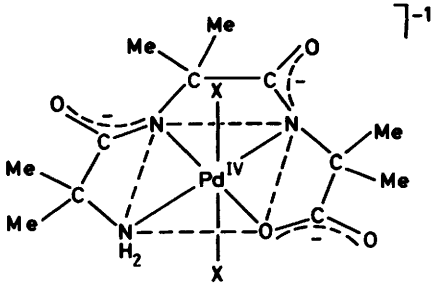


(28)

axial halide coordination at low pH and axial hydroxide at neutral pH. Multidentate ligands with amidate nitrogen donors have previously been extensively used to stabilise high oxidation states of a variety of transition metals, e.g. Cu(III), Ni(III), Ag(III), Os(VI) and Co(IV).

A number of studies have appeared dealing with nickel(III)-peptide complexes. Addition of excess tripeptide (L^-) to solutions of (tripeptido)nickel(III), $Ni^{III}(H_{-2}L)$, gives bis-(tripeptido)nickelate(III) complexes.[206] In the initial reaction $[Ni^{III}(H_{-2}L)L]^-$ forms, but this is a transitory species which rapidly loses a proton to give $[Ni^{III}(H_{-2}L)(H_{-1}L)]^{2-}$ with five nitrogen donors coordinated to nickel. The five-nitrogen donor complex is relatively stable from pH 6 to 11, but converts to a six-nitrogen tetragonally distorted complex $[Ni^{III}(H_{-2}L)_2]^{3-}$ above pH 11.

The terminal N(peptide) bonds to nickel in complexes of tetraglycine, $[Ni^{III}(H_{-3}G_4)(H_2O)_2]^-$, and tetraglycinamide, $[Ni^{III}(H_{-3}G_4a)(H_2O)_2]$, cleave rapidly in acidic solution with first-order rate constants that range from 0.1 to 15 s^{-1} as the hydrogen-ion concentration increases (0.004 to 1.0 mol dm^{-3}).[207] However, the other three equatorial Ni(III)-N bonds are relatively inert to substitution. The ternary complexes $[Ni^{III}(H_{-2}peptide)(phen)(H_2O)]$ and $[Ni^{III}(H_{-2}peptide)(terpy)]$ readily form in dilute acid with phen chelated to an axial and an equatorial site and terpy coordinated to two axial and one equatorial site on nickel(III). Further work[208] has shown that the Gly-Gly-Gly, Gly-Ala-Gly and Gly-Gly-Ala complexes of nickel(III) undergo substitution reactions with en, dien, bipy, phen and terpy to form relatively stable chelated ternary complexes. Peptides with α-aminoisobutyric acid (Aib) in the third residue are sterically hindered in the formation of chelate adducts. Thus,

$Ni^{III}(H_{-2}Aib_3)(H_2O)_2$ does not form polypyridine adducts and forms only monodentate, axially coordinated polyamine adducts.

Solution Equilibria. - A considerable volume of literature has appeared dealing with the interaction of metal ions with peptides in solution. Pettit and coworkers[209] have studied the interaction of Cu(II) with the series of tetrapeptides X-Gly-Gly-Gly, Gly-X-Gly-Gly, Gly-Gly-X-Gly and Gly-Gly-Gly-X (X = Pro or Sar). This study demonstrates the formation of a large chelate ring when tetrapeptides containing Pro (and, to a lesser extent, Sar) in the second or third position coordinate to copper(II). The ring spans the terminal residues of the peptide chain and locks the peptide into a 'bent' or 'horseshoe' conformation. It has been suggested that copper(II) could play a role in activating oligopeptides (e.g. neuropeptides) containing proline.[210] The proline residue in the second or third position of a tetrapeptide chain acts as a 'break point' to copper(II) coordination, dividing the peptide chain into two parts which coordinate independently. Proline also encourages a β-conformation of the peptide chain, presenting the terminal residues in a suitable conformation to form an abnormally large chelate ring. As many neuropeptides contain proline residues it is likely that Cu(II) ions assist in holding these peptide molecules in the biologically favourable β-conformation by bridging across the ends of the chain.

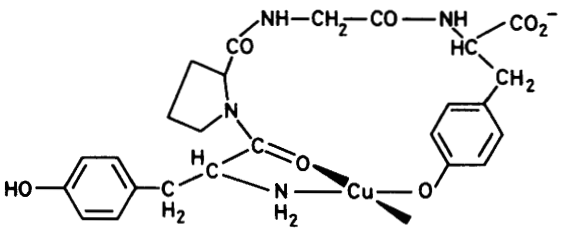
A potentiometric and spectrophotometric study of copper(II) complexes of methionine enkephalin and four related pentapeptides which show greater biological activity than their parent enkephalin has been reported.[211] All of the peptides form stable copper(II) complexes comparable with those formed by pentaglycine, with the peptide chain locked in a folded conformation by NNN or NNNN coordination to Cu(II). Bonding via the tyrosine-phenolate oxygen atoms on the methionine sulphurs does not occur.

The synthesis of β-casomorphin-5 (Tyr-Pro-Phe-Pro-Gly = H_2L) and a number of its peptide fragments has been described. A combination of potentiometric and spectroscopic measurements have been used to study the copper(II) complexes of these peptides.[212] With tyrosine as the N-terminal residue, the major complex formed at pH 7 is the dimeric complex $[Cu_2L_2]$ bonded via the phenolic O^- of the Tyr residue of one ligand and the N-terminal amino nitrogen

of the second ligand. Interestingly there is no evidence for coordination via the peptide nitrogens unless the terminal Tyr group is removed.

The peptides Gly-Gly-Pro-Gly, $(Gly\text{-}Gly\text{-}Pro\text{-}Gly)_2$, Gly-Gly-Pro-Lys and $(Gly\text{-}Gly\text{-}Pro\text{-}Lys)_2$ are models for the biologically active antitumor octapeptide dog tuftsinyltuftsin. Studies[213] of the interaction of Cu(II) with the tetra- and octapeptides clearly demonstrate the involvement of the $\varepsilon\text{-}NH_2$ group of -Lys- in coordination, indicating the presence of a bent conformation with a large chelate ring spanning the two ends of the peptide chain.

The interaction of Cu(II) with Tyr-Pro-Gly-Tyr and Tyr-Gly-Pro-Tyr (H_3L) has also been studied by potentiometric and spectrophotometric techniques.[214] In the first peptide the Pro residue imposes a bent conformation and the formation of an unusually stable [CuHL] complex involving coordination via the terminal amino group, the neighbouring peptide C=O and O^- of the C-terminal Tyr as shown in (29) giving a 17-membered chelate ring.



(29) Proposed structure for the [CuHL] species of Tyr - Pro - Gly - Tyr

In the case of Tyr-Gly-Pro-Tyr there is also O^- bonding via Tyr, which gives rise to the dimer $[(CuL)_2]^{2-}$.

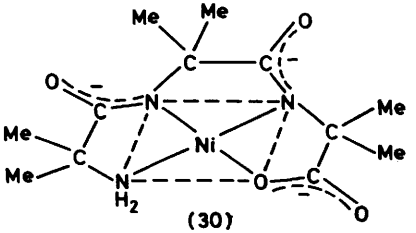
The interaction of Cu(II) with tyrosine dipeptides L-Tyr-X (X = L- or D-Ala, Arg, Tyr, Trp, His, L-Phe and L- or D-Glu) has been investigated by spectroscopic and potentiometric measurements.[215] All of the peptides react with Cu(II) in a manner analogous to L-Tyr-Gly, but the deprotonation of the peptide NH group is affected by the nature of the C-terminal side-chain groups. All of the dipeptides with the exception of L-Tyr-L/D-His give a dimeric species at pH 8-11 at 1:1 ligand-to-metal ratios. The equilibrium constants for the 2(monomer)$\rightleftharpoons$dimer equilibria are in the range 2.04 to 3.70 log units. The complexes $[Cu(L\text{-}Tyr\text{-}Gly)]0.5\ H_2O$ and $Na_2[Cu_2(L\text{-}Tyr\text{-}Gly)_2]7.5\ H_2O$ were characterised in the solid state.

The tripeptide Gly-His-Lys is co-isolated with copper and iron from human plasma, and it has been suggested that the peptide is the low-molecular-weight carrier for the transfer of copper(II) to human serum albumin. Complex formation between Gly-His-Lys and related dipeptides with copper(II) has recently been investigated.[216] A somewhat higher affinity of the tripeptide for Cu(II) compared with the dipeptides L-His-L-Ala and L-His-L-Leu is observed. The dipeptide L-Lys-L-His gives a dimeric copper complex which displays an e.s.r. spectrum at room temperature.

Formation constants for Cu(II), Ni(II) and Zn(II) complexes of the cyclic dipeptides cyclo(-Gly-L-His-) and cyclo(-L-Met-L-His-) have recently been reported.[217] ^{1}H n.m.r. studies establish that the metal ions bind exclusively to the nitrogen atom of the imidazolyl group of cyclo(-L-Met-L-His-).

The binding of zinc(II) by the tripeptide Gly-L-His-L-Lys (GHL) has been studied by ^{1}H n.m.r. and potentiometric titration.[218] At physiological pH, resonances were observed for two kinetically stable complexes. One of these complexes is tridentate with Zn(II) coordinated by the glycyl amino nitrogen, the deprotonated amide nitrogen of the glycyl-histidyl peptide bond and the imidazole 1-nitrogen. The amino group of the lysine side chain is protonated. The same group has also studied the binding of Zn(II) by Gly-L-His and L-Ala-L-His by ^{1}H n.m.r. and potentiometry.[219] At physiological pH and above, complexes form which are in slow exchange with the free peptide on the n.m.r. time-scale. Zinc is bound by the N-terminal amino group, the deprotonated amide nitrogen and the imidazole 1-nitrogen.

The α-aminoisobutyryl (Aib) residue is present in peptides which act as microbial antibiotics.[220] The presence of adjacent Aib residues in peptides restricts their conformation, due to steric effects of the two methyl groups on the α-carbons. The absence of α-hydrogens is also important in the stabilisation of Aib_3 complexes of Cu(III) and Ni(III).[221] The $[Ni^{II}(H_{-2}Aib_3)]^-$ complex, which has the structure (30), is unusually stable and is relatively sluggish in its reactions with both acid and cyanide



(30)

ion. Recent measurements[222] have established that the formation constant for $Ni^{2+} + L^- \rightleftharpoons Ni(H_{-2}L)^- + 2H^+$ is $10^{-9.65}$, some 1500 times larger than for the analogous triglycine complex. In addition, the rate of acid dissociation of $[Ni^{II}(H_{-2}Aib_3)]^-$ is 3.4×10^3 to 3.9×10^5 times slower than for the triglycine complex. These effects are believed to be due to the increase in the nickel-N(peptide) bond strength arising due to the α-methyl groups.

Triglycine (G_3^-) has been shown[223] to react with $PtCl_4^{2-}$ to give complexes with two deprotonated-N(peptide) bonds to Pt(II). The pK_a of the peptide group when bonded to Pt(II) is ca. 1 to 2. N.m.r. studies (^{13}C, ^1H and ^{195}Pt) indicate three complexes, with relative concentrations $[Pt(H_{-2}G_3)Cl]^{2-} > [Pt(H_{-2}G_3)]^- > [Pt(H_{-2}G_3)(OH)]^{2-}$.

A variety of bis(tripeptido)nickelate(III) complexes have been characterised in aqueous solution.[224] Addition of excess tripeptide (H_2L^-) to solutions of NiL gives initially $[NiL(H_2L)]^-$, which rapidly loses a proton to give $[NiL(HL)]^{2-}$ with five nitrogen atoms coordinated to nickel. The complex $[NiL(HL)]^{2-}$ is relatively stable at pH 6 to 11 but is converted to the tetragonally distorted $[NiL_2]^{3-}$ at pH > 11. This latter complex has six nitrogens coordinated to nickel.

Mixed-ligand complexes of copper(II) with peptides and adenosine-5'-triphosphate have been studied.[225] A correlation was noted between the pK_a value for the deprotonated peptide amide group coordinated to Cu(II) and the tendency to bind the ATP^{4-} ligand. Hydrophobic substituents enhanced the stability of the mixed-ligand complex as they affect the pK_a of the deprotonated amide groups of the peptides.

Oxygen-17 and nitrogen-14 n.m.r. studies of the binding of cobalt(II) to some cyclic dipeptides via the peptide oxygen have been reported.[226] Improvements in the use of ligand-exchange chromatography on copper(II) modified silica gel have been described.[227] The convenient u.v. detection at 254nm of amino acids and peptides as their copper(II) complexes and the use of gradients make ligand-exchange chromatography a simple method for the qualitative screening of protein hydrolysates and quantification of free amino acids and dipeptides in complex polypeptide mixtures.

The reaction of $[Co(py)]^{2+}$ (py ▫ pyridine) with dipeptides has been studied under inert atmosphere conditions.[228] The formation constants (log $\underline{K}$) for complexes formed by six different dipeptides fall in the range 2.37 to 2.98 at 25° and $\underline{I}$ = 0.1 mol dm^{-3}. The oxygenation of $[Co(bipy)(HL)(H_2O)_2]^+$ complexes (bipy ▫ 2,2'-bipyridyl, H_2L = Gly-Gly, Gly-DL-α-Ala, Gly-DL-norvaline, Gly-DL-Val and Gly-L-Leu) has been studied at 25°C and ionic strength 0.1 mol dm^{-3} by potentiometric and manometric techniques.[229] Increasing chain length and degree of branching of the C-terminal amino acid in the dipeptide lowers the stability of the μ-peroxo complex $[Co(bipy)L]_2O_2$.

The Cd(II) complexes of Gly-Gly, Gly-Gly-Gly, Gly-γ-aminobutyric acid and β-Ala-Gly have been studied by ^{113}Cd and ^{13}C n.m.r.[230] The minima observed in plots of the ^{113}Cd chemical shifts versus pH are consistent with Cd(II) binding first at the carboxylate group and then at the amino group. The results with β-Ala-Gly are different from those with the other dipeptides and suggest the formation of a five-membered chelate ring. Cd(II) binding to the NH group of the peptide bond does not occur. In addition, a reported pH-dependent ^{113}Cd resonance for parvalbumin has been reassigned.

The synthesis of Cadystin A and B, major unit peptides of cadmium-binding proteins, has been described.[231] The structures are H-γ-Glu-Cys-γ-Glu-Cys-γ-Glu-Cys-Gly-OH (Cadystin A) and H-γ-Glu-Cys-γ-Glu-Cys-Gly-OH (Cadystin B).

A simple and reliable method for the measurement of Ca(II) binding to proteins and peptides has been developed.[232] The procedure involves filtration through a nitrocellulose membrane filter and estimation of ^{45}Ca retained on the membrane. The routine assay can be completed in a few minutes and only microgram amounts of the samples are necessary. The technique also allowed the detection of very weak interactions ($\underline{K}_d \approx 10^{-3}$ mol dm^{-3}).

The low-molecular-weight ligands bound to zinc in soyabean seeds have been investigated.[233] The Zn appears to be bound to nucleotides and to peptides. Two mercury(II) peptides have been isolated from the sediments of a pond near the Itomuka mercury mine in eastern Hokkaido, Japan.[234] The two peptides were characterised by TLC, i.r. and electrophoresis and have estimated molecular weights of 1.5×10^6 and 1.2×10^5 daltons.

The effect of mixed-ligand complex formation on the deproto-

nation of amide groups in copper(II) complexes of amides and peptides has been investigated in some detail.[235] Deprotonation and coordination of the amide group was not detected in complexes of N-acetyl-L-histidine, but the presence of other ligands containing aromatic nitrogen donors caused deprotonation of the amide group. Lanthanide(III) complexes with aspartate and glutamate residues have been studied using n.m.r. techniques.[236] Two types of peptide backbones were employed, the methyl esters of N-acetylamino acids and cyclic dipeptides. The Yb(III)-induced chemical shifts were used to determine binding constants and the geometry of the complexes. Both 1:1 and 2:1 (peptide:cation) complexes were detected. Only one oxygen of the carboxylate group is involved in bonding and the Yb(III)-O distances are different with the aspartate (2.70 to 2.75 Å) and the glutamate (2.50 to 2.55 Å) residues. The predominant conformer has an extended side chain so that interaction with the peptide backbone does not occur.

The mode of Ca(II) and Eu(III) binding to 4'-amino-Phe^4 and Leu^5-enkephalin in MeCN has been studied spectrofluorometrically.[237] Complexation is accompanied by an increase in the distance between the aromatic rings [Tyr^1 and (4'-amino)-Phe^4] in the peptide. The ^{1}H n.m.r. of the peptide in water in the presence of increasing amounts of Eu(III) was used to identify the binding sites as the Leu^5-carboxyl group and probably the (4'-amino)Phe^4 amide carbonyl group.

Crystallographic and Other Structural Studies. - The crystal structure of a zinc(II) complex with cyclo(L-methionyl-L-histidyl), having the formula $Zn(C_{11}H_{16}N_4O_2S)_4.SO_4.10\ H_2O$, establishes that the zinc(II) is coordinated tetrahedrally by four N atoms of the imidazolyl groups of the histidyl residues.[238] The N-Zn-N bond angles are all within 3.5° from 109.5° and bonding occurs via the pyrole nitrogen.

The phenolate-to-iron(III) charge-transfer transition in a series of iron(III) phenolate complexes has been investigated as a model system for iron-tyrosinate proteins.[239] The n.m.r. contact shifts for the phenolate protons are well correlated with the visible absorption maxima of the complexes and the Fe(III)/Fe(II) redox potentials. The energy of the charge-transfer band is sensitive to the crystal field strength of the other ligands

bonded to iron(III). The stronger the other ligands, the higher the energy of the phenolate charge-transfer band.

The ^{13}C and ^{1}H n.m.r. relaxation rates for the 2:1 complex of glutathione with oxovanadium(IV) have been determined.[240] The two carboxyl groups are shown to be the main binding sites and the kinetics of the dissociation of the peptide ligand were delineated. Copper(II) complexes of His, Gly-His and Gly-Gly-His and their interaction with L(+)-ascorbic acid have been studied by ^{1}H n.m.r.[241] It has been observed that ascorbate is cytotoxic against tumour cells in the presence of copper(II), possibly due to the production of OH· radicals by a Fenton-type reaction between copper(I) complexes and H_2O_2.

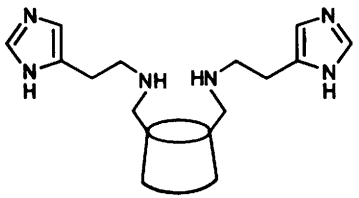
The interaction between $[Fe(CN)_6]^{3-}$ and the copper(I) complexes of Gly-His and Gly-Phe has been investigated by electronic and e.p.r. spectroscopy and magnetic measurements.[242] Evidence for a specific metal-metal interaction probably via a cyanide bridge was obtained. An X-ray absorption spectroscopic study of the interaction of Cu(II)-dipeptide complexes with imidazole, 2-methylimidazole, 2,2-bipy and 1,10-phen has been published.[243]

Reactivity. - Earlier studies[244-246] by Isied and collaborators concentrated on metal-to-metal intramolecular electron transfer across amino acids, flexible and rigid polypeptides and modified proteins. A recent paper[247] discusses the synthesis and the intramolecular electron transfer in the series of complexes $[(NH_3)_5Os^{III}\text{-L-}Co^{III}(NH_3)_5]^{5+}$, where L ▫ iso(pro)$_{0-4}$, iso(Phe)$_2$ and iso(Gly)$_2$, iso ▫ isonicotinyl. The peptide bridges are spacers which separate the Os(III) centre from the Co(III) centre. On one electron transfer using pulse radiolysis techniques the $[(NH_3)_5Os^{II}\text{-L-}Co^{III}(NH_3)_5]^{4+}$ complexes are formed. These precursor complexes undergo intramolecular electron transfer on time-scales varying from microseconds to many seconds. The Os(II) site in all these complexes has approximately the same redox potential (-0.26V vs. NHE). Because the driving force and re-organisational energy in these complexes are very similar, variations in the rate of electron transfer can be related directly to the properties of the peptide bridge. The rates of intramolecular electron transfer are more rapid than the rate of trans-to cis-proline isomerisation, and therefore electron transfer takes place while the proline is in the extended trans configuration, the predominant isomer under these conditions.

Glutathione (γ-Glu-Cys-Gly) is an intracellular peptide important in the maintenance of redox status, and is found in millimolar concentrations in typical mammalian cells. The carcinogenicity and mutagenicity of chromium(VI) (e.g. CrO_4^{2-}) are well established, and a recent paper[248] establishes that Cr(V) species of considerable stability can be generated in the reduction of Cr(VI) by glutathione. The relevance of these observations to chromate toxicity is discussed.

The effect of charge on the racemisation of L-alanine in cobalt(III) complexes has been studied.[249] Racemisation rates are directly proportional to the charge on the complex as it varies from -2 through 0 to +2. Complexation of dipeptides to Co(III) reduces the rates of racemisation; however, the larger the positive charge on the complex the faster is the rate of racemisation. The catalytic oxidation of benzoin by 1,4-benzoquinone in the presence of cysteine-containing peptides or bulky thiolate/Fe_4S_4 complexes has been reported.[250]

A carbonic anhydrase model bis(histamino)-β-cyclodextrin, $(His)_2CD$, has been prepared[251] from a benzophenone-3,3'-disulphonate capped cyclodextrin. The association constant of Zn(II) with $(His)_2CD$ (31) was determined to be $(4.5 \pm 2) \times 10^2$ dm^3 mol^{-1} at



(31)

pH 7. The detailed kinetic analysis of the CO_2 hydration in imidazole buffer showed that $(His)_2CD.Zn.imidazole$ was a potent catalyst with a catalytic rate constant of ca. 10^3 dm^3 mol^{-1} s^{-1} at 25°C.

It has previously been shown that the base-catalysed condensation of the dipeptide ligand of mer-$[Co(glygly)NO_2(en)]$ with acetaldehyde takes place at the N-terminal CH_2 group of the dipeptide. A further paper[252] describes the preparation of mer-$[Co(glygly)L(en)]^{n+}$ complexes (L = H_2O, CN^-, NCS^-, H_2glygly ▫ glycylglycine and en = 1,2-diaminoethane). The crystal structure of mer-$[Co(glygly)NCS(en)].H_2O$ was also determined and the condensation of the neutral complexes with acetaldehyde examined. A complex series of products were obtained involving reaction with both the dipeptide and ethylenediamine.

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