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Amino Acids and Peptides

VOLUME 20

ROYAL SOCIETY OF CHEMISTRY

Natural Product Reports

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Volume 20

A Specialist Periodical Report

Amino Acids and Peptides

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A Review of the Literature Published during 1987

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Preface

Our work this year has been overshadowed by the loss of Ian Galpin. After a peptide apprenticeship at Exeter, he extended his experience as an ICI Postdoctoral Fellow with the late Professor George Kenner at Liverpool. He joined the academic staff there in 1974, and it was his professional base for the rest of his life. A still rising but already leading figure in UK peptide chemistry, he had contributed the chapter on peptide synthesis to every volume of these specialist reports since the eleventh, and would have done so again for the twentieth. He had also recently been elected Secretary-Treasurer of the Peptide and Protein Group of the Royal Society of Chemistry and Biochemical Society. He will therefore be greatly missed, not only in his own country but also abroad. Many of our readers will have met him at European or American Peptide Symposia, and will remember him as a convivial conference companion as well as an outstanding peptide chemist. He listed hill-walking as one of his main relaxations: he met his death as the result of sudden bad weather on an expedition in Wales with a colleague on 8 February 1988, aged only 41.

Balliol College, Oxford *John Jones*

August 1988

Contents

Abbreviations

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

1
Amino Acids

BY G. C. BARRETT

1 Introduction

The occurrence, chemistry and analysis of amino acids contained in the literature of 1987 are reviewed in this Chapter, which is arranged in the sections as used in all previous Volumes in this Specialist Periodical Report.

Access to the literature for creating this Chapter has been by way of Chemical Abstracts (to Volume 108, issue 9) and Biological Abstracts (to issue 8 of Volume 85), supplemented by scanning a selection of major journals so as to cover the 1987 literature adequately. The abstracts coverage also nets a few citations published in 1986, and these are included to give continuity for the topic over the years.

2 Textbooks and Reviews

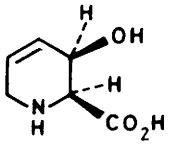
Uses of amino acids and simple derivatives in synthesis are surveyed in recent texts.[1,2] Reviews of every conceivable amino acid with a sulphur functional group in the side chain comprise a complete Volume of Methods in Enzymology.[3] A Symposium has covered roles of amino acids in various disorders.[4] A similarly thorough treatment has been given to the biosynthesis of protein amino acids.[5]

Other textbooks and reviews are located in the relevant sections of this Chapter.

3 Naturally Occurring Amino Acids

3.1 Occurrence of Known Amino Acids.- Amino acids in the Murchison meteorite show unusually high abundance of ^{2}H,[6] ^{15}N,[6] and ^{13}C,[7] giving further evidence for extraterrestrial origins for these amino acids (as opposed to contamination of the meteorite after arrival).

Amino acids and peptides in algae have been reviewed.[8] Bacterial sources of less common amino acids as protein constituents (phycobiliproteins[9,10]) include Mastigocladus laminosus and Calothrix (γ-N-methyl asparagine),[9] and Chromatium



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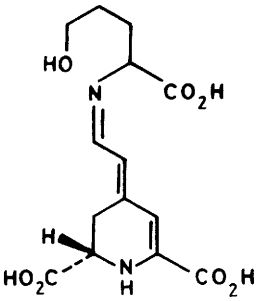

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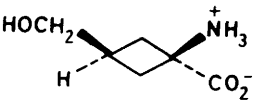

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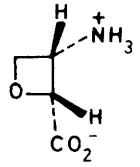

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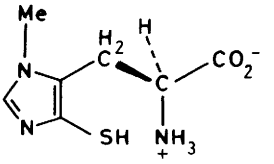

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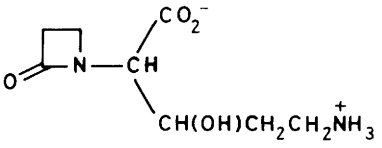

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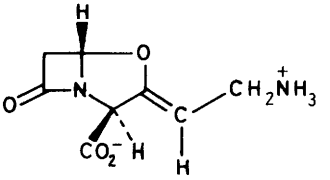

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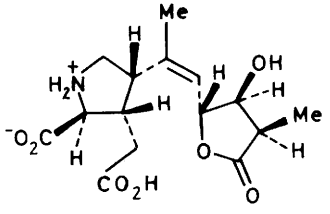

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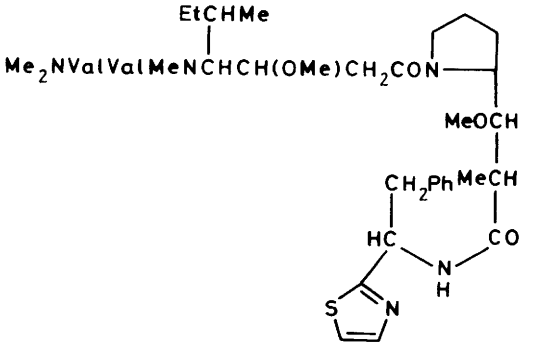

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vinosum (N^{ε},N^{ε}-dimethyl lysine).[11] p-Aminophenylalanine occurs in Vigna, as a growth inhibitor of Escherichia coli.[12]

Plant sources include tulip, with 4-methyleneglutamine identified as one constituent of its leaves.[13] Trees of the Copaifera genus, whose leaves contain N-methyl trans-4-hydroxyproline in substantial amounts (up to 3% dry weight and representing 10% of the nitrogen content), are thought to be protected from bruchid beetle larval attack by this amino acid.[14] The same amino acid occurs in Melaleuca, together with the corresponding betaine.[15]

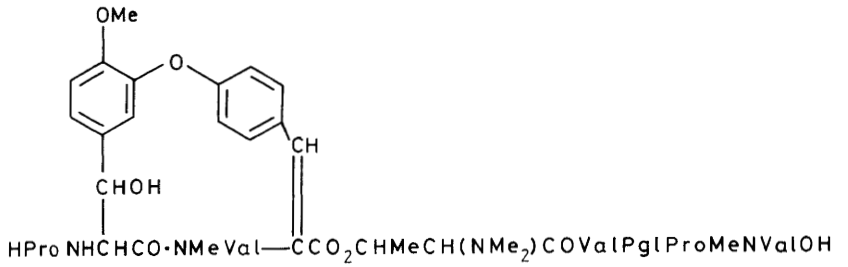
The free D-alanine content of bivalves is surprisingly high, frequently far exceeding that of its L-enantiomer. Several bivalve species also contain D-aspartic acid in concentrations approaching that of the L-isomer.[16] The absence of D-valine[16] in these animals must have significance that has not yet been a source of speculation.

The accumulation of D-arginine in rat liver mitochondria has been reported.[17] Another notable occurrence is of β-hydroxyasparagine (not previously known as a protein constituent), and β-hydroxyaspartic acid, in Vitamin K-dependent proteins[18] and in bovine low – density lipoprotein receptor and bovine thrombomodulin.[19] The introduction of isodityrosine crosslinking residues in extensin resulting from oxidative coupling in vivo cannot be repeated in vitro using H_2O_2 and peroxidase, which introduces dityrosine crosslinks instead into this protein.[20] N^{π}-Methyl histidine has been identified for the first time as a protein constituent (in rabbit skeletal-muscle light-chain kinase).[21]

3.2 New Natural Amino Acids.- The presence of L-2-amino-4-chloropent-4-enoic acid in fruit bodies of Amanita pseudoporphyria Hongo accounts for antibacterial properties of this fungus;[22] Amanita abrupta contains (2S,4Z)-2-amino-5-chloro-6-hydroxy-4-hexenoic acid (as well as three other unusual but previously known amino acids).[23] (2S,3R)-(-)-3-Hydroxybaikiain (1) is a constituent of the toxic mushroom Russula subnigricans Hongo.[24]

Fruits of Rivina humilis contain the new betalain (2), a 5-hydroxy-norvaline derivative.[25] Smaller ring moieties appear in cis-1-amino-3-hydroxymethyl-cyclobutane-1-carboxylic acid (3), found in Atelia herbert smithii Pittier (Sophoreae, Leguminoseae),[26] and (2R,3S)-oxetin (4).[27,28] L-Ovothiol A and its N,N-dimethyl analogue (L-ovothiol B) have been proved to possess structure (5)by virtue of its synthesis from (12).[29] The authors suggest that previously described marine 3-mercaptohistidines should be revised to the 1-methyl structures.[29] The novel hydroxyornithine derivative proclavaminic acid (6) from the mycelium of the clavulanic acid-producing organism Streptomyces clavuligerus ATCC 27064 undergoes enzymatic cyclization in cell-free extracts to clavaminic acid (7).[30]

A further example of the well populated class of natural N-carboxyalkyl amino



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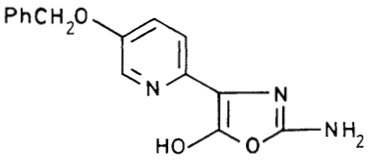

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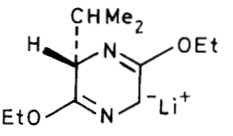

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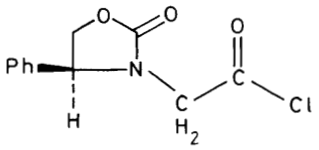

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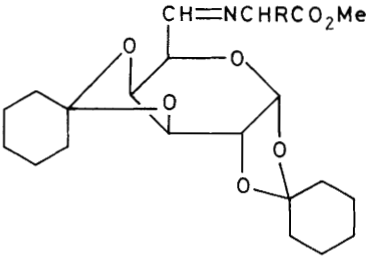

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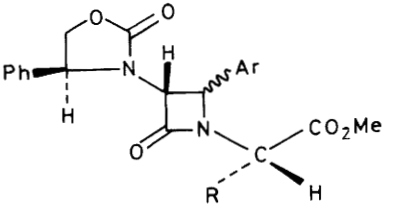

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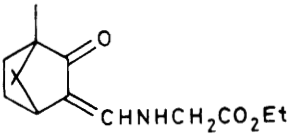
(i) NaOH(aq) | (iii) Birch reduction
(ii) H_2/Pd | (iv) hydrolysis



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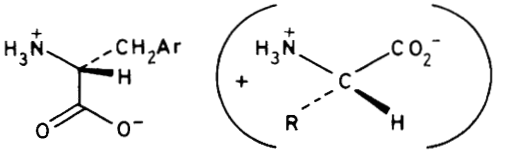


acids is (2S,7S)-N^6-(1-carboxyethyl)ornithine, from Streptococcus lactis.[31]

More complete information is now available[32] on new amino acids from the red alga Chondria armata, previously noted in this Specialist Periodical Report (Vol.19, p.2) to contain seven new amino acids. However, only domoilactone A (8; a correction of the structure given in Vol.19, p.2) and domiolactone B (epimer of A at the chiral centre carrying the hydroxy group) have been described so far.[31,32]

3.3 New Amino Acids from Hydrolysates. - The earlier section (3.1) included reports on the occurrence of known amino acids as noteworthy protein constituents. This section develops the same topic but with new and therefore even more noteworthy analogues.

Bovine ligament elastin contains a cross-linking amino acid related to isodesmosine, insofar as it is a pyridinium compound, but it also carries a C=C bond in an extra side chain.[33] It has been christened "pentasine", as it is derived from the condensation of five lysine residues.

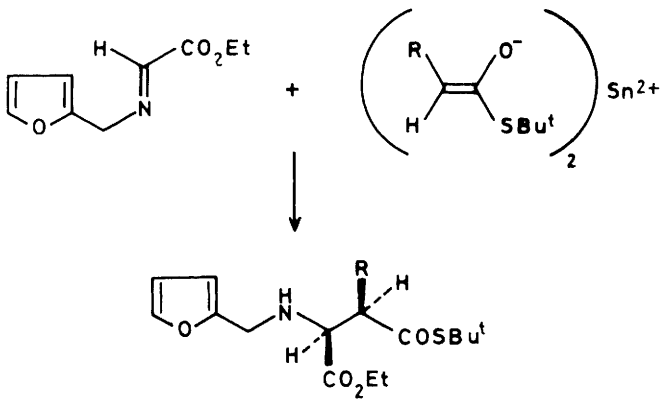
Fifteen years' work leads to the structure assigned (without stereochemical details) to dolastatin 10 (9).[34] It is a "pentapeptide" from the sea hare Dolabella auricularia and is the most potent antineoplastic compound known, containing four amino acids not previously known in Nature.[34] A corrected abstract has been published[35] for an unusual peptide from a strain of Streptomyces AM-2504 that contains a β-hydroxydopa derivative (mild hydrolysis of AM-2504 gives (10) and an amino acid of relative molecular mass 141).

The vapour-phase hydrolysis regime for peptides and proteins, employing 7M hydrochloric acid containing 10% trifluoroacetic acid at 150° during 22 - 45 minutes, will be of considerable interest.[36]

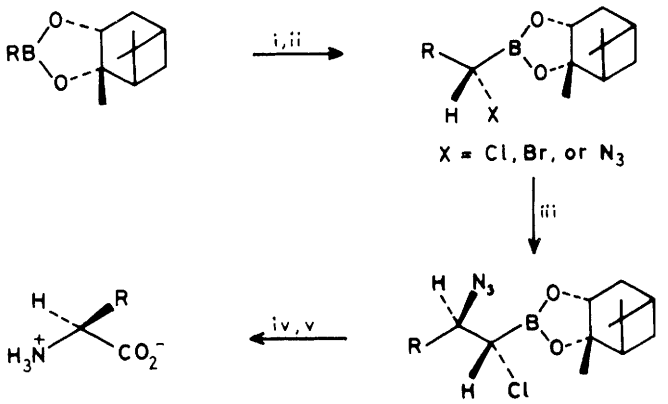
4 Chemical Synthesis and Resolution of Amino Acids

4.1 General Methods. - A broad review of amino acid synthesis [37] and a more limited coverage of contributions to synthesis of unusual natural amino acids [38] have appeared. Formation of N-acylamino acids by the amidocarbonylation of aldehydes (CO/amide/homogeneous mixed-metal catalyst systems)[39] has been extensively reviewed.[40]

This section is divided into applications, either of well established or of lesser-known general methods. Later sections include several examples of variations of standard general methods. In the former category, the Bucherer-Bergs synthesis has been used for the synthesis of α-(5-hydroxy-2-pyridyl)glycine starting from (5-benzyloxy)pyridine-2-carboxaldehyde, though with the unexpected intermediacy of the oxazole (11),[42] rather than the usual hydantoin. Treatment of (11) with 2M sodium hydroxide at 120° during 8 hours gave the required product. Other hydantoin-based syntheses include carboxylation of α-aminonitriles

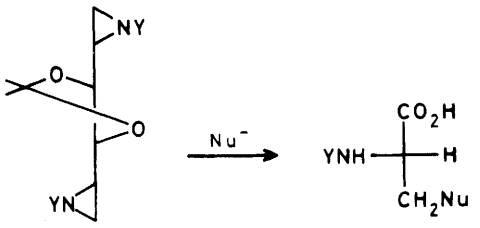


Scheme 1



Reagents: i, $LiCHCl_2$ or $LiCHBr_2$; ii, NaN_3; iii, $LiCHCl_2$; iv, $NaClO_2$; v, H_2/Pt or Pd

Scheme 2



(17) (18)

(via carbamate, oxazolidinone, and α-isocyanato-acid amide).[42]

The Strecker synthesis has been applied to the preparation of α-hydroxymethyl-α-(3,4-disubstituted phenyl)glycines.[43] The acetamidomalonate synthesis continues to be widely used in all its variations, e.g. for 3-(3-pyridyl)- and 3-(3-benzo[b]thienyl)-D-alanines (including enzymic resolution),[44] and for 3-(2-carboxy-4-pyridyl)- and 3-(6-carboxy-3-pyridyl)alanines.[45]

Alkylation of glycine derivatives has also become widely used in its many variants. The predominance of the route based on alkylation of glycine Schiff bases continues, to which novel routes occasionally arise (e.g. to t-butyl *N*-(t-butyloxycarbonyl)iminoacetate from $BocNHCHBrCO_2{}^tBu$, this being alkylated with either a Grignard reagent, or an enamine, or morpholinostyrene[46]). 6-Fluorodopa has been prepared from veratric acid (via the derived benzylic bromide) and $Ph_2C{=}NCH_2CO_2Et$.[47] Similar alkylations of *N*,*N*-dibenzylglycine esters,[48] methyl nitro-acetate,[49] and reduction of α-diazo-acetoacetates $RCOC(N_2)CO_2Et$ with H_2/Pd in acetic acid to give α-acetamidoacetoacetates [50] further illustrate applications of established methods.

Tin(II) enolates of thiolesters undergo diastereoselective addition to *N*-furfuryl imines (Scheme 1).[51] Another example of a novel route that might develop into a useful general method involves photochemical addition of N_3CO_2Et to silylenols $R^1R^2C{=}C(OR^3)OSiMe_3$, giving *N*-(ethoxycarbonyl)amino acid esters in 45 - 75% yields.[52]

4.2 Asymmetric Synthesis.- Some of the general methods in the amino acid field offer useful enantiospecific synthesis opportunities in the α-amino acid area. The later Section 4.17 deals both with general methods and asymmetric synthesis of β- and higher homologous amino acids. The requirement for enantiomerically pure α-amino acids in biological and synthetic studies has sustained the development of stereoselective synthetic methods, frequently feeding back improvements into established general methods.

The "bis-lactim ether" method, introduced by Schöllkopf and his co-workers, continues to be used profitably for asymmetric synthesis of α-amino acids. Typically, L-Ovothiol A (5) and its *N*,*N*-dimethyl analogue (L-ovothiol B) have been prepared by this method, from (12).[29] The method involves alkylation of the chiral carbanion of (12), commonly using an alkyl halide as electrophile[29] [as in a synthesis of enantiomers of phosphinothricin, $MeP(O)(OH)CH_2CH_2CH(NH_3{}^+)CO_2{}^-$[53]]. However, Michael additions (better than 99% stereoselectivity with methyl 2-alkenoates[54]), synthesis of α-amino-γ-nitroalkanoates using nitro-alkenes,[55] and "non-Michael additions" [bis-lactim ether (12) - $Ti(NEt_2)_3$ derivatives add diastereoselectively to $R^2CH{=}CR^1CHO$ to give (2*R*,3*S*)-$R^2CH{=}CR^1CH(OH)CH(NH_3{}^+)CO_2{}^-$[56]] have been illustrated. Alkylation by oxiranes[57] has been used for synthesis of 2-methoxyethoxymethyl-protected (*R*)-homoserine methyl esters. α-Methylated cyclic amino acids have been synthesised

by this method.[58]

The bis-lactim ether method is a "hidden" form of Schiff base alkylation, a widely used approach with a longer history. This is represented in the recent literature with alkylations of glycine chiral Schiff bases, giving proline homologues through alkylation with $I(CH_2)_nHal$,[59] or α-alkyl-α-amino acids using the D-galactodi-aldehyde imines (13) with from 23 to >95% asymmetric induction.[60] The "Evans - Sjoegren" ketene derived from the glycyl chloride (14) gives β-lactams by cycloaddition to benzylidene-amino acids.[61] On alkaline hydrolysis and hydrogenolysis (H_2/Pd), the adduct (15) gives optically pure amino acids. Michael addition of the (S)-(o)-[(N-benzylprolyl)amino]benzophenone-derived Schiff base of glycine to acrylates gives L-glutamic acid (and acrolein gives L-proline) after cleavage using aqueous hydrochloric acid, with almost complete diastereoselection.[62] A related method uses a chiral alkylating agent, e.g. alkyl sulphates of D-glucose (0-76% enantioselection).[63]

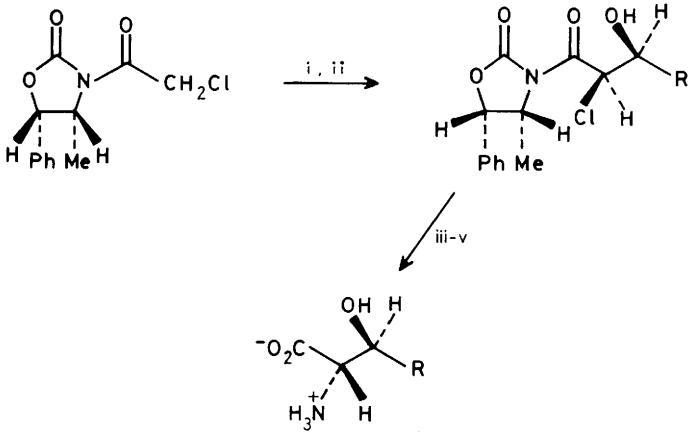
Alkylation of the imine (16) from 3-hydroxymethylenecamphor and ethyl glycinate with alkyl chlorides after carbanion formation with lithium di-isopropylamide gives only low to moderate enantiomer excesses; better diastereoselectivity is seen for sarcosine analogues.[64]

The Schiff base from β-D-galactopyranosylamine is a source of D-amino acids through diastereoselectivity of Strecker reactions based on it.[65] Aldehydes react with the tetra-O-pivaloyl derivative catalyzed by Me_3SiCN and $ZnCl_2$ or $SnCl_4$ to give diastereoisomer mixtures favouring the R-epimer by 7-13:1.[65]

Introduction of the nitrogen function diastereoselectively is a feature of well established aminolysis procedures. Serine β-lactone prepared from protected serines by the Mitsunobu method reacts with organometallic reagents, R_2CuLi or $R_2Cu(CN)Li_2$, to give optically pure β-hydroxyamino acids resulting from alkylation of the original serine methylene group.[66] Lesser enantioselectivity is found for the lactone derived from N-benzylserine; conversely, optical purity better than 99.4% is achieved through copper(I)-catalyzed Grignard alkylation.[66] Reductive aminolysis of oxazolinones with (S)-(-)-phenylethylamine and $H_2/PdCl_2$ gives corresponding (S)-amino acid derivatives.[67] Azidolysis of (S)-1-halogenoalkylboronates of (S)-pinanediol is followed by generation of the carboxy group in the target L-amino acids by a novel method involving insertion of a chloromethylene group through reaction with $LiCHCl_2$ (Scheme 2).[68]

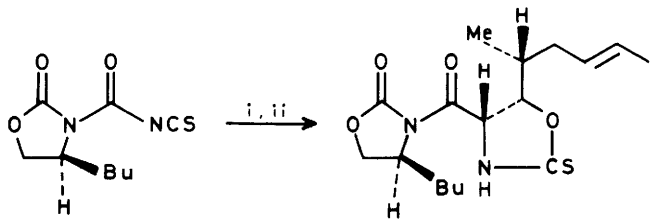
Chiral bis-aziridines obtained from D-mannitol can undergo nucleophilic ring-opening in one of two ways; it has been found that the pathway leading to (S)-amino acids is followed [(17)→(18)] when R_2CuLi is used.[69]

Alkylation of chiral oxazolidinones or imidazolinones has been developed further from its initial exploration as a route to α-amino-β-hydroxy acids.[70-73] The hydroxy group originates in alkylation by a carbonyl compound - i.e. a diastereoselective aldol condensation in its original form[70] - and complete stereoselectivity leading to the anti-relationship of the hydroxy and amino groups



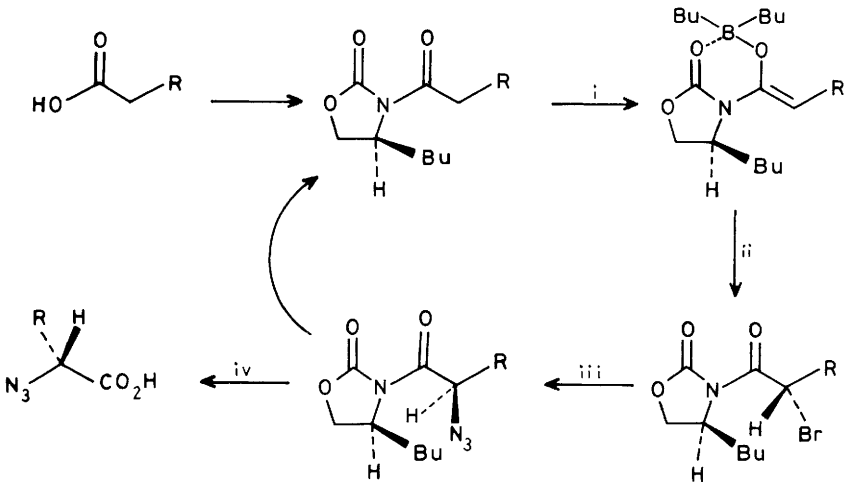
Reagents: i, Bu_2BOTf/NEt_3; ii, RCHO; iii, $NaN_3/DMSO$; iv, OH^-; v, $H_2/Pd—C$

Scheme 3



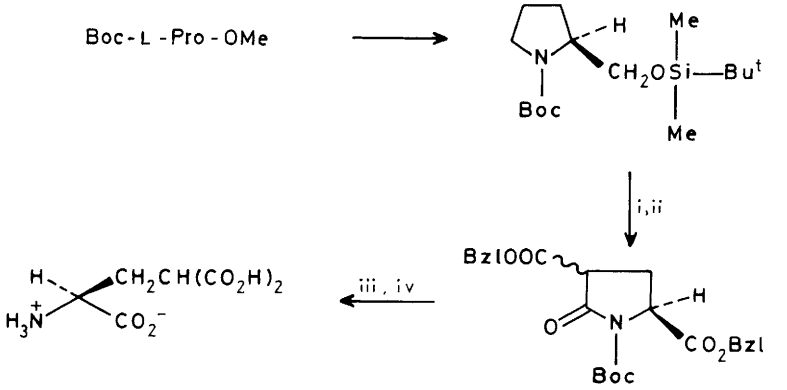
Reagents: i, $Sn(OTf)_2$; ii (R)—OHC—$CHMeCH_2CH$=$CHMe$

Scheme 4



Reagents: i, Bu_2BOTf, R_3N; ii, NBS; iii, NaN_3; iv, LiOH

Scheme 5



Reagents: i, RuO_4; ii, base, ZCl; iii, H_3O^+; iv, H_2/Pd

Scheme 6

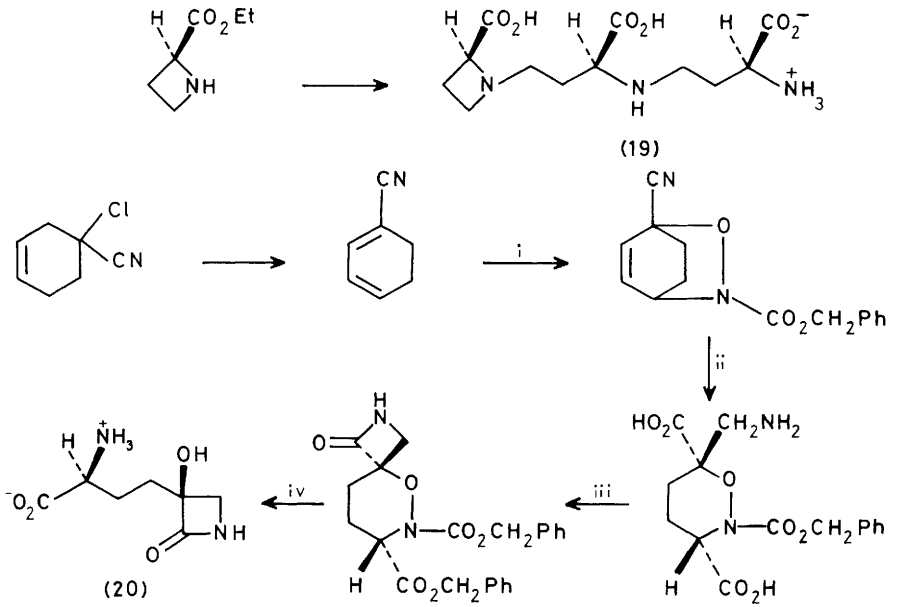
calls for a longer route (Scheme 3).[71] This variation has been used[72] in alternative approaches to the synthesis of unusual constituents of the peptide Echinocandin D (aldolization of the starting material in Scheme 4 with (R)-2-methyl-4-hexenal and tin(II) triflate).[72] [Both these amino acids were synthesized by Ohfune in 1986 (Vol.19, p.10).] Chiral N-acyloxazolidinones, after conversion into dibutyl boron enolates, undergo successive diastereoselective bromination (N-bromosuccinimide) and azidolysis, offering a general asymmetric synthesis (e.e. better than 98%) suitable for multifunctional amino acids (Scheme 5) and involving recovery of the chiral auxiliary.[73]

There is a regular supply of papers describing asymmetric hydrogenation of acetamidocinnamic acid (82-93% enantiomeric excess using chiral Rh or Re complexes as homogeneous catalysts)[74] and its close relatives.[75] Regular hydrogenation systems lead to rather low enantiomeric excesses when operating on unsaturated amino acid (S)-phenylethylamides [5-cyano-2-(hydroxyimino)valeric acid gives the D-lysine derivative in 6-12% diastereoisomeric excess with H_2/Pt[76]] or on 2-trifluoromethyl-4-alkylidene oxazolinones (H_2/Pt/(S)-phenylethylamine).[77]

A brief reference to enzyme-catalyzed syntheses of L-amino acids is usually located in this Chapter in the later section covering protein amino acids, but unusual enzymic methods are found a place here. The availability of relatively large quantities of cloned E.coli aspartate transaminase for mediating the conversion of α-keto-acids into corresponding α-amino acids offers a practical route to a wide range of aliphatic and aromatic side chains. The essential role of large relative amounts of enzyme in these asymmetric syntheses and use of aspartic or glutamic acids as nitrogen source are notable aspects of this unselective application of an enzyme.[78] Dimethyl meso-N-benzylpyrrolidine-2,5-dicarboxylate gives N-benzyl-D-proline methyl ester through selective hydrolysis catalyzed by pig liver esterase, followed by radical decarboxylation of the N-hydroxypyridine-2-thione ester.[79]

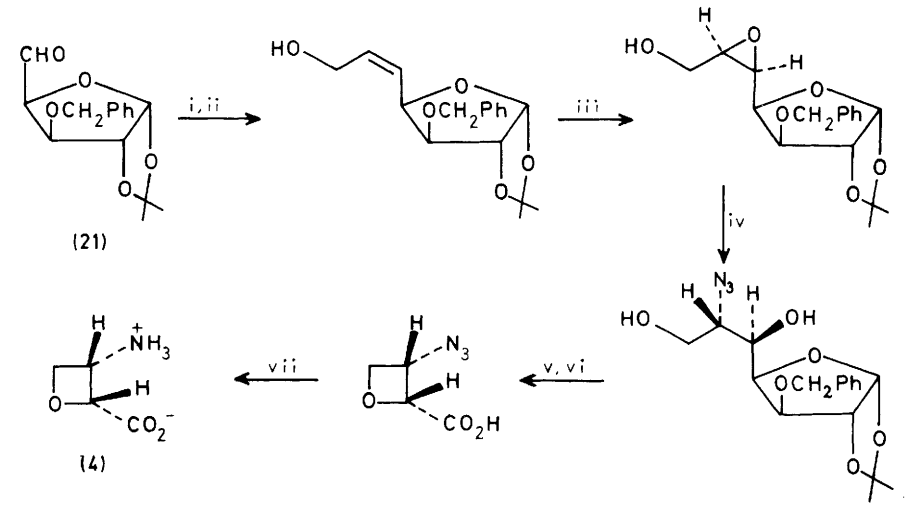
4.3 Synthesis of Protein Amino Acids and Other Naturally Occurring α-Amino Acids. - The protein amino acids feature incidentally in exploration and development of new synthetic methods (see preceding sections). Many of the reactions are covered under Section 6.3 'Specific reactions of amino acids' with side-chain modifications, and often amount to the synthesis of one protein amino acid from another. Having drawn attention to these other locations, the main interest as far as this Section is concerned lies in developments in enzymatic and bacterial methods applicable to large-scale production of protein amino acids. However, limitations of space permit only representative citations of these papers (readers are directed inter alia to Section 16 'Fermentation and Bio-industrial Chemistry' in Chemical Abstracts for more complete access to this literature). Biosynthetic studies for protein amino acids are otherwise excluded.

Several reviews[80] have appeared, dealing with low-cost enzymatic synthesis of



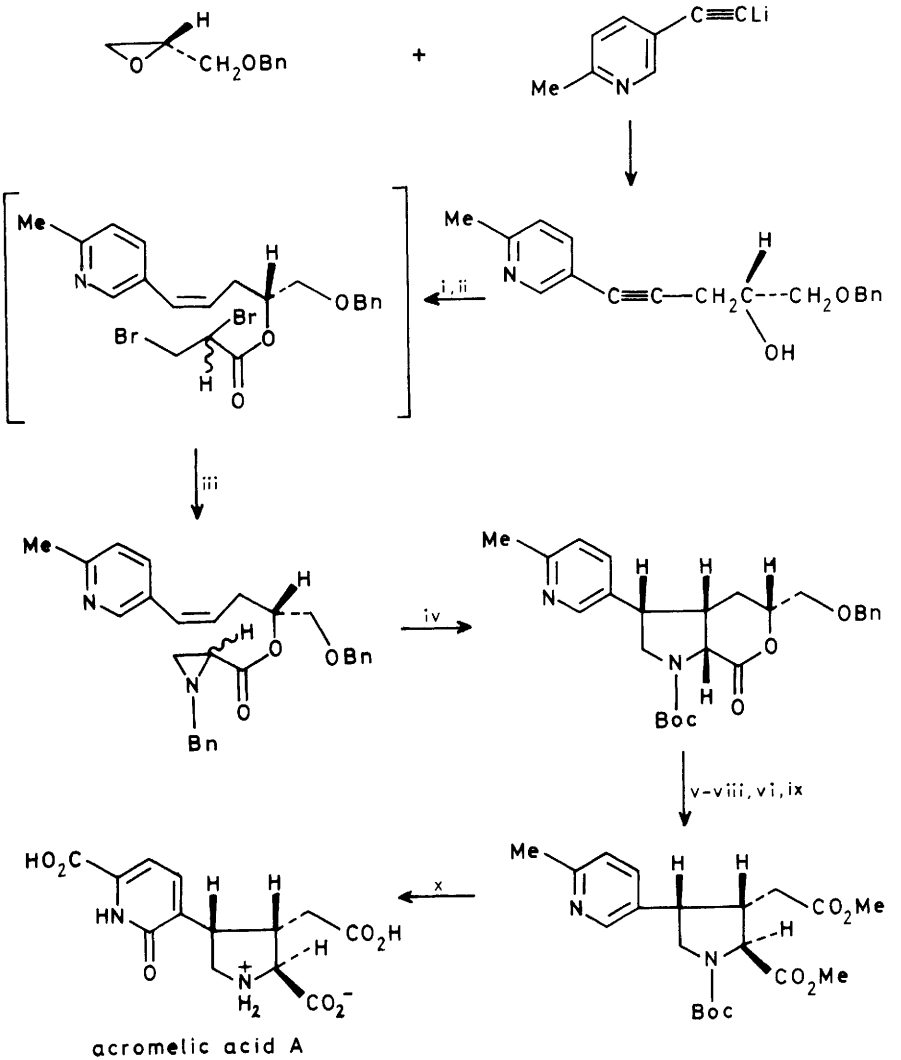
Reagents: i, $PhCH_2OCONO$; ii, [H] and [O]; iii, ZCl, then $Ph_3P/(pyS)_2$; iv, H_2O, H_2/Pd

Scheme 7



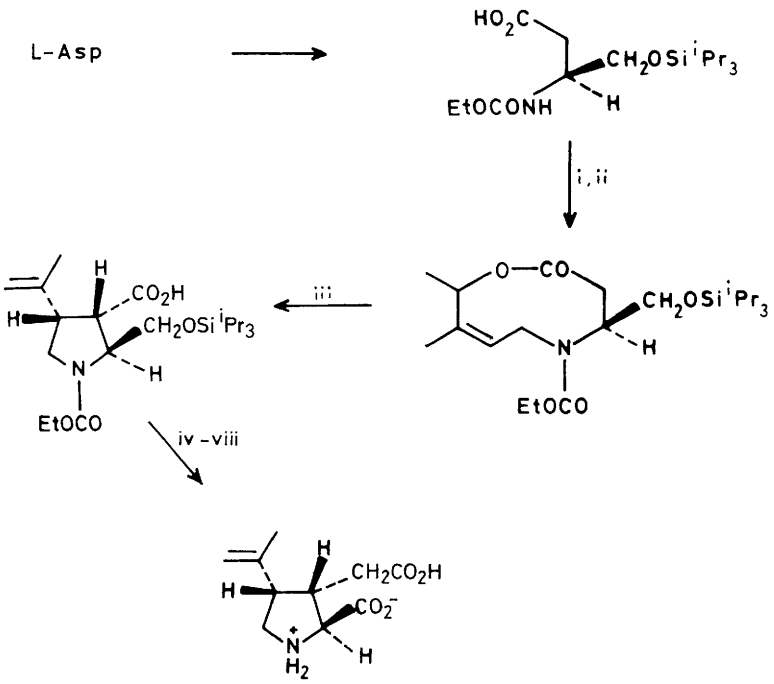
Reagents: i, $Ph_3P{=}CHCO_2Et$; ii, DIBAL/*cis*-ester; iii, MCPBA; iv, NaN_3; v, TosCl; vi, $KOBu^t$; vii, [H]

Scheme 8



Reagents: i, H_2/Lindlar catalyst; ii, $BrCH_2CHBrCOCl$; iii, $PhCH_2NH_2$; iv, 200 °C; v, H_2/Pd—C; vi, $(Boc)_2O$; vii, $NaIO_4$ then $KMnO_4$; viii, Conc. H_2SO_4 (trace), MeOH; ix, NaH; x, established procedures

Scheme 9



Reagents: i, $Pr^i_3SiOCH_2CMe{=}CHCl$ / BuLi; ii, pyridinium toluene-*p*-sulphonate, then 2-chloro-1-methylpyridinium iodide; iii, LDA / Bu^tMe_2SiCl, K_2CO_3; iv, $(COCl)_2$, then CH_2N_2, then $PhCO_2Ag$; v, 40% aq. HF / 20°C; vi, CrO_3 / acetone; vii, Me_3SiI; viii, aq. KOH, then neutralize

Scheme 10

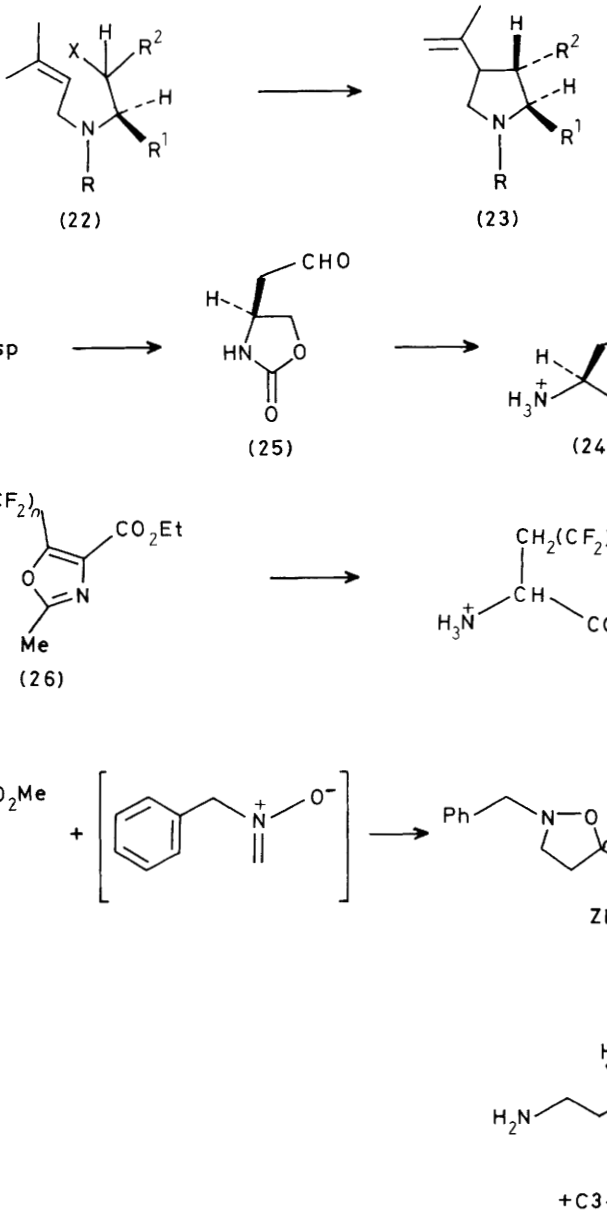
L-amino acids from racemic raw materials. A Volume of Methods in Enzymology is assigned to applications of immobilized aminotransferases in amino acid production.[91] The specific topic of conversion of 5-substituted DL-hydantoins into corresponding L-amino acids using soil bacteria[92] or into D-amino acids using Pseudomonas AJ[93] continues to be studied very thoroughly. Papers covering enzyme reactor technology for production of L-phenylalanine from DL-phenyl-lactic acid,[94] conversion of DL-2-oxo-oxazolidine-4-carboxylic acid into L-serine by Pseudomonas testosteronii[95] and of ethyl α-acetamido-acetoacetate into a mixture of N-acetyl-D-threonine and N-acetyl-L-allothreonine ethyl esters using Saccharomyces rouxii,[96] and a proposed α-proton abstraction mechanism for the biosynthesis of L-azetidinecarboxylic acid from S-adenosylmethionine in Actinoplanes ferrogineus[97] are representative of this topic area.

Syntheses of serine and γ-carboxy-L-glutamic acid, by cathodic reduction of methyl (hydroxyimino)malonamate[98] and by RuO_4 oxidation of a protected L-prolinol (Scheme 6),[99] illustrate specific (non-general) routes to protein amino acids. In the wider context of non-protein natural α-amino acids, continuing interest in canavanine [2-amino-4-(guanidinoxy)butyric acid] is represented in quantitative synthesis from cyanamide and copper(II) canaline in the presence of zinc(II) salts.[90]

The more complex natural products located in this Section all happen to be saturated heterocyclic compounds. Analogues of nicotianamine (19) have been synthesized by condensation of (S)-2-ethoxycarbonylazetidine with ethyl (S)-4-oxo-2-(trifluoroacetylamino)butanoate or ethyl 4-oxobutanoate.[91] A synthesis of tabtoxinine β-lactam ((20) in Scheme 7) in which a nitrone cycloaddition is a key step has been published.[92] Continuing with four-membered ring heterocycles, synthesis of (2R,3S)-oxetin (4) from D-glucose via the derived pyranosylaldehyde ((21) in Scheme 8) has been extended to all 3 stereoisomers.[27] α-D-Glucuronolactone serves as starting point for syntheses of (2R,3R,4R)-2,3-dihydroxyproline and (2R,3R,4R,5R)-3,4,5-trihydroxypipecolic acid and its 2S-epimer using routes briefly described in last year's report (Vol.19, p.10).[93] A related synthesis from the same group is described later (ref.131).

Efficient routes to acromelic acids A and B have been described,[94,95] that by Matsumoto's group[94] confirming structural and stereochemical details (see also Vol.19, p.10). An enantioselective route[95] that is more straightforward than Matsumoto's[94] (which requires L-α-kainic acid as starting material) leading to acromelic acid A is shown in Scheme 9.

A new enantiospecific route (Scheme 10) to (-)-α-kainic acid[96] uses a Claisen enolate rearrangement that achieves the correct stereochemistry at all three chiral centres. This depends of course on chirality already established in the compound subjected to this rearrangement, and this is built into the L-aspartic acid used as starting material. In a related approach, optically pure kainoids with the required (2S,3S)-stereochemistry have been made available through



Reagents: i, LiOH; ii, H_2/Pearlman's catalyst [$Pd(OH)_2$/C]

Scheme 11

cyclization of N-alken-2-yl-L-amino acids in which the carboxy group is first elaborated into a haloalkyl group (22)→(23).[97] Chlorocobaloxime(I) formed in situ is used as the cyclization agent in this route.

4.4 α-Alkyl Analogues of Protein Amino Acids.- Some of the standard general methods are appropriate for satisfying the need for potential enzyme inhibitors of this general class. For example, α-substituted serines were prepared for study as irreversible inhibitors of serine hydroxymethyl transferase through the acetamidomalonate route or through acetoxymethylation of methyl 2-(benzylideneamino)but-2-enoate to give α-vinylserine.[98] Further examples of the latter approach are Michael additions to N-alkylidene alanine and its homologues [99] and preparation of (S)-α-alkylaspartic acids by alkylation by ethyl α-bromoacetate of chiral enolates of oxazolidinones formed from Schiff bases and benzoyl chloride.[100] α-Methylphenylalanine is easily prepared from N-benzylidene-alanine methyl ester by C-benzylation.[101] An example of many applications of the Strecker synthesis to an interesting variation of the present purpose uses a ketone (R^1COR^2), KCN, NH_4OH, and H_2S to give α-amino acid thioamides ($NH_2CR^1R^2CSNH_2$).[102]

A related method, formation of α-spirocyclopropyl amino acids by addition of diazomethane to an activated αβ-unsaturated α-amino acid, has been developed further.[103] The nitrogen atom within the ring of 3-dimethylamino-2,2-dimethyl-2H-azirine is readily acylated by a carboxylic acid accompanied by ring-opening to give N-acyl-α-aminoisobutyroyl dimethylamides, a route that in principle can be extended to any αα-disubstituted α-amino acid.[104]

A review has appeared covering syntheses and uses in peptide synthesis of α-amino-isobutyric acid.[105]

4.5 Models for Prebiotic Synthesis of Amino Acids.- Amino acids continue to be discovered in reaction mixtures that will be familiar to readers of this Chapter over the years. There is still scope for novel variations, as illustrated by the formation of amino acids and amines in aqueous aliphatic carboxylic acid solutions into which an argon-nitrogen plasma is passed.[106] The authors note that this actually represents a new type of catalyst-free nitrogen fixation. Atomic carbon generated in the presence of water and ammonia at 77K leads to glycine, sarcosine, α- and β-alanine, aspartic acid, and serine.[107] This is surmised to account for extra-terrestrial generation of amino acids found in meteorites (in the absence of any other explanation).

Laser irradiation of aqueous ammonium acrylate at 266nm generates mainly α- and β-alanine.[108] Less energetic irradiation (xenon lamp) of solutions of ammonium glycollate containing suspended particulate CdS yields glycine and methylamine.[109]

(Imidazol-4-yl)acetaldehyde and the corresponding glycol are generated in solutions of erythrose, formaldehyde, and ammonia, suggesting a likely prebiotic route to histidine via Strecker reactions.[110] The absence of some simple amino

acids is noticeable when surveying all the experiments of these types. This has been discussed in the context of a hypothesis to the effect that the development of the genetic code and the prebiotic generation of amino acids occurred concurrently.[111]

Further reviews of the topic of this Section have appeared.[112]

4.6 Aliphatic α-Amino Acids.- Sections that follow directly after this cover amino acids for which additional interest resides in side-chain functional groups. This Section serves to collect aliphatic amino acids deserving mention but which are not catered for elsewhere.

After numerous conference papers describing its uses, details of the preparation of 2-amino-4,4-dimethylpentanoic acid (neopentylglycine) have appeared.[113]

Diels-Alder addition of cyclopentadiene to N-acyl dehydroalanine esters gives mixtures of stereoisomers of the expected alicyclic amino acids based on bicyclohept[2.2.1]ene.[114]

Conversion of di-N$^{\alpha}$N$^{\varepsilon}$-t-butoxycarbonyl-L-lysine 2,2,2-trichloroethyl ester into di-N$^{\alpha}$N$^{\varepsilon}$-t-butoxycarbonyl-L-homoglutamine can be achieved by RuO_4 oxidation.[115]

Considerable interest will be generated by the finding that incorporation of the acetal (24) as a histidine replacement in biologically active peptides can give useful analogues. It has been synthesized from L-aspartic acid via its semi-aldehyde derivative (25), formed by Swern oxidation of the corresponding 2-amino alkanol,[116] and the same intermediate has been used in a synthesis of the equivalent dithioacetal and extended through routine steps leading to a novel statine analogue (see Section 4.17).[117]

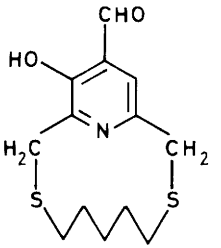
4.7 Alkoxy-α-amino Acids.- Further studies of electrochemical α-methoxylation have concentrated on N-acetyl 4-hydroxyproline esters.[118]

γ-Alkoxy-α-aminonitriles are acceptable substrates for *Brevibacterium* sp.R312 and undergo hydrolysis to the corresponding L-amino acids.[119]

4.8 Halogenoalkyl α-Amino Acids.- β-Fluoroalkyl α-amino acids have been reviewed.[120]

In studies of enzymic amination by 3-methylaspartate ammonia lyase, chloro- and bromofumaric acids were converted into (2R,3S)-3-halogenoaspartates.[121] This implies that enzymic amination of the natural substrate (mesaconic acid) involves re-attack at C2. Amination by ammonia is the final step in a synthesis of DL-hexafluorovaline benzyl ester from benzyl bromoacetate, converted by Ph_3P and hexafluoroacetone into $(CF_3)_2C{=}CHCH_2CO_2CH_2Ph$.[122]

Acylation of ethyl azidoacetate by $CHF_2(CF_2)_nCOCl$ and photocyclization gives the oxazole (26), from which the 2-aminofluoroalkanoic acid is obtained by catalytic hydrogenation and acid hydrolysis.[123]



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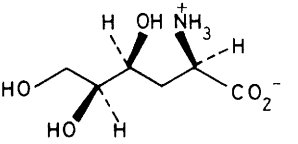

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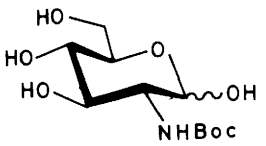

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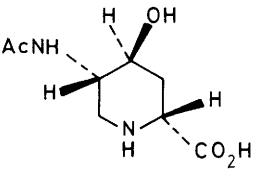

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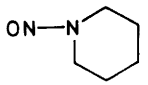

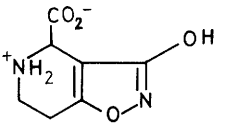

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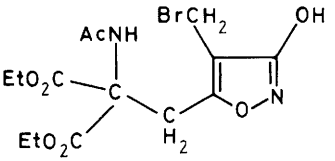

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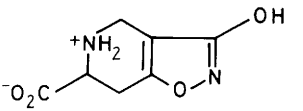

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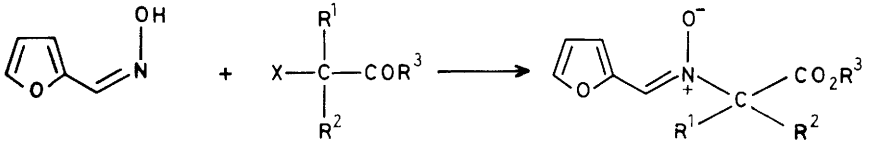

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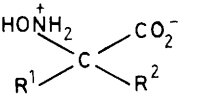

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4.9 Hydroxyalkyl α-Amino Acids.- This class can be divided into acyclic and alicyclic types. Some examples[70-73] have been covered in an earlier section to illustrate asymmetric synthesis.

Electrochemical reduction of L-asparagine[124] and of pyroglutamic acid (pyrrolidone-5-carboxylic acid)[125] yields L-homoserine and (S)-2-amino-5-hydroxypentanoic acid, respectively. A 1.7:1 mixture of allo-L-threonine and L-threonine in 88 and 74% enantiomeric yields, respectively, is obtained through a biomimetic aldol condensation of acetaldehyde with the zinc(II) chelate of the Schiff base of glycine with the pyridoxal-like pyridinophane (27).[126] (3R)- and (3S)-Hydroxy-2S-arginines have been prepared from the corresponding ornithines [these are formed by 1,3-dipolar cycloaddition of the nitrone from $PhCH_2NHOH$ and formaldehyde to methyl N-benzyloxcarbonyl (S)-vinylglycine methyl ester (Scheme 11)].[127]

Established routes to the four ɣ-hydroxyisoleucine diastereoisomers, through photochlorination of L-isoleucine and D-alloisoleucine followed by hydrolysis, have been surveyed.[128] (4,5,6)-Trihydroxynorleucines ((28), and its enantiomer and C-5 epimer) have been prepared[129] from 5,6-O-benzylidene L-ascorbic acid through steps that are, by now, becoming routine and well understood. This is because of an absorption of knowledge and confidence by organic chemists in manipulating simple monosaccharides, illustrated in routes from N-t-butoxycarbonyl-D-glucosamine (29) to (2R,4S,5S)-5-acetamido-4-hydroxypipecolic acid (30),[130] and from D-glucuronolactone via amination of (4S,5S)-5,6-dihydroxyhex-2-en-4-olide en route to (2S,4S,5S)-dihydroxypipecolic acid and bulgecine (see also refs. 93, 337).[131]

Enzymic transamination (immobilized glutamic oxaloacetic aminotransferase) effects the conversion of ɣ-hydroxy-α-ketoglutaric acid to ɣ-hydroxy-L-glutamic acid. An interesting explanation for the success of this otherwise sluggish conversion lies in the choice of the amination agent, cysteinesulphinic acid, which generates a driving force for the reaction through the instability of the corresponding keto-acid that readily breaks down into SO_2 and pyruvic acid.[132]

4.10 α-Amino Acids with Unsaturated Side Chains.- α-Azidocinnamates, formed by condensation of ethyl azidoacetate with aromatic aldehydes, yield N-carboxy anhydrides of dehydro-phenylalanine and tyrosine through reaction with phosgene.[133] This general process has been extended[134] to ornithine and lysine analogues through Wittig-type condensation of N-benzyloxycarbonylamino aldehydes with methyl N-benzyloxycarbonyl α-diethoxyphosphinylglycinate in the presence of ^{t}BuOK.

L-Vinylglycine has a number of practicable syntheses on offer, supplemented now by elimination from the L-methionine-derived Seebach oxazolidinone (cf. ref 70).[135] 3-Chlorovinylglycine has been prepared through chlorination of N-benzyloxycarbonyl vinylglycine methyl ester,[136] though the same workers choose to

prepare the 3-fluoro analogue through Strecker synthesis starting with 2-fluoroacrolein.[136] These turn out to be some 800 times more effective as irreversible inhibitors of alanine racemase from E.coli, compared with β-fluoro-substituted alanines. Since β-fluoro-D-alanine is a potent broad-spectrum orally active antibiotic, much pharmaceutical promise is offered by these halovinylglycines.

Allyl selenides in the presence of amines undergo oxidative [2,3]-sigmatropic rearrangement accompanied by nucleophilic addition, and this offers an entry to D-allylglycines starting from αβ-unsaturated alkanoates.[137]

Similar methods have led to the synthesis of (E)-3,4-dehydroglutamic acid, in a continuing search for potential Vitamin K-dependent carboxylase inhibitors.[138] γ-Methylene-L-glutamic acid and the (E)-ethylidene analogue have been prepared through ring-opening of ethyl R-[N-(p-nitrobenzoyl)]aziridinecarboxylate with $Ph_3P{=}CHCO_2Et$ (to give an isolable ylide), followed by Wittig reaction with formaldehyde or acetaldehyde, respectively.[139]

4.11 α-Amino Acids with Aromatic and Heteroaromatic Side-chain Groups.- There are many examples in the later Section 6.3, describing specific reactions of amino acids (i.e. reactions of side-chain functional groups), which amount to the conversion of one of the familiar aromatic or heterocyclic amino acids into others of the same class.

DL-o-Carboxyphenylglycine (noticed to be a potential glutamic acid substitute) has been synthesized from phthalonic acid ($o\text{-}HO_2C.C_6H_4.CO.CO_2H$) and NH_3 followed by $NaBH_4$ reduction of the resulting Schiff base.[140] Two well explored enantioselective routes have been explored for the synthesis of homotyrosines, one involving conventional azlactone synthesis followed by asymmetric hydrogenation [H_2/chiral Rh(I) - phosphine catalyst] and the other involving Friedel-Crafts acylation of chloroanisoles with (R)-aspartic anhydride.[141]

β-(N-Indolyl)-L-alanine has been isolated from fermentation of L-serine with indoline and E.coli.[142]

Syntheses of ibotenic acid analogues (31), through construction of the isoxazole ring on the pipecolic acid moiety, and (32), through cyclization of the substituted acetamidomalonate (33), have been reported.[143]

Regiospecific alkylation of N^{τ}-N^{α}-di-t-butoxycarbonyl-L-histidine by alkyl triflates or mesylates yields N^{τ}-alkyl-L-histidines.[144]

4.12 N-Substituted α-Amino Acids.- This Section excludes N-protected amino acids (representative coverage of these will be found in Section 6.2) but deals with N-hydroxamino acids, hydrazino acids, and their close relatives that are of interest, inter alia, in the synthesis of modified peptides.

N-Alkylation of furan-2-aldoxime by an α-halogeno-ester, followed either by acid hydrolysis or by reaction with hydroxylamine, yields an α-N-hydroxamino acid ((34)→(35)).[145] Alkylation of O-benzyl- or O-trityloximes of α-keto acids using

an alkyl-lithium gives the corresponding hydroxyamino acid.[146] These compounds may also be obtained from α-hydroxy esters by treatment with diethyl azodicarboxylate and Ph_3P in the presence of N-trichloroethoxycarbonyl- or N-benzyloxycarbonyl-O-benzylhydroxylamine [147] or by conversion into triflate esters and S_N2 displacement by O-benzylhydroxylamine.[148] HCN reacts with nitrones derived from secondary amines by oxidation with SeO_2 or by H_2O_2 and Na_2WO_4 and leads to α-cyano-hydroxylamines, from which the corresponding hydroxy-imino acid is readily obtained by hydrolysis of the cyanide group.[149] A similar side-chain N-hydroxylation procedure with N^{α}-benzyloxycarbonyl-N^{ϵ}-acetyl lysine t-butyl ester, using benzoyl peroxide, has been described.[150]

Hydrazino acids have been prepared by Shestakov rearrangement of hydantoic acids $H_2NCONHCHRCO_2H$ through treatment with KOCl.[151] Conversion of norbornyl esters into silyl enolates followed by treatment with di-t-butyl azodicarboxylate and $TiCl_4$ gives (S)-bis(Boc)hydrazino esters of high enantiomeric purity with efficient recovery of the norbornyl alcohol auxiliary.[152]

Guanidino acids may be prepared in good yields by reaction of amino acids with guanidine C-sulphonic acids $R'N{=}C(NHR^2)SO_3H$ (obtained from the corresponding thiourea and performic acid).[153]

Betaines are made easily from simple amino acids by alkylation, but an N^{α}-protection strategy is needed for lysine for the synthesis of the N^{ϵ}-trimethyl betaine analogue.[154]

4.13 α-Amino Acids containing Sulphur or Selenium.- Michael addition of thiols to α-cyano-ethenyl esters, followed by amination with NH_3, leads to S-alkyl-cysteines.[155] Similar application of established methods has led to series of α-monosubstituted and ββ-disubstituted cysteines.[156]

An improved synthesis of S-adenosylmethionine involves reaction of the sodium salt of homocysteine thiolate with 5'-chloro-5'-deoxyadenosine followed by S-methylation using trimethylsulphonium iodide.[157]

N-Acetylselenocysteine N-methylamide has been prepared by standard methodology.[158] New approaches to the synthesis of L-selenocystine and L-selenomethionine involve the carbon radical approach in which N-Boc-L-glutamic and aspartic α-benzyl esters are converted into N-hydroxypyridine-2-thione esters, and these are irradiated in the presence of dimethyl diselenide.[159]

4.14 Phosphorus-containing α-Amino Acids.- Analogues of the amino acids in which the carboxy group is replaced by a phosphorus oxyacid grouping are excluded.

Mention has been made earlier of amino acids containing side-chain phosphorus functional groups,[53] and a further example that is representative of straightforward synthetic approaches is found in the reaction of oximinophosphonate esters: $(EtO)_2P(O)C(CO_2Et){=}NOCOR$ yield insertion products with diazomethane which on Al/Hg reduction and hydrolysis give β-phosphonylalanine.[160]

4.15 α-Amino Acids Synthesized for the First Time.- Inclusion of this Section should not be taken to suggest that this Chapter offers a complete listing of all new α-amino acids in the period under review. Its purpose is to net examples that have not found a place in other parts, and its presence helps to avoid further proliferation of the Chapter into small sub-sections. The Journal of Medicinal Chemistry is well known as a location for compact presentation of research leading to synthesis of large numbers of closely related analogues, and this information is not comprehensively repeated in this Chapter.

2-Amino-3-boronopropionic acid, an analogue of aspartic acid in which the side-chain function is replaced by a boronic acid grouping, has been prepared by acetamidomalonate and Curtius routes.[161] 3'-Deoxy-modified S-adenosyl-L-homocysteines have been described.[162]

4.16 Labelled Amino Acids.- All examples are based on familiar protein amino acids and are cited in order of increasing atomic number of the labelling isotope. Most attention has been given to introduction of hydrogen and carbon labels, of course, and the special demands for rapid synthesis and purification imposed by the short half-life of ^{11}C offer fascinating and more widely useful insights into the exploitation of the organic chemist's skills.

The simplest labelling of the simplest α-amino acid is represented in reduction by Saccharomyces cerevisiae of [2 - ^{2}H]furfural to provide a substrate for production of (R)-[2 - ^{2}H]glycine.[163] (R)-[2 - ^{2}H]serine (from which the corresponding β-fluoroalanine was prepared by SF_4/HF) has been synthesized by ^{2}H exchange.[164] This was achieved by conversion of DL-serine isopropyl ester into 2-phenyl 4-isopropyloxycarbonyl oxazoline using methyl benzimidate and deprotonation with Ph_3CLi followed by quenching with $AcO^{2}H$, and classical resolution of the labelled serine as its (-)-bromocamphorsulphonate. Doubly ^{2}H-labelled L-phenylalanine (the α-proton and the ring o-proton) has been prepared from E-[2,2' - ^{2}H]-cinnamic acid through standard manipulations.[165]

Tritium labelling has been accomplished using $^{3}H_2$ to give L-[β,γ - ^{3}H]lysine, starting from the side-chain chlorination product of L-lysine.[166] More controlled approaches employing $^{3}H_2$ - Pd/C are embodied in syntheses of [3,4,5,5 - $^{3}H_4$]-L-ornithine (from which the correspondingly labelled L-arginine was obtained using NH_2CN and MeSH) and of [3,4 - $^{3}H_2$]-DL-glutamic acid, using β-(β-cyanovinyl) acetamidomalonate.[167] Similar approaches from β-(4-chlorobut-2-ynyl) acetamidomalonate lead to [4,5,6,6 - $^{3}H_4$]-L-lysine, [4,5,5,5 - $^{3}H_4$]-L-leucine, and [2,2,3,3 - $^{3}H_4$]-α-aminoisobutyric acid.[168]

The 1- and 3-[^{11}C]-labelling studies described in a surprisingly large number of papers this year illustrate several different standard methods of α-amino acid synthesis. [1 - ^{11}C]Glycine, prepared within 30-35 minutes by the Bucherer-Strecker synthesis from $^{11}CN^-$, formaldehyde and ammonium carbonate,[169] represents

a slow rate of production compared with that for D- and L-[1 - ^{11}C]tyrosines, prepared by the same route within 20 minutes.[170] Further Strecker reaction studies using H^{11}CN,[171] including their acceleration with an apparatus permitting operations at high temperatures combined with high pressures,[172] have been described. Carboxylation of α-lithioisocyanides with $^{11}CO_2$ offers an alternative route to [1 - ^{11}C]amino acids and has been exemplified with a synthesis of DL-[1 - ^{11}C]methionine.[173]

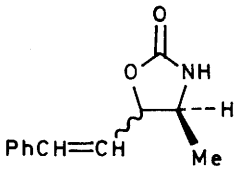
[3 - ^{11}C]-L-Alanine has been prepared by asymmetric alkylation with $^{11}CH_3I$ of [(+)-2-hydroxypinan-3-ylidene]glycine t-butyl ester, deprotonated with 2,2,6,6-tetramethylpiperidyl-lithium.[174] A total of 85 minutes is required for the synthesis of [3 - ^{11}C]-L-valine, using phase-transfer alkylation of $(Ph_2CH)_2NCH_2CO_2Bu^t$ by $Me_2{}^{11}CHMgI$ and resolution using D-amino acid oxidase (35 minutes).[175]

[3 - ^{11}C]-DL-Phenylalanine has been prepared from the azlactone of [1 - ^{11}C]benzaldehyde, not by the obvious hydrogenation and hydrolysis route but via the less well-known alkaline hydrolysis to the labelled phenylpyruvic acid, followed by amination.[176] The same role is given to an azlactone in a synthesis of [^{15}N, 2 - ^{13}C]-L-tyrosine from [2 - ^{13}C]glycine; the α-keto-acid is aminated to L-tyrosine using aspartate transaminase.[177] Detailed study of biosynthetic pathways is also the purpose of synthesizing [^{15}N, ^{13}C]-L-glutamic acid by Brevibacterium flavum using differently labelled acetate to provide appropriate patterns of ^{13}C-labelling.[178] [2' - ^{13}C]-DL-Tryptophan has been prepared by alkylation of formamidomalonate with the Mannich reaction product of [2 - ^{13}C]indole, dimethylamine, and formaldehyde.[179] [^{15}N, 2 - ^{13}C]-L-Phenylalanine[165] and [1 - ^{13}C]-DL-1-amino-4-carboxycyclohexane-1-carboxylic acid[180] have been prepared also by applications of standard amino acid syntheses.

Conventional routes have been published for [3 - ^{14}C]-3-(2-naphthyl)-D-alanine [asymmetric hydrogenation of $AcNHC(=^{14}CH\text{-}C_{10}H_7)CO_2R$],[181] [1 - ^{14}C]-L-phenylalanine [Strecker synthesis and enzymic resolution (thermolysin)],[182] and [methyl - ^{14}C]-L-methionine ($^{14}CO_2$ to $^{14}CH_3I$ by $LiAlH_4$ and HI, and methylation of protected L-homocysteine).[183]

As mentioned above, introduction of nitrogen isotopes in the synthesis of labelled amino acids has the special option of enzymic amination available,[78,132] and syntheses of ^{13}N-γ-aminobutyric acid from α-ketoglutaric acid using immobilized glutamate decarboxylase[184] and of ^{15}N-L-alanine from lactic acid, $^{15}NH_4Cl$, NADH, and immobilized L-alanine dehydrogenase[185] are further examples. Synthesis of (π-^{15}N)- and (π,τ-$^{15}N_2$)-labelled histidines involves construction of the imidazole ring on to appropriately labelled 2,5-diamino-4-oxopentanoic acids by standard methods.[186]

To the large number of papers already published on ^{18}F-labelled 6-fluorodopa [6-fluoro-(3,4-dihydroxyphenyl)-L-alanine] are added two more,[187,188] both using [^{18}F]acetyl hypofluorite for electrophilic substitution of protected dopas. In



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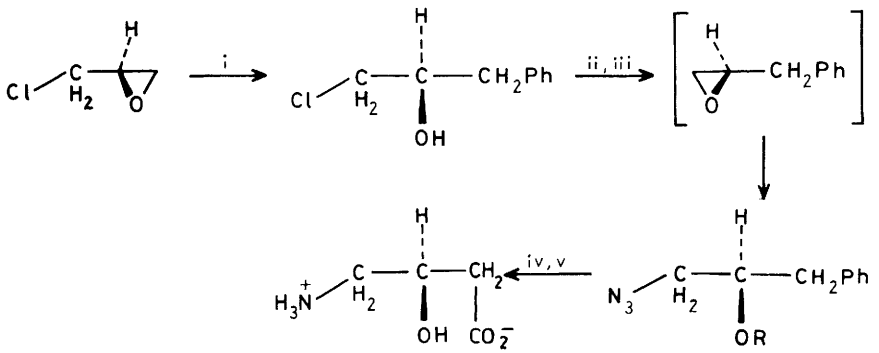
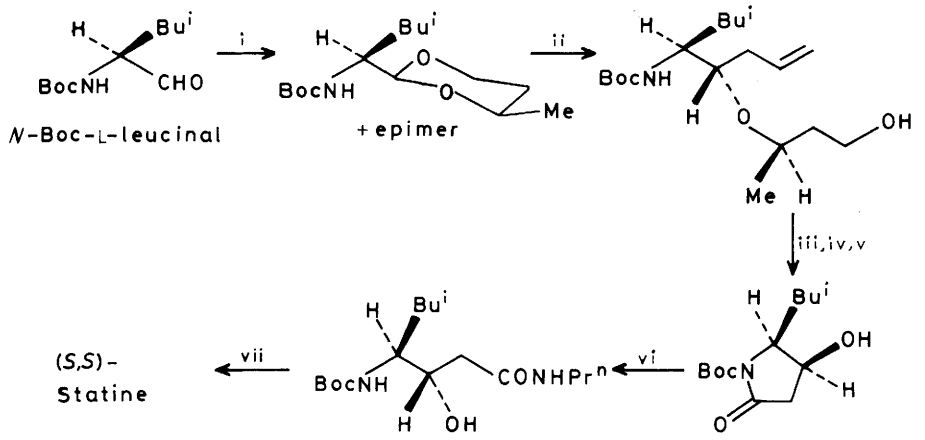


Reagents: i, PhLi; ii, NaN_3; iii, Boc_2O; iv, Sharpless oxidation, then TFA; v, H_2/Pt

Scheme 12



Reagents: i, (*R*)-1,3-dihydroxypropane, TsOH, CH_2Cl_2/reflux; ii, TMS-$CH_2CH{=}CH_2/TiCl_4$; iii, O_3; iv, pyridinium chlorochromate; v, $(PhCH_2)_2\overset{+}{N}H_2$ $CF_3CO_2^-$; vi, Pr^nNH_2; vii, deprotection

Scheme 13

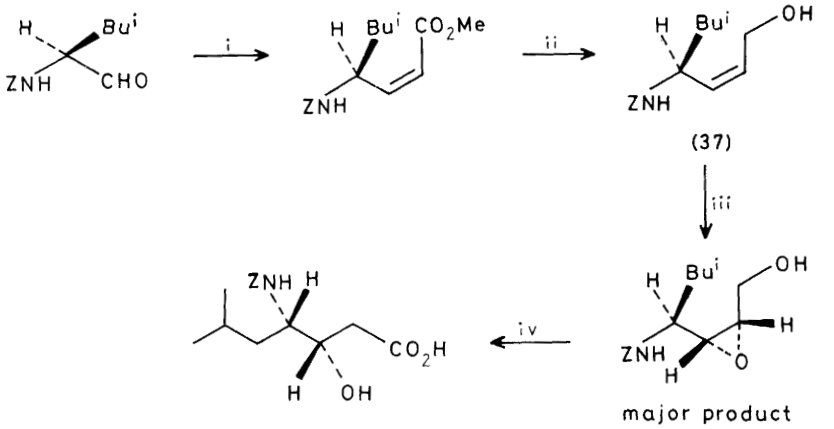
one of these, particular attention was paid to achieving synthesis and h.p.l.c. purification in the minimum time so as to achieve maximum radiochemical yield, though this was only 8% at the end of 100 minutes' work.[188]

Syntheses of [^{35}S]-adenosylhomocysteine[189] and [^{75}Se]-methionine[190,191] employ simple reagents for introduction of the labels (demethylation of commercially available [^{35}S]-L-methionine for the synthesis of homocysteine, followed by alkylation;[189] $Me^{75}SeNa$[190] and $Me^{75}SeLi$[191] for reaction with L-2-amino-4-bromobutanoic acid).

4.17 Synthesis of β-Amino Acids and Higher Homologous Amino Acids.- Hydroxylated β-amino acids that were discovered as unusual components of peptide antibiotics have started to dominate this Section, which is usually occupied by less spectacular ω-amino acids, such as γ-aminolaevulinic acid - shown to be formed from glutamic acid in plant chloroplasts.[192] Studies of glutamic acid α-semi-aldehyde as a potential intermediate in the C-5 tetrapyrrole biosynthetic pathway have been published in a paper that also describes surprising failures in applications of standard synthetic manipulations in exploration of routes to this γ-amino acid.[193]

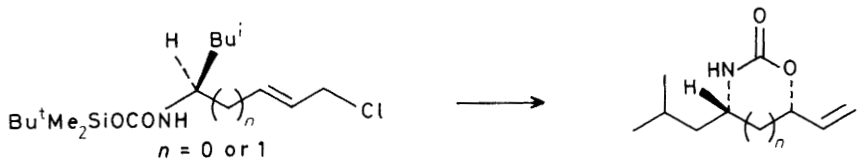
Asymmetric syntheses of intrinsic interest have been published for other close relatives of protein amino acids. These apply to several hydroxylated ω-amino acids, including (R)-isoserine, synthesized in good yield from di-azidomannitol through conventional stages in a route that is readily adaptable to lead to the (S)-enantiomer.[194] "D-isothreonine" and "L-allo-isothreonine" [(2R,3S)- and (2S,3S)-3-amino-2-hydroxybutanoic acids, respectively] have been prepared by $RuCl_3/NaIO_4$ oxidation of the styryloxazolidin-2-one (36) obtained from L-alanine.[195] The homologue, γ-amino-β-hydroxybutanoic acid ("GABOB"), of interest as a GABA analogue, has been prepared from (R)-epichlorhydrin in 57% overall yield through six steps (Scheme 12), representing a distinct improvement over existing methods.[196] Perhaps most obvious of all as a route to (S)-2- or -4-amino-5-hydroxypentanoic acids, the selective reduction of L-glutamic acid derivatives, has been achieved by $LiAlH_4$ reduction of N-trityl α- or γ-methyl esters.[197] At the other end of the scale, sophisticated strategies are being developed for synthesis of statine and, particularly, "MeBmT" [2S,3R,4R,6E-3-hydroxy-4-methyl-2-(methylamino)-6-octenoic acid].

Statine [(3S,4R)-3-hydroxy-4-amino-6-methylheptanoic acid] is approachable by chain extension of the carboxy group of an α-amino acid, and Boc-D-leucine offers a suitable starting point.[198] Conversion into the acylimidazole and nucleophilic substitution with $LiCH_2CO_2R$ gives the β-keto-ester $^tBuCH(NHBoc)COCH_2CO_2R$, which gives a mixture of statine and 3R-epimer on hydride reduction and deprotection. The same chain extension technique is used in the synthesis of a statine analogue in which the two methyl groups of the isobutyl group are replaced by sulphur atoms as part of a dithiolane moiety.[117] Here, N-benzyloxycarbonyl-L-aspartic acid is



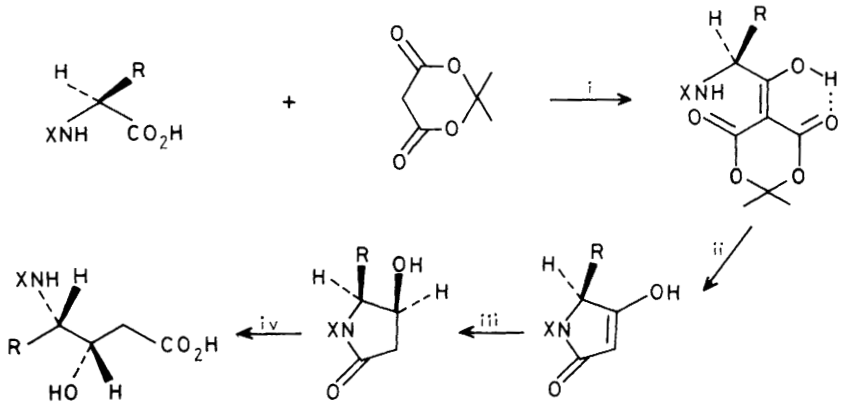
Reagents: i, $(CF_3CH_2O)_2P(O)CH_2CO_2Me$; ii, DIBAL; iii, MCPBA; iv, O_2/Pt

Scheme 14



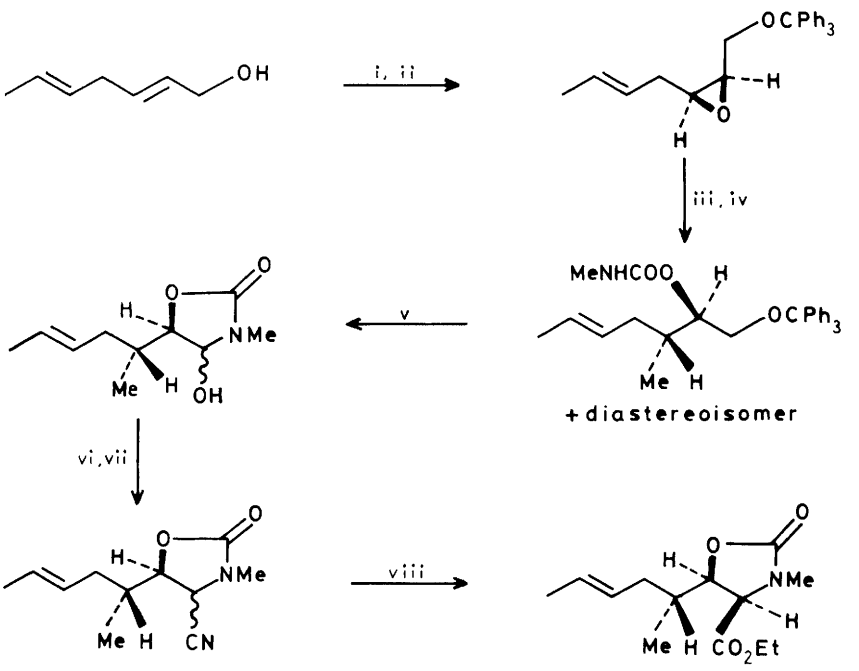
Reagents: i, AgF-Pd^{II}

Scheme 15



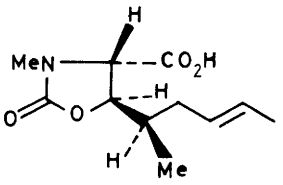
Reagents: i, isopropenyl chloroformate, DMAP; ii, reflux in MeCN or EtOAc, 20 min; iii, $NaBH_4$; iv, 1M NaOH

Scheme 16



Reagents: i, Bu^tOOH; ii, Ph_3CCl; iii, Me_2CuLi, $BF_3 \cdot Et_2O$; iv, MeNCO; v, $(COCl)_2$; vi, Ac_2O; vii, Me_3SiCN; viii, K_2CO_3 then EtOH

Scheme 17



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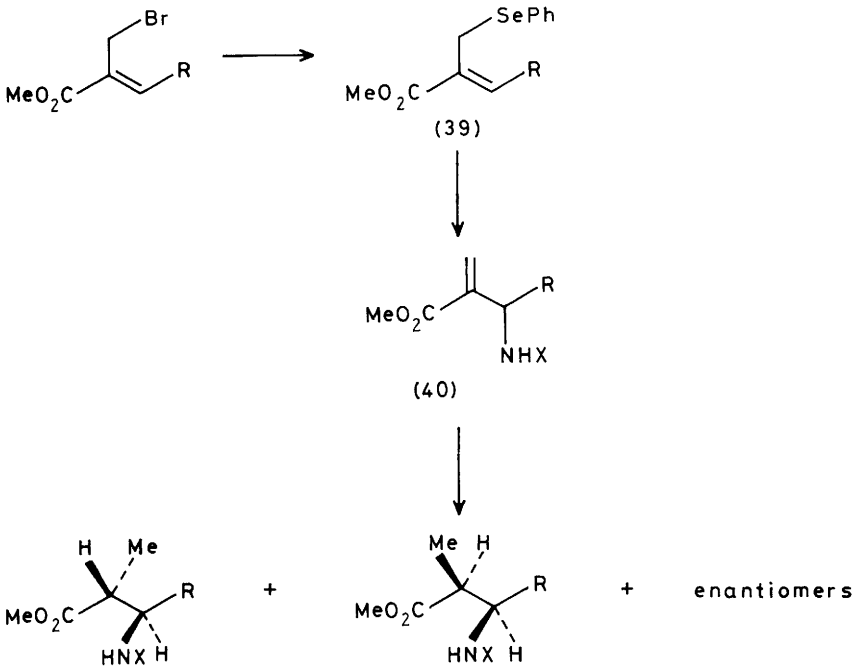
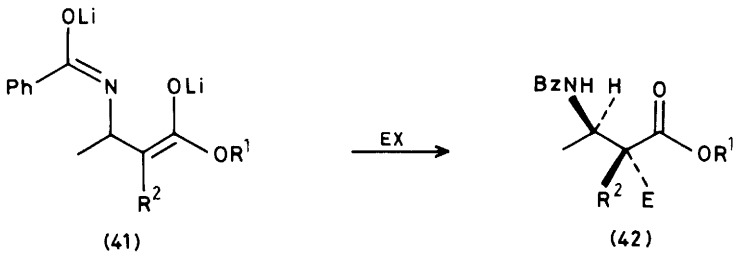

Br
MeO2C
R
SePh
(39)
NHX
(40)
H
Me
HNX
+
enantiomers

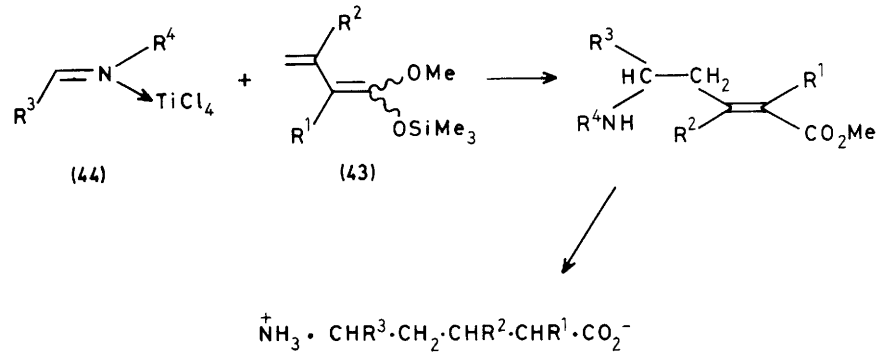
Scheme 18


OLi
Ph
N
OR1
R2
(41)
EX
BzNH
H
O
E
(42)

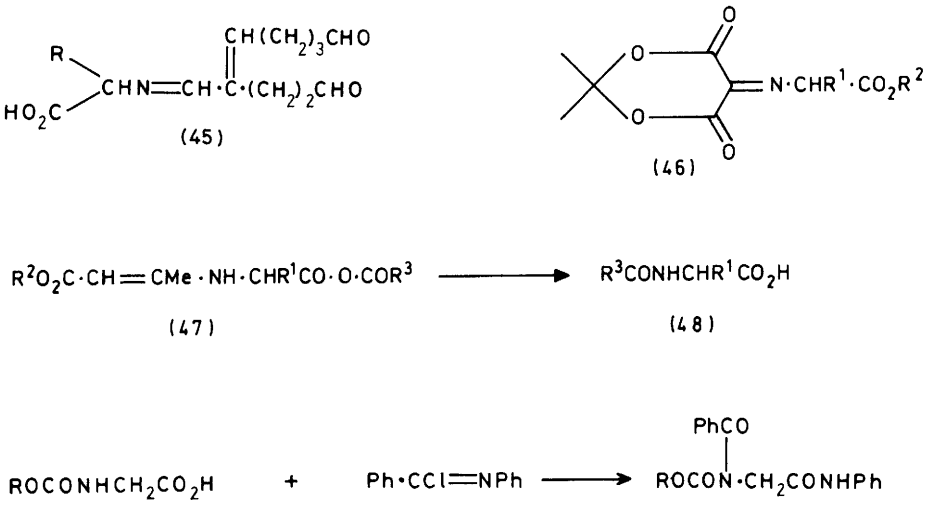
the starting material, the derived chiral oxazolidinone (25) being opened with $LiCH_2CO_2R$. The other routes to statines in this year's literature employ nucleophilic attack on an N-Boc-γ-lactam (Scheme 13),[199] diastereoselective epoxidation of the appropriate cis-4-(benzyloxycarbonylamino)allyl alcohol ((37) in Scheme 14),[200] and a related use of an allyl chloride (Scheme 15).[201] The last-mentioned route explores the usefulness of the silylcarbamate group in stereoselective access to 1,2- and 1,3-amino-hydroxyl systems, both in the statine case and also for the synthesis of (3S,4S)-4-amino-3-hydroxy-5-phenylpentanoic acid. Activation of an N-protected amino acid through mixed-anhydride formation with isopropenyl chloroformate has been used[202] to prepare a suitable derivative for coupling to Meldrum's acid. Of several bases tried, only 4-dimethylaminopyridine was a suitable catalyst for this process, the start of a route to N-Boc-statines via N-protected tetramic acids (Scheme 16).[202]

The problems of synthesis of "MeBmT", the cyclosporin constituent, seem much less formidable as a result of several recent reports. Earlier routes involved up to 24 separate steps; this has been cut back considerably for new routes by Rich and co-workers, in one of which[203] the lithium enolate of N-(p-methoxybenzyl)-sarcosine is added to (2R,4E)-$CH_3CH{=}CHCH_2CHMeCHO$ to give (38) and its diastereoisomer after ethanolic KOH treatment. Resolution of (38) with (+)-ephedrine followed by hydrolysis gives MeBmT. Routes by Evans[204] and by Schmidt[205] use the same 2-methylhexanal as starting material; the latter authors add to it the lithium enolate of N,N-bis(trimethylsilyl)glycine trimethylsilyl ester. As an alternative to Rich's route just described[203] (which the authors have christened a 'short synthesis'), a route starting with allylic epoxidation of hepta-2,5-dienol has been reported by Rich's group (Scheme 17).[206]

More generally applicable methods for the synthesis of β-amino acids continue to be developed. Tin(II) carboxylic thiolester enolates formed *in situ* from tin(II) 2-methyl-2-propanethiolate and ketenes react stereoselectively (anti-addition) with imines in the presence of tin(II) triflate to give β-amino acid thiolesters.[207] Amination of allyl selenides gives protected β-amino-α-methylene-alkanoic acid esters ((39)→(40) in Scheme 18); homogeneous-catalyzed hydrogenation of these esters is strongly diastereoselective, depending on the nature of the N-substituent.[208] More routine results arise from the modification of the α-carboxy function of N-formyl dibenzyl aspartate to give β-amino-γ-ketobutyric acid derivatives, including Dakin-West manipulation of 4-benzyloxycarbonylmethyloxazolin-5-one derived from this aspartic acid derivative.[209] The other obvious way to build up αβ-disubstitution patterns on a β-amino acid is by alkylation, and methyl or ethyl N-benzoyl-3-aminobutanoates can be stereoselectively alkylated after dilithiation with LDA.[210] The incoming group is directed to create products of l- and u,u - configuration, through alkylation with lk-1,2-induction (to use Seebach's terminology[210]), i.e. the electrophile approaches the enolate β-carbon atom from the least-hindered direction ((41)→(42)).



Scheme 19



γ-Amino acids may be obtained from corresponding keto-esters by Meerwein-Ponndorf-Verley reduction, substitution of OH by Cl with $SOCl_2$, and amination (NH_3).[211] A more sophisticated approach leading to δ-amino acids is based on the reaction of vinylketene silylacetals (43) with imine - $TiCl_4$ complexes ((44) in Scheme 19).[212]

4.18 Resolution of Amino Acids.- Examples of classical methods based on diastereoisomeric salt formation of a DL-amino acid or an N-substituted derivative with a chiral amine or acid are embedded in some of the papers located in other sections of this Chapter. A further example of this type, with the added interest of an accompaniment of asymmetric transformation (DL-p-hydroxyphenylglycine is converted into its D-enantiomer to the extent of 80%), employs (+)-phenylethanesulphonic acid as chiral reagent.[213] Equilibration of N-(2-naphthyl)-D-alanine 1-undec-10-enyl ester with triethylamine in the presence of the stereolabile 2,4-dinitrobenzoyl-DL-leucine n-butylthiolester gives 80% enantiomeric excess of the D-leucine isomer. This interesting example of asymmetric induction points to a stabilizing enantioselective association between the respective D-enantiomers.[214]

The preferential-crystallization technique depends, perhaps arbitrarily, upon the intimate details of crystal habit. Quaternary ammonium salts of N-formyl-DL-amino acids, for example, have been found to crystallize in the essential characteristic form (each crystal composed exclusively of one enantiomer) for preferential crystallization. Among several further examples in this year's literature, 1,1,3,3-tetramethylbutylammonium salts of N-formyl-DL-α-phenylglycine,[215] DL-tyrosine,[216] or DL-phenylalanine,[217] ammonium N-acetyl-DL-norleucinate,[217] and DL-thiazolidine-4-carboxylic acid[218] have been resolved by this technique.

Column chromatographic methods are simple and often very effective, for example for resolution of DL-amino acids over cellulose.[220] Discriminatory ratios have been calculated to be close to 1% for adsorption of D-alanine relative to its L-enantiomer on crystalline cellulose,[221] though it must be said that practitioners would establish characteristics such as these on an empirical basis when upscaling their laboratory experiments. Optically active cationic complexes, such as copper(II)-L-lysine, adsorbed on clays (montmorillonites) show modest selectivity coefficients for DL-amino acids, the L-enantiomer being more strongly adsorbed from solutions at neutral pH.[222] There are numerous papers on the h.p.l.c. variant of this approach, using a variety of chiral phases, and those on analytical resolutions are described in Section 7.4.

The demands of clean separation on the analytical scale have led to the development of new chiral stationary phases that are suitable for preparative resolution. Silica linked to (R,R)-tartramide through undecamethylene spacer chains provides a substantial discriminatory ratio between D- and L-amino acids

based on hydrogen-bonding interactions,[223] and an (R,R)-tartaric acid mono-n-octylamide-copper(II) modified stationary phase operating on the ligand exchange mechanism for resolution of DL-amino acids has been described.[224] The Pirkle-Pochapsky chiral stationary phases (CSPs) based on N-(3,5-dinitrobenzoyl)-L-amino acids[225] continue to be studied, showing substantial chiral recognition for enantiomers of N-aryl-α-amino acid esters[226,226] with discriminatory ratios larger than 18:1 at room temperature for 10-undecenyl esters of N-2-(naphthyl)amino acids.[226] A new chiral stationary phase, formed by attaching [N-(S)-(1-α-naphthylethyl)amino-carbonyl]-L-valine to γ-aminopropylsilanized silica, shows promise for chromatographic resolutions of amino acids.[227]

Tailor-made polymers, represented by acrylamide - divinylbenzene - ε-itaconyl-L-lysine copolymer and by methacrylic acid - ε-methacryloyl-L-lysine NN'-methylene bis(acrylamide) copolymer, have been used for resolution of DL-lysine; the former copolymer permits emergence of the D- before the L-isomer, and the reverse sequence is seen with the latter copolymer for the same amino acid.[228] A simple approach in which silica is coated with proteins in this way is successful for the resolution of N-benzoyl- or -dansyl-amino acids,[229] and a related approach,[230] based on zwitterion pairing of a DL-amino acid with a di- or tripeptide in solution, has been studied using DL-tryptophan.[230]

Diastereoisomeric salt or complex formation, as in the last-mentioned example, is represented in chromatographic separation of a chiral crown ether - DL-amino acid pair, using aqueous perchloric acid as mobile phase.[231] The same principle is exploited in ligand exchange resolution of DL-amino acids using (1R,2S)- or (1S,2S)-2-carboxymethylamino-1,2-diphenylethanol as chiral additive.[232] More examples are discussed in the context of h.p.l.c. analysis in the later Section 7.4 of this Chapter.

Enzymic methods are represented (see also ref.44) in a use of α-chymotrypsin for the stereoselective hydrolysis of N-acetyl erythro-β-(p-nitrophenyl)serine esters to give the (2S,3S)-amino acid.[233] N-Acetyl-D-amino acids accumulate through the action of fermenting yeast proteinases on corresponding DL-esters.[234] All four enantiomers of δ-hydroxylysine have been secured through mould acylase-catalyzed hydrolysis of N^{α}-formyl-N^{ϵ}-benzyloxycarbonyl-DL-lysine esters and their allo isomers.[235] Applications of enzymes in the production of protein L-amino acids have been reviewed in the earlier Section 4.3, the principle being applicable for the synthesis of unusual L-amino acids (e.g. from keto-acids using aspartic aminotransferase[70]).

No year passes without a few references in the literature to experiments (and many references to speculations) on the predominance in the present biosphere of the L-enantiomers of amino acids. Reviews have appeared on this topic, a general coverage,[236] and specific attention to parity violation in chemical reactions, with special reference to the formation of α-aminonitriles ($CN^- + CH_3CH{=}N^+H_2$).[237] In the latter context, the combination of differential degradation by chiral β-

radiation with the weak parity violation inherent in an individual enantiomer[238] is concluded to require a very long time-scale (1500 y) before any noticeable selection could occur.[239]

It has been surmised that prebiotic conditions could not have been favourable for the enantiospecific role given to chiral β-radiation in the enantioselective degradation of racemates.[240] An experiment that will undoubtedly be the subject of further scrutiny, like all others of its type that make fundamental claims, has shown that chiral positrons from ^{22}Na decay interact differently with the enantiomers of alanine.[241]

Frank's model[242] has been given further theoretical support.[243] The model assumes local deviations from equilibrium in reactions of the separate enantiomers of a racemate with large excesses of achiral compounds and the merging of these localities so as to tend to "extinguish" one enantiomer over long periods of time. Weak neutral currents influence rate constants differently for reactions of each enantiomer and determine that those localities rich in one enantiomer will tend to accumulate more rapidly than localities rich in the other enantiomer.

5 Physical and Stereochemical Studies of Amino Acids

5.1 Crystal Structures.- Amino acids subjected to X-ray crystal structure determination, as reported in the recent literature, are β-alanine,[244] L-citrulline,[245] and L-methionine sulphoximine [the "natural" isomer, shown to possess the (S,S)-configuration[246]].

A substantial crop of simple amino acid derivatives is represented in the other crystal structure papers this year, covering L-phenylalanine benzyl ester hydrochloride,[247] S-benzyl-L-cysteine methyl ester hydrochloride,[248] N-(β-phenylpropionyl)glycine ethyl dithioester,[249] N-phenylacetyl-L-phenylalanine,[250] N^{α}-acetyl-L-arginine ethylamide perchlorate,[251] N-acetyl-N-hydroxy-DL-alanine,[252] N-trityl-L-aspartic acid dibenzyl ester and N-trityl-L-leucine benzyl ester,[253] N-benzyloxycarbonyl-γ-carboxy-L-glutamic acid γγ-di-t-butyl ester α-methyl ester,[254] and N-acetyl-2,4-methanoproline methylamide.[255] Interest in the topic of the last-mentioned paper lies in the fact that the formally achiral molecule adopts mirror-image enantiomeric forms in the crystal, due to distortions accompanying packing.

5.2 Nuclear Magnetic Resonance Spectrometry.- Attention continues to spread to other nuclei - i.e. beyond ^{1}H and ^{13}C - but novel and relevant applications continue to be explored for all n.m.r.-active nuclei.

Configurational assignments may be made to α-amino acids and their esters on the basis of consistent shift differences of the α-proton resonance for each enantiomer in the presence of either of the chiral shift reagents (R)-propylenediaminetetra-acetato europium(III)[256] or Eu(d-facam)$_3$.[257] D- and L-

enantiomers of 22 amino acids were studied in aqueous solutions containing the former of these reagents.[256] In all cases, the Hα-resonance showed larger upfield shifts for an L-enantiomer than for its D-isomer. The method is not suitable for estimation of enantiomer ratios for amino acids since accurate integration of the relevant peaks was not possible.[256]

Some doubt must be cast on the practicability of both quantitative and qualitative analysis of amino acids and other organic acids in urine, proposed on the basis of ^{1}H-n.m.r.,[258] though only limited information is offered in the abstracts for these papers. Another conventional use for n.m.r. is for the distinction between tautomers, and 4-nitrohistidine has been shown to exist in solution as the N-1 H form.[259]

Quantitative analysis of ^{13}C-labelled amino acids by n.m.r. has been reviewed.[260] Selective labelling of tryptophan by photo-deuteriation has settled a controversy surrounding ^{13}C-assignments, now established as: C-4, 118.4; C-5, 118.2; C-6, 120.6 ppm.[261]

^{14}N-N.m.r. quadrupole coupling tensors have been determined for crystalline L-histidine hydrochloride monohydrate,[262] and corresponding resonance parameters have been calculated for representative amino acids.[263] More routine applications for ^{15}N-n.m.r. include establishment of a 65:35 E/Z ratio for geometrical isomers of N^{α}-acetyl-N^{in}-nitroso-tryptophan.[264]

5.3 Optical Rotatory Dispersion and Circular Dichroism.- C.d. spectra have been reported for biliverdin-substituted L-amino acids that reveal the formation of bilatriene helices, with c.d. parameters that are very sensitive to the structure of the amino acids involved.[265] While this paper is typical of an application of c.d. that could by now be called classical, the remaining paper for inclusion in this section represents an evolving technique, Raman optical activity spectrometry, for studies of aqueous solutions of L-lysine, L-proline, and L-hydroxyproline.[266] Two specific points of interest arise from these data; interpretation of a strong scissoring vibration mode for lysine and proline, and a significant observation that the Raman optical activity pattern at frequencies greater than 1300 cm^{-1} seems characteristic of the L-configuration.

5.4 Mass Spectrometry.- An idiosyncratic review of the role of m.s. in structure determination of new amino acids from marine algae has appeared.[267]

With all other non-routine papers on m.s. of amino acids located in the appropriate analytical sections later in this Chapter, there remain: molecular-beam studies of the decomposition of glycine in the vapour phase on contact with solid surfaces at 420-800K;[268] secondary-ion emission from amino acids evaporating from metal surfaces[269] or from glycerol solutions containing also a thallium(I) salt;[270] and fast-atom-bombardment MIKE spectra for 20 common L-α-amino acids to provide an order of proton affinities.[271] The solution SIMS study is substantial,

showing that sulpholane is a poor solvent for the purpose and that protonation by trifluoromethanesulphonic acid gives better results than metal ion complex formation.[270] The MIKE study is conceptually simple: the most abundant ion in MIKE of a cluster ion of two different amino acids corresponds to the amino acid with the higher proton affinity. The order that emerges is from glycine (lowest proton affinity) through a generally predictable intermediate order to arginine (highest proton affinity).[271]

5.5 Other Spectroscopic Studies.- Intramolecular hydrogen bonding and formation of hydrogen-bonded dimers is revealed by infrared (i.r.) spectrometry of *N*-acylglycines in solution and dispersed in CsBr.[272] Similar studies of *N*-acetyl glycine, L-alanine, and L-leucine methylamides in chloroform solutions[273] show the co-existence of intramolecularly hydrogen-bonded 5-membered rings and non-hydrogen-bonded species, while i.r. and Raman studies for *N*-acetyl-L-phenylalanine, L-tyrosine and L-tryptophan methylamides have concentrated on solid samples and (for the Raman study) methanol solutions.[274]

Fluorescence spectra of *N*-acetyl-1-pyrenyl-DL-alanine methyl ester reveal intermolecular excimer formation with a smaller rate constant for the dissociation of the D,L-excimer to the locally excited state at room temperature than that for the L,L- (or D,D-) excimer.[275] This confirms earlier work of relevance to theories of prebiotic enantiomeric discrimination.

5.6 Other Physical Studies.- Reviews have appeared on extraction of amino acids from aqueous solutions using reversed micelles [276] and of amino acid transport across liquid membranes.[277] The latter topic is a substantial research area in its biochemical context, for which a representative paper[278] describes L-alanine transport in renal luminal membrane vesicles. There may be physiological relevance to the demonstration of transport of simple monovalent and divalent metal ions and heavy metal ions through $CHCl_3$ membranes by long-chain *N*-acylamino acids.[279]

Solution studies are illustrated further in partition coefficient data for *N*-acetyl dialkylglycinamides[280] and adsorption of valine on a carbon electrode[281] and of alanine or γ-aminobutyric acid on platinum[282] during electro-oxidation.

Thermometric studies include solid-liquid phase transitions of *N*-acylamino acids by differential scanning calorimetry and by i.r. spectrometry,[283] heats of dilution of *N*-acetylalaninamide solutions,[284] and emission thermophotometry of amino acids in a flowing O_2 atmosphere.[285] There is an uncertainty in the results of the latter study, in which it is acknowledged that light emission might arise from oxygenation of unstable pyrolysis products.

Gas-phase electron diffraction data for L-alanine methyl ester are consistent with a *syn*-periplanar N-C-CO torsion angle imposed by formation of a bifurcated intramolecular hydrogen bond between NH_2 and CO groupings.[286]

5.7 Theoretical Studies of Amino Acids.- Molecular-orbital calculations for proline, N-formylprolinamide, and N-acetylprolinamide reveal the formation of an intramolecular hydrogen bond between the acyl oxygen and amide groups that is seen in the peptide γ-turn conformation.[287] Theoretical studies with less obviously applicable results concern calculations of free-energy changes, relevant to aqueous solutions, that accompany the structural changes glycine → alanine and alanine → phenylalanine [288] and calculations of three-dimensional details of the best fit between two interacting amino acids.[289]

6 Chemical Studies of Amino Acids

6.1 Racemization.- This topic can be divided into a number of sub-divisions, one of which uses rate data for racemization of protein amino acids for fossil dating. This has become a controversial subject with doubts being cast on the validity of the results. The uncertainty arises in the role of the immediate environment of the individual amino acid enantiomer that undergoes racemization; while the rate constant data under defined conditions link the degree of racemization with age, it has been found that the process is non-linear (and was shown to be reversed!) from one fossil shell to another, dependent on age and genus of the sample.[290] The deviations for valine and isoleucine racemization are less than for aspartic acid, which has been most commonly used for fossil dating because of its high racemization rate constant (see also ref.420).

The isoleucine:alloisoleucine ratio for snail shells from Holocene sediments of the northern Negev desert is a useful index of age variations within a layer, but not for absolute chronology, for which the ^{14}C technique was used.[291] Racemization of bone collagen has been subjected to a laboratory study, showing that within the time-scale of a practicable research project the onset of racemization can only be detected at elevated temperatures (>40°C).[292] The topic of amino acid dating has been reviewed.[293]

Strong basic anion exchange resins in the OH^- form partly neutralized with 5-sulphosalicylaldehyde have been found to bring about racemization of amino acids in the presence of a copper(II) salt at a faster rate than the well-known equivalent solution reaction.[294] Leucine, phenylalanine and tryptophan amides undergo the equivalent racemization process in the presence of their parent amino acids and formylated phenol - formaldehyde resins or polystyrene - divinylbenzene - salicylaldehyde condensation products.[295] A similar-looking process in which L-methionine undergoes racemization in acetic acid containing a catalytic amount of salicylic acid[296] may have another mechanistic explanation, since an N-acetylamino acid is known to undergo racemization in the presence of acetic anhydride. The other well-known Schiff-base-mediated racemization procedure employing pyridoxal has been shown to be accelerated by phosphate ion.[297]

Certain N-protected α-amino aldehydes are prone to racemization during chromatography on silica gel, though bulky N-[9-(9-phenylfluorenyl)]-L-alaninal has been shown to be configurationally stable in this respect and in the presence of some simple reagents.[298]

6.2 General Reactions of Amino Acids.- This substantial section collects reports on reactions in which amino and carboxy groups and their simple derivatives are involved, either together or separately. In the latter category, the emphasis is on papers of wider interest than the synthesis of protected amino acids for peptide synthesis.

Carpino has reviewed the chemistry of Fmoc and related base-sensitive N-protecting groups that are useful in the amino acid and peptide field.[299] Reagents explored for introduction of the Fmoc (using Fmoc N-hydroxysuccinimide ester[300]) and other standard N-protecting groups include pentafluorophenyl formate (for N-formylation),[301] BocN=C(CN)Ph for the synthesis of Boc-L-phenylalanine,[302] crosslinked polystyrene carrying CF_3COSCH_2- or $CF_3CO_2CH_2$- groups for N-trifluoracetylation,[303] and 1-(N-benzyloxycarbonyl)benzotriazole and dibenzyl dicarbonate for Z-amino acid formation.[304] The use of the alternative reagent for the synthesis of Fmoc derivatives[300] minimizes the formation of Fmoc-dipeptide side products. Unwanted side reactions have also been noted in the reaction of palmitoyl chloride with L-cystine di-t-butyl ester, giving some N-$Me(CH_2)_{13}COCH[(CH_2)_{13}Me]CO$-substituted side products,[305] and in the formation of different condensation products between an amino acid and samples of glutaraldehyde of varying quality.[306] One of these is assigned the somewhat unlikely structure (45), based on the propensity of Schiff bases to participate in aldol-type reactions.[306]

Trimethylsilylation of amino acids in the presence of CO_2 giving trimethylsilyl derivatives of N-carboxyamino acids,[307] copper(II)-promoted acylation of leucine with a β-ketothiolester,[308] and N-alkylation of amino acid esters with 5-methoxymethylene-1,3-dioxan-4,6-dione ("methoxymethylene Meldrum's acid") to give (46)[309] have featured in recent papers. Asymmetric hydrogenation of N-pyruvoyl-L-proline esters gives N-(S)-lactoyl analogues with diastereoisomeric excesses of up to 59%.[310] D-α-Hydroxy acids have also been prepared from L-α-amino acids via halogeno-acids (formed using $NaNO_2$/HX).[311]

Kinetics of nitrosation of thioproline have been determined.[312]

Catalytic hydrogen-transfer deprotection of N-benzyl-[312] and N-Z-amino acids[313] using ammonium formate and 10%Pd-C is rapid at reflux temperatures. The formation of adenine in heated solutions of N-(4-purinyl)glutamic acid containing a copper(II) salt is a notable C - N cleavage reaction.[315] A curious C - C cleavage process is seen in the formation of Z-β-alanininamide as a result of an attempted Hofmann-type rearrangement of 1-aminocyclopropanamide using

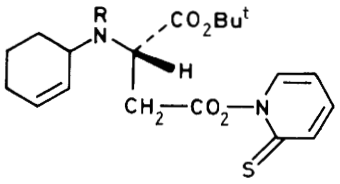
$PhI(OCOCF_3)_2$.[316]

Esterification of amino acids using a mono-alkyl sulphate[317] and an optimized route to L-histidine benzyl ester ditosylate[318] have been reported. Esterification of N-protected amino acids catalyzed by papain[319] (only the α-carboxy group of aspartic and glutamic acid derivatives is esterified) is successful with Boc-amino acids and a wide range of alcohols and diols in a two-phase procedure.[320] Details of the preparation of 4-(Boc-aminoacyloxymethyl)phenylacetic acids for making PAM resins for solid-phase peptide synthesis have been described.[321] Esterification of N-protected amino acids and peptides, but using 15-crown-5 in DMF with 1M sodium hydroxide and an alkyl halide, is claimed as a mild method.[322] However, the method involves distilling off water (from a mixture containing sodium hydroxide) and some DMF prior to adding the alkyl halide, and then the mixture is kept at 40°C for 2 hours before evaporation, so there seems to be considerable scope for racemization and side reactions. 1,2,2,2-Tetrachloroethyl esters are formed from N-protected amino acids and an alkyl 1,2,2,2-tetrachloroethyl carbonate.[323]

Activation of an N-protected amino acid through mixed-anhydride formation with isopropenyl chloroformate permits easy esterification by alcohols.[324] Mixed anhydrides formed from N-alkoxycarbonylamino acids[325] can undergo intramolecular acylation of the urethane nitrogen atom as a side reaction during aminolysis by an amino acid[326] or by an amino acid ester.[327] Similar acyl transfer is seen in the conversion of α-enamino acid anhydrides (47) to corresponding acylamino acids (48) under the influence of aqueous hydrochloric acid.[328] The same process operates in the reaction of a chloro-imine with an N-alkoxycarbonylamino acid [(49) → (50)], previously thought to lead to the N-phenyl-N-benzylamide.[329]

Reactions of carboxy-group derivatives of amino acids include Friedel-Crafts acylation by N-protected L-prolyl chlorides giving corresponding aryl ketones,[330] oxidative decarboxylation of N-methoxycarbonyl-[O-(t-butyldimethylsilyl)]-L-hydroxyproline to give the corresponding 5-methoxypyrrolidine by electrolysis in MeOH,[331] a new route to α-amino aldehydes through $LiAlH_4$ reduction of a Z-amino acid 3,5-dimethylpyrazolide,[332] and conversion of Z-D- or L-alanine into corresponding trans-2,5-dimethylpyrrolidine enantiomers via $ZNH\text{-}CHMe\text{-}CH_2CH_2CH{=}CH_2$.[333] Oxazolin-5-ones are a form of activated ester, and continuing interest in their oxidative cleavage into acylamides (see also Vol.19, p.29) is shown in O_2 - Pd peroxidation[334] and in radical anion formation with K or KO_2.[335]

Ever more numerous papers reflect the considerable interest in enantioselective hydrolysis of N-acylamino acid p-nitrophenyl esters, either involving micellar media containing an L-histidine-containing peptide[336,337] or imidazole in the presence of a chiral surfactant,[338] or involving α- or β-cyclodextrins.[339] One of these papers[337] records the "perfectly enantioselective hydrolysis" of N-dodecanoyl-D- and L-phenylalanine p-nitrophenyl esters in micelles containing Z-L-Phe-L-His-L-Leu-OH.



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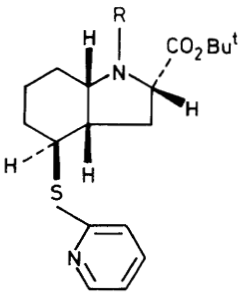

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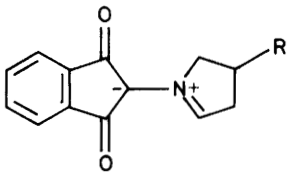
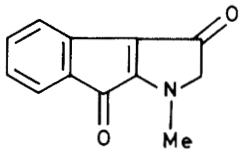


(53) R = H or OH



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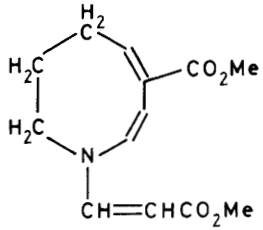

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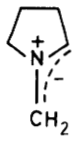

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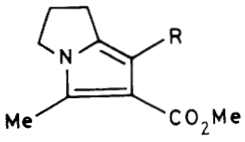

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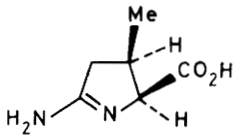

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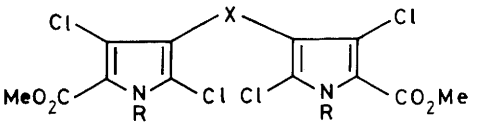
Representative papers for the many mechanistic studies that have appeared this year for oxidation of individual amino acids describe bromamine-T/glutamic or aspartic acid,[340] alkaline hexacyanoferrate(III)/tryptophan,[341] and chloramine-T/alanine, 2-aminobutyric acid, valine, serine and threonine.[342] Chloramine-T oxidation of arginine in acid media is catalyzed by Cl^- (which is thought to become Cl^+ in the oxidizing medium).[343] Since one of these papers[342] describes a 're-investigation' of the well studied chloramine-T system, there will no doubt continue to be expenditure of much effort on this topic.

Uses of amino acids as chiral auxiliaries in asymmetric synthesis are cited elsewhere in this Chapter; interesting synthetic developments in nitrogen-heterocyclic chemistry starting with amino acids continue to call for space in this Section. Maillard condensation of [1- or 2-^{13}C]glycine or [^{15}N]glycine with D-xylose or its [1-^{13}C] isotopomer leading to melanoidins (structural details not established) is a paper[344] of an important research topic. The generation of a carbon-centred radical from an N-hydroxypyridine-2-thione ester has been studied in the context of the L-aspartic acid derivative (51), leading to the condensed proline (52) and its diastereoisomer.[345] Azomethine ylides are intermediates in the formation of oxazolidines from secondary amines and aldehydes (see Vol.19, p.29) and include the yellow ninhydrin - proline (or hydroxyproline) condensation product ((53); reaction in ethanol at 0°C), recognised for the first time to be a stable azomethine ylide.[346] In contrast, sarcosine and ninhydrin give (54).[347] Further work on this enlightened view on the mechanism of the Strecker decarboxylation of amino acids covers the formation of the ring-expanded proline (55) arising via the azomethine ylide (56) through the reaction of proline, formaldehyde, and methyl propiolate.[348] A full paper has appeared on cycloaddition of proline to methyl 2-alkynoates in acetic anhydride, leading to pyrrolizines (57).[349] The adduct from the intermediate mesoionic oxazolinone, formed from N-acetylproline by the action of acetic anhydride, yields the final product through retrocycloadditive decarboxylation.

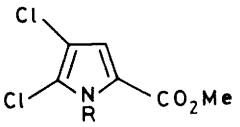
A representative paper[350] on the formation of β-lactams from β-amino acids describes the use of bis(5'-nitro-2'-pyridyl)-2,2,2-trichloroethyl phosphate for the purpose.

6.3 Specific Reactions of Amino Acids.- The literature on chemical studies that result in side-chain modifications to amino acids is reviewed in this Section. Readers seeking a full overview should also read both the preceding section and the earlier sections covering synthesis (where papers describing the use of one amino acid to synthesise another are mostly located).

There are relatively few reactions in which changes to aliphatic amino acid hydrocarbon side chains are accomplished without affecting the amino and carboxy functions or the α-carbon atom, but suitably protected examples undergo γ-proton



(59) X = S, SO, SS, or SO_2



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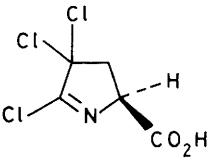

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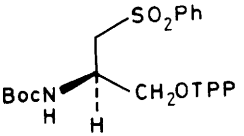

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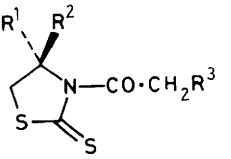

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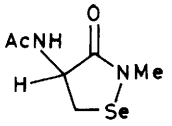

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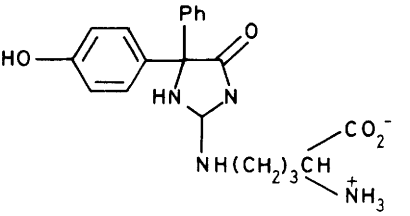

(65)

abstraction by hydroxyl radicals (from $TiCl_3$ - H_2O_2) rather than the more common α-attack.[361] 1-Aminocyclopropanecarboxylic acid, not surprisingly, reacts more readily, and its biological role as ethylene source has been modelled using the O_2 - Mn^{2+} - 1-benzyl-3-carbamoyl-1,4-dihydropyridine - flavin mononucleotide system.[362]

Oxidation of N-alkoxycarbonylprolines gives the corresponding pyroglutamates,[363] which have a role in a synthesis of chiral pyrroline-5-carboxylic acids (58).[364] Aspartic and glutamic acids are perhaps the most hard-worked of the protein amino acids as chiral starting materials in synthesis, a further example (see also, inter alia, refs.96, 116) being a three-step route from L-aspartic acid to N-trityl-L-homoserine lactone (via selective reduction in 50-60% yield of the N-trityl dibenzyl ester using DIBAH).[365] A convenient preparation of L-aspartic β-semialdehyde from L-methionine via its sulphonium salt, and pyridinium chlorochromate oxidation of the derived homoserine, leads on to other synthetic possibilities [Wittig elaboration to L-2-amino-hept-4,6-dienoic acid; a new synthesis of bulgecinine (see also ref.121)].[366]

Uranyl-sensitized photo-oxidation of aspartic acid in aqueous acidic media gives malonic acid.[367] This amino acid is included with lysine and glutamic acid in an e.s.r. study of free-radical formation on pyrolysis through the temperature range 473-873K.[368] More routine results, useful however in synthesis, concern the preparation of β-aspartates and γ-glutamates from esterification of the amino acids by alkanols in the presence of Na_2SO_4 and HBF_4,Et_2O [369] and the formation of the same mono-esters through alcalase-catalyzed hydrolysis of the di-esters.[370] L-Glutamic acid is the source of (3S)-pyrrolidinol, via L-(-)-$H_2NCH_2CH_2CH(OH)CO_2H$.[371] Pyrroles are formed from pyroglutamate esters and thionyl chloride [which leads to (59)] or PCl_5 [which leads to (60) and the 3,3-dichloropyrroline (61)].[372] All three functional groups of DL-threonine are modified by PCl_5 in dioxan to give $MeCHClCH(COCl)N{=}PCl_3$, and in benzene the product is $Cl_2P(O)OCMe{=}CHCOCl$.[373] The hydroxy group in serine is modified in an enantiospecific synthesis of representative β-alkylated alanines (D- and L-homophenylalanine, norvaline, and norleucine), in which Boc-L-serine is converted into the phenylsulphonylmethyl derivative ((62) and its enantiomer) by routine methods.[374] Activation and alkylation of the α-methylene grouping permits introduction of a chosen β-substituent. Removal of the tetrahydropyranyl protecting group followed by oxidation (pyridinium chlorochromate) converts the original serine side chain into the carboxy group of the target amino acid.[374] Stereoselective alkylation of benzene with N-phthaloyl-(2S,3R)-threonine O-trifluoromethanesulphonate (in CF_3SO_3H at 80° during 10 h) gives N-phthaloyl-(2S,3S)-3-methylphenylalanine,[375] while the (2S,3S)-threonine derivative gives a 40:60 mixture of (2S,3R)- and (2S,3S)-diastereoisomers in the same reaction.[375]

The hydroxy and carboxy groups of N-palmitoyl-hydroxyproline can be condensed intermolecularly to form novel polyesters,[376] and a similar approach based on O-

cyanylation of tyrosine followed by poly(iminocarbonate) formation is described in the same paper. Attack at aromatic and heteroaromatic groupings feature in a study showing the relatively greater propensity of the tyrosine rather than the tryptophan side chain to undergo free-radical hydroxylation (by ClO_2 or NO_2 in alkaline aqueous media, in which HO$^{\bullet}$ is generated).[367] L-Tyrosine offers a starting point for synthesis of substituted dopas through Friedel-Crafts acetylation, reduction of the acetyl group, and rearrangement.[368] $S_{RN}1$ Reactions of protected *S*-mercapto-phenylalanines and -di-iodotyrosines to give phenylthio analogues have been described.[369] The phenyl moiety of phenylalanine is unchanged through reaction of *N*-protected esters with 4-t-butyliodylbenzene, 4-$Bu^tC_6H_4IO_2$, whereas the familiar oxidative changes for tyrosine (→ dopaquinone), tryptophan (→ kynurenine), and histidine (→ γ-formamidoglutamine) are seen for the heteroaromatic amino acids.[370] Conversion of tryptophan into aspartic acid (and of valine into isobutyric acid, and phenylalanine into phenylacetic acid) is the outcome of ruthenium(VIII) oxidation (via $RuCl_3$).[371] Degradation of tryptophan into α-amino-β-carboxymuconate ε-semialdehyde by nicotinamide enzymes is followed by non-enzymic condensation into pyridine-2,3-dicarboxylate in aqueous media at pH > 4.5.[372] D-Tryptophan (formed from the L-enantiomer) is the biosynthetic source for indole-3-acetic acid in *Pisum sativum*.[373]

Conflicting advice about the stability and optimum storage conditions for *S*-adenosyl-L-methionine has been clarified through a study suggesting that epimerization and other processes do not occur in solutions at pH values 3-5.[374] Several papers have appeared on sulphur-containing amino acids (see also ref.3) dealing with conversion of *S*-carboxymethyl-L-cysteine into a diastereoisomeric mixture of sulphoxides with H_2O_2,[375] kinetics of HS^-/cystine reduction and competitive processes,[376] $Br_2/^{18}OH_3^+$ oxidation of L-cystine to cysteic acid (all oxygen atoms are labelled, revealing the intermediacy of a sulphenic-carboxylic anhydride in this reaction),[377] and synthesis of a useful chiral auxiliary thiazolidinethione (63) from L-cysteine (analogous to the oxazolidinethione from L-serine) for use in boron- and tin-mediated aldol reactions (see Section 4.2).[378] Another study of u.v. photolysis of aqueous L-cysteine (254 nm/24 h) has been reported, in which 36 volatile products extractable into dichloromethane were identified.[379] 2-Methyl-thiazole predominated in the group of 5-membered heterocyclic compounds in the product mixture.

Selenocysteine has been converted via its *N*-acetyl-Se-benzyl methylamide into the cyclic seleninamide (64), adding to knowledge of selenium functional-group interconversions.[380]

The guanidine side chain of arginine hydrochloride has been condensed with 4-hydroxybenzil to give the imidazolinone (65).[381]

Introduction of protecting groups is either intended to select for a side-chain function, as in the synthesis of *S*-Fmoc-L-cysteine (deprotection by 50% piperidine in DMF),[382] *S*-acetamidomethyl-*N*-Fmoc-L-cysteine (introduced using $AcNHCH_2OH$,

prepared from $AcNH_2$ + HCHO in aq. KOH),[383] N$^{\omega}$-2,2,5,7,8-pentamethyl chroman-6-sulphonyl-L-arginine (cleavable by 50% trifluoroacetic acid in CH_2Cl_2), [384] and N$^{\tau}$-alkylation of N$^{\tau}$-phenacyl-histidine (removal of the phenacyl group with Zn/AcOH),[385] or is found, unexpectedly, to involve the side chain, as in the formation of N-Fmoc pyroglutamic acid during the reaction of FmocCl with glutamic acid.[386]

6.4 Non-Enzymic Models of Biochemical Processes involving Amino Acids.- Specific interactions of anticodon dinucleoside monophosphates with cognate amino acids, as detected by precise u.v. difference absorbance spectroscopy, are of similar magnitude to base-base stacking interactions.[387]

6.5 Effects of Electromagnetic Radiation on Amino Acids.- The emphasis on photolysis and fluorescence studies for tryptophan continues to provide most of the material for this Section, including haematoporphyrin-sensitized 630nm pulsed laser[388] and related[389] photo-oxidation studies. Fluorescent light irradiation of tryptophan in the presence of riboflavin gives kynurenine and its N-formyl derivative, most rapidly at pH 7.5,[390] as seen also in irradiation in the presence of "oxidizing methyl linoleate"[391] (cf. Vol.19, p.29) and of selectively sulphonated gallium phthalocyanines.[392] Photosensitized oxidation of N-acetyltryptophanamide in neutral aqueous solutions in the presence of various dyes has been shown to involve only singlet oxygen.[393]

The one-electron reduction potential of the oxidized tryptophan radical at neutral pH is greater than that of the oxidized tyrosine radical.[394] Photoinduced electron transfer to cytochrome c from kynurenine and its N-formyl derivative is revealed in reduction under both aerobic and anaerobic conditions.[395]

Fluorescence behaviour of tryptophan has been reviewed.[396] The intermolecular equivalent of one of the tryptophan quenching mechanisms, the fluorescence quenching of indoles by simple amino acids, has been studied from the point of view of the effect of either anionic or cationic micelles.[397]

There is a range of radioprotective and radiosensitizing effects for *E.coli* and mice, shown by sulphur-containing amino acids.[398] Further studies of 265nm laser flash photolysis of dopa and its 5S-cysteinyl and 2,5-S,S-dicysteinyl derivatives have been reported.[399]

7 Analytical Methods

Current methodology in all aspects of amino acid analysis has been reviewed.[400]

7.1 Gas-Liquid Chromatography.- A three-volume set[401] contains thorough coverage of amino acid analysis by g.c. Detailed case studies are included to illustrate the general trends towards derivatization using N(O)-perfluoracylated amino acid

alkyl esters, trends also revealed in the current primary literature.

N-Trifluoroacetyl n-butyl esters have been chosen for the derivatization protocol for analysis of representative amino acids[402–405] and specifically for [1-^{15}N-5,β,β-$^{2}H_3$]- and [1,3-$^{15}N_2$-5,α,β,β-$^{2}H_4$]-N^{im}-carbethoxyhistidine.[406] The purpose of a particularly detailed study[403–405] using these derivatives was to clarify long-standing anxieties concerning artefacts and reliability in g.c. analysis of amino acids. Careful attention to aspects of a protein hydrolysis regime is recommended;[403] if this advice is heeded, excellent agreement with results of ion exchange amino acid analysis is generally achieved.[404,405] N-Heptafluoroisobutyroylamino acid isobutyl esters continue to feature regularly in the literature,[405] and a substantial review of their analytical use has appeared.[407] G.c.-m.s. analysis of 4-hydroxyproline and 5-hydroxypipecolic acid in brain and blood samples using their heptafluoroisobutyroyl methyl esters is applicable at 3-6 nmol cm^{-3} and 20-30 pmol cm^{-3} levels, respectively.[408]

Silylated amino acids have been considered to be less reliable for the present purpose, but the discovery that N(O)-dimethyl-t-butylsilylated amino acids possess satisfactory stability under both preparative and g.c. conditions has led to renewed interest in them.[409–412] One of these studies[410] involves g.c.-m.s. analysis of cysteine, methionine, and homocysteine in serum samples, and another[411] also grasps the nettle of their use for the analysis of the polyfunctional amino acids, lysine, arginine, and histidine. Introduction of the dimethyl-t-butylsilyl moiety into these amino acids using N-methyl N-(dimethyl-t-butylsilyl)trifluoroacetamide by reaction at 150° during 2.5 h does not cause multiple silylation because of the bulk inherent in the group being introduced (the usual reaction protocol involves reaction in MeCN at room temperature).[411] However, two peaks have been reported for g.c. of arginine derivatized in this way,[412] and further refinement of derivative preparation is needed.

Simple acylated derivatives continue to be used in some cases,[413–415] such as N-acetylamino acid n-propyl[413] or isopropyl[414] esters, and these studies include comparisons with perfluorinated analogues. Treatment of a peptide with t-butyl isocyanate and subsequent N-terminal cleavage with HCl in isopropanol provide a new use for g.c. with the oldest N-terminal amino acid analysis method and gives both structure and configuration of the N-terminal residue when Chirasil® (a chiral stationary phase) is employed for the g.c. analysis.[416] Estimation of enantiomeric excesses through this form of "chiral g.c." continues to be developed, with an example closely related to that just described. Transesterification of L-proline benzyl ester with isopropanol and formation of the ureide is followed by g.c. over Chirasil-Val® for enantiomeric analysis of the product.[417] Further examples[418–420] of the use of these commercial phases include glass capillary g.c. of N-acylamino acid isopropyl esters [419] and an application to dating of quaternary mammal fossil teeth based on enantiomer ratios for the eleven amino acids identified in these samples (particularly concentrating on the

D:L ratio for aspartic acid; see also Section 6.1).[420] Exploration of new chiral g.c. phases has been reported through a study of the separation of *N*-trifluoroacetyl-DL-amino acid isopropyl esters over immobilized (*S*)-*N*-phenyl-5-isopropylthiohydantoin,[421] a stationary phase which would normally have been thought to be difficult to prepare in enantiomerically pure form. The alternative approach to g.c. resolution involves creation of diastereoisomeric pairs of derivatives. *N*-Trifluoroacetyl menthyl esters[422] have been used in a study of the D-amino acid content of single-cell proteins, showing that significant amounts are found only for alanine and glutamic acid residues.[423] Further exploration of the variation of this approach in which the partially racemic sample is converted into its *N*-trifluoroacetyl-L-prolyl derivative has been reported.[424] A g.c. study of the conversion of D/L-aspartic acid mixtures into their isoindoles through reaction with *o*-phthaldialdehyde and *N*-acetyl-L-cysteine (a method that has shown promise in the equivalent h.p.l.c. process) concludes that it is superior in this application to the more usual chiral-phase g.c. resolution of *N*-trifluoracetyl isopropyl esters.[425]

7.2 Ion Exchange Chromatography.- Assessment of an improved amino acid analyzer that uses 3μm resin beads and can detect to 10 pmol (using ninhydrin) or 500 fmol levels (using fluorescamine) has been reported.[426] Aspects of existing commercial methodology are being assessed single-handedly,[427-431] for factors that accelerate deterioration,[427] including an alternative solvent for flushing ninhydrin,[428] and the performance of the integrator.[429] A non-instrumental objective is contained in one of these papers, a spurious peak in histidine analyses that is concluded to have been introduced by the buffer.[430]

Application of the Beckman amino acid analyzer for the determination of cystine, cysteine-penicillamine disulphide, and pipecolic acid has been described.[432]

The effect of n-propanol in the cation exchange separation of amino acid esters has been assessed.[433]

7.3 Thin-Layer Chromatography and Related Separation Methods.- Standard applications of t.l.c. have been described in separations of dansylamino acids,[434,435] including nanomole analysis of neurotransmitter amino acids [435] and picomole separations of dansylamino acid methyl esters.[436] Analysis of phenylthiohydantoins by t.l.c. over silica impregnated with zinc, cadmium, and mercury salts,[437] with transition metal ions,[438] or with (+)-tartaric acid[439] has been investigated.

Cysteine and cystine show up as yellow spots on t.l.c., when sprayed with Ellman's reagent [5,5'-bis(dinitrobenzoic acid) disulphide], a useful distinction between the two being the slow time for revelation of the colour with cystine (the cysteine colour is rapidly formed).[440] Cation exchange foils have been used in

t.l.c. of ^{15}N-labelled amino acids.[441] Another less-commonly used variation is over-pressured layer chromatography, which carries a number of advantages in terms of speed and resolution, e.g. in separations of sulphur-containing amino acids.[442]

Calculations of relationships between t.l.c. retention data with amino acid structure have appeared.[443]

Paper chromatography rarely gains an entry to the literature these days, but it has its uses, such as for the analysis of diaminopimelic acid in bacterial cells.[444] Paper electrophoresis offers improved identification of opines (nopaline and octopine) in plant tissues, these N-carboxyalkylamino acids being revealed by u.v.-fluorescent red-purple products formed with phenanthrenequinone.[445]

7.4 High-Performance Liquid Chromatography.- Reviews include a chapter on h.p.l.c. of amino acids and peptides in a useful small book,[446] another on amino acids in a larger treatise,[447] and amperometric detection methods.[448]

Supercritical liquid CO_2 h.p.l.c. is a fundamentally novel approach that is being widely explored in analysis and has been shown to give fast, efficient separation of N-acetylamino acid t-butyl esters on a chiral stationary phase.[449]

H.p.l.c. separation of amino acids and their post-column derivatisation is favoured only by a minority of h.p.l.c. practitioners, though there are benefits associated with the delivery of amino acids free from interfering species into the detector. There are interesting possibilities for pre-column purification or concentration of amino acid samples. The use of a short boronate affinity column for purifying samples before passage to an h.p.l.c. column (estimation of 5-S-cysteinyldopa in urine) [450] and enrichment of tryptophan and tyrosine by pre-column silica bis(thiocarbamate) treatment of samples to which a copper(II) salt had been added [451] are representative of these approaches.

Separation of underivatized amino acids using the ion pair (or "ion interaction") principle, in which the buffer contains a long-chain alkanesulphonic acid,[452] has been compared with discrimination based on crown ether complexation.[453] A related method employing copper(II) alkanesulphonates[454] seems to offer improved resolution. The requirements for the mobile phase in the separation of underivatized amino acids need, and will undoubtedly receive, further study.

The detection of amino acids emerging in such media from the h.p.l.c. column imposes no special problems, and electrochemical methods have been described for cysteine[455] and subnanomole analysis of other amino acids.[456] Estimation of serum methionine[457] using an anodically treated glassy carbon electrode illustrates the continuing efforts towards the creation of tailor-made analytical packages. Post-column o-phthaldialdehyde-mercaptoethanol derivatization of amino acids separated using a sodium alkanesulphonate buffer is typical of the standard general approach in this category of h.p.l.c. amino acid analysis,[458] including a use for the

analysis of total hydrolysates from samples of polyacrylamide gel carrying 5μg or more of protein.[459] The gel + protein sample is hydrolysed in the usual way, with thioglycollic acid added to prevent degradation of tyrosine, cysteine, and methionine.

Brief mention can be made of estimation of tryptophan (219nm absorption),[460] N^{G}-hydroxymethyl-L-arginines (as newly discovered amino acids in serum in urine arising from reaction with endogenous formaldehyde),[461] tyrosine and phenylalanine in dried blood spots after extraction with ethanol,[462] and desmosine and isodesmosine in elastin[463] and other tissue hydrolysates.[464-466] Methylated amino acids and 5-hydroxylysine are also included in one of these reports,[464] which provides picomole level data.

Pre-column derivatization, while requiring more reference citations, needs only limited space here because most of the papers build upon methods that are becoming standard. Out of the ordinary, however, is an assay for N-acetyl-S-(N-alkylthiocarbamoyl)-L-cysteine, the principal plant metabolite from an alkyl isothiocyanate.[467] The process by which the metabolite is formed is reversed for the purpose of analysis, and the resulting isothiocyanate is derivatized into a thiourea using butylamine.

Dansylamino acids[468-470,495] and their dabsyl analogues[471,472] continue to be advocated for routine amino acid analysis. In a comparative study,[472] results for the latter derivatives are shown to be in good agreement with those employing the o-phthaldialdehyde - mercaptoethanol procedure, which is among the most widely used derivatization regimes at the moment.[473-485] Standard applications of the last-mentioned method to 3-methylhistidine,[473,474] 1-aminocyclopropanecarboxylic acid,[475] N^{ε}-trimethyllysine,[476] branched-chain α-amino acids,[477] and samples containing large numbers of amino acids[478] have been described. Papers on this topic with specific attention to practical aspects deal with resolution (narrow-bore versus normal column h.p.l.c.),[479] improved gradient elution,[480] and automated o-phthaldialdehyde-mercaptoethanol analysis.[481]

The other burgeoning application of h.p.l.c. in amino acid analysis concerns pre-column phenylthiocarbamoylation using phenyl isothiocyanate.[486-494] Artefacts arise during (and subsequent to) this operation, and it has been found that ether extraction of the derivatized sample prior to h.p.l.c. leads (paradoxically) to "fewer losses".[486] The literature continues to describe applications of this method, which can now be called routine,[487-490] including estimations of 4-hydroxyproline,[487] phenylalanine and tyrosine.[489] The method has been extended successfully to more problematic cases, phosphorylated[491,492] and sulphated[492] amino acids, N-mono- and N,N-di-methylarginines, methylated lysines, and 3-methylhistidine.[493] The phosphoserine content of proteins[491] is not actually estimated as such, but as S-ethylcysteine (formed by operating on the protein, hydrolysis, and phenylthiocarbamoylation). The tryptophan content of proteins can be estimated by this method without interference from the 0.4% mercaptoethanol

that is routinely included in the 6M hydrochloric acid hydrolysis medium.[494]

There are relatively few papers covering h.p.l.c. analysis of phenylthiohydantoins in non-routine contexts. Among these,[495-498] chemical ionization m.s. studies[496] and analysis at 20-100 fmol levels[498] are featured. There are additions to the experience accumulated over several years of the use of fluorescent derivatives formed with 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole[499] and its 7-fluoro-4-sulphonate analogue.[500] Fmoc derivatives offer several advantages for h.p.l.c. analysis, being introduced under mild conditions and showing readily measurable fluorescence at 313nm (excitation at 260nm), allowing pmol level detection.[501,502] A new form of derivative links the chemiluminescent phthalhydrazide group to the amino group of an amino acid through an isothiocyanate group, and can be detected at the level of 10 fmol per 20μl injected sample.[503] A specific fluorescence-forming reaction between dopa and 1,2-diphenylethylenediamine permits detection at 10 fmol levels.[504]

Estimation of enantiomer ratios using h.p.l.c. continues to develop along lines that have become established over several years. The o-phthaldialdehyde procedure using a chiral thiol is represented in several papers,[505-509] employing N-acetyl-L-cysteine[505-507] in most cases (but 2,3,4,6-tetra-O-acetyl 1-thio-β-D-glucopyranoside has been tried in one study, establishing a fmol level assay[509]). The other main "chiral mobile phase" approach employs the copper(II) - L-proline system for enantiomeric analysis of α-alkyl-lysines and -ornithines (but not ornithine itself)[510] and for simple α-amino acids (but not for γ-aminobutyric acid)[511] or the copper(II) - $(CH_2)_n(L\text{-}NHCHRCO_2H)_2$[512] and simpler copper(II) - L-amino acid systems[513,514] for the resolution of dansylamino acids[512,513] and o-phthaldialdehyde-mercaptoethanol - DL-amino acid condensation products.[513] Chiral stationary phases made in the simplest way by adsorbing a protein on to silica[515] (cf.ref.228) (for the resolution of N-benzoyl-DL-amino acids) or N-alkoxycarbonyl (S)- or (R)-naphthylethylamides[516,517] or related α-arylglycine derivatives[518] or proprietary products ('Chiral-Pak WH'®)517 represent the other major approach.

Comparison of four systems, o-phthaldialdehyde - N-acetyl-L-cysteine, β-cyclodextrin-containing mobile phase, D-phenylglycine-based stationary phase, or Chiral-Pak WH, leads to the conclusion that each has its virtues (and each has its shortcomings).[517]

7.5 Other Analytical Methods.- A multicompartment apparatus for isoelectric-focussing analysis of amino acids has been described.[519]

7.6 Estimation of Specific Amino Acids.- The title of this Section would suggest enzymic methods, but individual amino acids can also be analysed without interference from others by several classical chemical methods.

Oxidative de-amination of branched-chain α-amino acids (valine, leucine, or isoleucine) by leucine dehydrogenase is quantitatively linked to fluorescence

generated by NADH that is part of the reagent.[520] A similar approach but a different physical basis is involved in an L-lysine assay using L-lysine decarboxylase immobilized on a CO_2 sensor,[521] in an L-glutamic acid assay using immobilized L-glutamine synthetase/NH_4^+/ATP (based on the change in pH due to H^+ liberated in the transformation),[522] or conversely in an L-glutamine assay employing an immobilized glutaminase/glutamate oxidase electrode.[523]

γ-Carboxyglutamic acid has been estimated in urine using capillary isotachophoresis.[524]

Colorimetric methods are represented in the estimation of citrulline and N-carbamoyl-β-alanine [525] and spectrophotometric assays for cysteine (as its methylglyoxal derivative)[526] and cystine.[527] Serine, formed from sarcosine as a result of oxidative demethylation by Pseudomonas WRE, can be estimated by conversion into formaldehyde (arising from the β-carbon atom through the Nash reaction) by spectrophotometric methods.[528]

Cystine gives a linear response down to about 10^{-8}M levels in hydrodynamic modulation voltammetry.[529] G.c. estimation of selenomethionine can be based on the quantitative character of the cyanogen bromide reaction that leads to MeSeCN with this amino acid.[530]

REFERENCES

1 "Asymmetric Synthesis: Construction of Chiral Molecules Using Amino Acids", G.M.Coppola and H.F.Schuster, Wiley, New York, 1987.

2 "α-Amino Acids, N-Carboxyanhydrides, and Related Heterocycles; Synthesis, Properties, Peptide Synthesis, and Polymerization", H.R.Kricheldorf, Springer Verlag, Berlin, 1987.

3 Methods in Enzymology, 1987, Vol.143. Representative Chapter contents: T.Ubuka, 17 (Reactions of S-(2-Amino-2-carboxyethylsulphonyl)-L-cysteine leading to S-sulpho-L-cysteine and L-alanine sulphodisulphane); J.D.H.Cooper and D.C.Turnell, 141 (Cysteine and Cystine); F.J.Leinweber and K.J.Monty, 160 (Cysteinesulphinic acid); R.K.Gordon, G.A.Miura, T.Alonso, and P.K.Chiang, 191 (S-Adenosyl-L-methionine); J.H.Fellman, 223; K.Soda, 453 (Microbial metabolism of sulphur-containing amino acids).

4 "Amino Acids in Health and Disease: New Perspectives", UCLA Symposia in Molecular and Cell Biology, New Series, Vol.55, Ed. S.Kaufman, A.R.Liss Inc, New York, 1987.

5 "Escherichia coli, Salmonella typhimurium", edited by F.C.Neidhardt, American Society of Microbiology, Washington DC, 1987, Vol.1. Reviews of biosynthesis of protein amino acids by: N.Glansdorff, 321 (arginine); T.Leisinger, 345 (proline); H.E.Umbarger, 352 (leucine, isoleucine); M.E.Winkler, 368 (histidine); A.J.Pittard, 368 (phenylalanine and tyrosine); G.V.Stauffer, 412 (serine and glycine); N.M.Kredich, 419 (cysteine); G.N.Cohen and I.Saint-Girons, 429 (threonine, lysine and methionine), etc.

6 J.R.Cronin, S.Pizzarello, G.U.Yuen, S.Epstein, and R.V.Krishnamurthy, Nature, 1987, **326**, 477.

7 S.Epstein and E.Zinner, Earth Planet.Sci.Lett., 1987, **84**, 359.

8 T.Wakamiya and T.Shiba, Kagaku, Zokan (Kyoto), 1987, 121.

9 R.Ruembeli, F.Suter, M.Wirth, W.Sidler, and H.Zuber, F.E.B.S. Lett., 1987, **221**, 1.

10 A.V.Klotz and A.N.Glazer, J.Biol.Chem., 1987, **262**, 17350.

11 W.H.Schaefer, T.J.Lukas, I.A.Blair, J.E.Schultz, and D.M.Watterson,

J.Biol.Chem., 1987, **262**, 1025.
12 A.N.E.Birch, L.E.Fellows, and K.Doherty, Phytochemistry, 1986, **25**, 2745.
13 T.Ohyama, Nippon Dojo Hiryogaku Zasshi, 1986, **57**, 300 (Chem.Abs., 1987, **106**, 135303).
14 R.Figlinolo, S.Naylor, J.Wang, and J.H.Langenheim, Phytochemistry, 1987, **26**, 3255.
15 G.P.Jones, B.P.Naidu, L.G.Paleg, E.R.T.Tiekink, and M.R.Snow, Phytochemistry, 1987, **26**, 3343.
16 H.Felbeck and S.Wiley, Biol.Bull.(Woods Hole, Mass.), 1987, **173**, 252.
17 R.Villalobos-Molina, J.P.Pardo, A.Saavedra-Molina, and E.Pina, Biochem. Cell. Biol., 1987, **65**, 1057.
18 J.Stenflo, A.Lundwall, and B.Dahlbaeck, Proc.Natl.Acad.Sci.U.S.A., 1987, **84**, 368.
19 J.Stenflo, A.K.Oehlin, W.G.Owen, and W.J.Schneider, J.Biol.Chem., 1988, **263**, 21.
20 S.C.Fry, J.Exp.Bot., 1987, **38**, 853.
21 H.E.Meyer and G.W.Mayr, Biol.Chem.(Hoppe Seyler's), 1987, **368**, 1607.
22 A.Moriguchi, Y.Hara, and S.Hatanaka, J.Antibiot., 1987, **40**, 904.
23 T.Ohta, S.Nakajima, I.Hatanaka, M.Yamamoto, Y.Shimmen, S.Nishimura, Z.Yamaizumi, and S.Nozoe, Phytochemistry, 1987, **26**, 565.
24 G.Kusano, H.Ogawa, A.Takahashi, S.Nozoe, and K.Yokoyama, Chem.Pharm.Bull., 1987, **35**, 3482.
25 D.Strack, D.Schmitt, H.Reznik, W.Boland, L.Grotjahn, and V.Wray, Phytochemistry, 1987, **26**, 2205.
26 G.N.Austin, P.D.Baird, H.F.Chow, L.E.Fellows, G.W.J.Fleet, R.J.Nash, J.M.Peach, R.J.Pryce, and C.H.Stirton, Tetrahedron, 1987, **43**, 1857.
27 Y.Kawahata, S.Takatsuto, N.Ikekawa, M.Murata, and S.Omura, Chem.Pharm.Bull., 1986, **34**, 3102.
28 S.Omura, M.Murata, N.Imamura, Y.Iwai, and H.Tanaka, J.Antibiot., 1984, **37**, 1324.
29 T.P.Holler, A.Spaltenstein, P.B.Hopkins, E.Turner, R.E.Klevit, and B.M.Shapiro, J.Org.Chem., 1987, **52**, 4420.
30 S.W.Elson, K.H.Baggaley, J.Gillett, S.Holland, N.H.Nicholson, J.T.Sime, and S.R.Woroniecki, J.Chem.Soc.,Chem.Commun., 1987, 1736; for synthesis of proclavanamic acid: K.H.Baggaley, J.T.Sime, N.H.Nicholson, S.W.Elson, J.Gillett, S.Holland, and S.R.Woroniecki, Ibid., p.1738.
31 S.P.F.Miller and J.Thompson, J.Biol.Chem., 1987, **262**, 16109.
32 M.Maeda, T.Kodama, T.Tanaka, H.Yoshizumi, T.Takemoto, K.Nomoto, and T.Fujita, Tetrahedron Lett., 1987, **28**, 633.
33 B.Starcher, G.Cook, P.M.Gallop, E.Hensen, and B.Shoulders, Connect.Tissue Res., 1987, **16**, 15.
34 G.R.Pettit, Y.Kamano, C.L.Herald, A.A.Tuinman, F.E.Boettner, H.Kizu, J.M.Schmidt, L.Baczynskj, K.B.Tomer, and R.J.Bontems, J.Am.Chem.Soc., 1987, **109**, 6883.
35 T.Teshima, M.Nishikawa, I.Kubota, T.Shiba, and S.Omura, Peptide Chemistry 1986, Proceedings of the 24th Symposium on Peptide Chemistry, Peptide Institute, Protein Research Foundation, Osaka, 1986, p.169 (Chem.Abs., 1988, **108**, 3113; 1986, **104**, 55380).
36 A.Tsugita, T.Uchida, H.W.Mewes, and T.Akata, J.Biochem. (Tokyo), 1987, **102**, 1593.
37 J.Taillades, J.Brugidou, R.Pascal, R.Sola, L.Mion, and A.Commeyras, Actual. Chim., 1986, 13
38 Y.Ohfune and N.Kurokawa, Yuki Gosei Kagaku Kyokaishi, 1986, **44**, 647.
39 See for example G.C.Barrett, in "Chemistry and Biochemistry of the Amino Acids", ed. G.C.Barrett, Chapman & Hall, London, 1985, p.276.
40 I.Ojima, J.Mol.Catal., 1986, **37**, 25; M.Tanaka and T.Kobayashi, Kagaku Kogyo Shiryo (Tsukuba-gun, Jpn), 1986, **21**, 35; A.M.Ajami and S.S.Yuan, "Synthesis and Applications of Isotopically-labelled Compounds", Ed.R.R.Muccino, Elsevier, Amsterdam, 1986, p.201.
41 C.Herdeis and R.Gebhard, Heterocycles, 1986, **234**, 1019.
42 J.Taillades, A.Rousset, M.Lasperas and A.Commeyras, Bull.Soc.Chim.Fr., 1986, 650.
43 M.Piovano, M.I.Gauido, P.Fiedler, and J.A.Garbarino, Rev.Latinoam.Quim.,

1987, **18**, 66.
44 P.N.Rao, J.E.Burdett, J.W.Cessac, C.M.DiNunno, D.M.Peterson, and H.K.Kim, Int.J.Pept.Prot.Res., 1987, **29**, 118.
45 H.Hilpert, Helv.Chim.Acta, 1987, **70**, 1307.
46 P.Münster and W.Steglich, Synthesis, 1987, 223.
47 J.R.Grierson and M.J.Adam, J.Labelled Compd.Radiopharm., 1986, **23**, 1019.
48 B.D.Gray and P.W.Jeffs, J.Chem.Soc.,Chem.Commun., 1987, 1329.
49 D.Dauzonne and R.Royer, Synthesis, 1987, 399.
50 S.Fujii, H.Kimoto, T.Okano, H.Muramatsu, and T.Ueda, Nagoya Kogyo Gijutsu Shikenso Hokuku, 1986, **35**, 228.
51 T.Mukaiyama, H.Suzuki, and T.Yamada, Chem.Lett., 1986, 915.
52 M.Mitani, O.Tachizawa, H.Takeuchi, and K.Koyama, Chem.Lett., 1987, 1029.
53 H.J.Zeiss, Tetrahedron Lett., 1987, **28**, 1255.
54 U.Schöllkopf, D.Pettig, U.Busse, E.Egert, and M.Dyrbusch, Synthesis, 1986, 737.
55 U.Schöllkopf, W.Kuehnle, E.Egert, and M.Dyrbusch, Angew.Chem., 1987, **99**, 480.
56 U.Schöllkopf and J.Bardenhagen, Liebigs Ann.Chem., 1987, 393.
57 R.Gull and U.Scholl kopf, Synthesis., 1985, 1052.
58 U.Schöllkopf, R.Hinrichs, and R.Lonsky, Angew.Chem., 1987, **99**, 137.
59 J.Bajgrowicz, A.El Achquar, M.L.Roumestant, C.Pigiere, and P.Viallefont, Heterocycles, 1986, **24**, 2165.
60 U.Schöllkopf, R.Tölle, E.Egert, and M.Nieger, Liebigs Ann.Chem., 1987, 399.
61 I.Ojima and H.-J.C.Chen, J.Chem.Soc.,Chem.Commun., 1987, 625.
62 Yu.N.Belokon, A.G.Bulychev, M.G.Ryzhov, S.V.Vitt, A.S.Batsanov, Yu.T.Struchkov, V.I.Bakhmutov, and V.M.Belikov, J.Chem.Soc.,Perkin Trans.I, 1986, 1865; Yu.N.Belokon, N.I.Chemoglazova, N.S.Garbalinskaya, M.B.Saporovskaya, K.A.Kochetkov, and V.M.Belikov, Izv.Akad.Nauk S.S.S.R., Ser.Khim., 1986, 2340.
63 P.Duhamel, J.J.Eddine, and J.-Y.Valnot, Tetrahedron Lett., 1987, **28**, 3801.
64 K.Suzuki, Y.Hirami, M.Taniai, T.Mohri, C.Goda, R.Fujiyama, and S.Kiyoota, Nippon Kagaku Zasshi, 1987, 186.
65 H.Kunz and W.Sager, Angew.Chem., 1987, **99**, 595.
66 L.D.Arnold, J.C.G.Drover, and J.C.Vederas, J.Am.Chem.Soc., 1987, **109**, 4649.
67 E.I.Karpeskaya, E.S.Levitina, L.F.Godunova, and E.I.Klabunovskii, J.Mol. Catal., 1986, **34**, 129.
68 D.S.Matteson and E.C.Beedle, Tetrahedron Lett., 1987, **28**, 4499.
69 A.Durealt, I.Tranchepain, C.Greck, and J.-C.Depezay, Tetrahedron Lett., 1987, **28**, 3341.
70 D.Seebach, E.Juaristi, D.D.Miller, C.Schickli, and T.Weber, Helv.Chim.Acta, 1987, **70**, 237.
71 D.A.Evans, E.B.Sjögren, A.E.Weber, and R.Elom, Tetrahedron Lett., 1987, **28**, 39.
72 D.A.Evans and A.E.Weber, J.Am.Chem.Soc., 1987, **109**, 7151.
73 D.A.Evans, J.A.Ellman, and R.L.Dorow, Tetrahedron Lett., 1987, **28**, 1123.
74 B.D.Zwick, A.M.Arif, A.T.Patton, and J.A.Gladysz, Angew.Chem., 1987, **99**, 921.
75 A.Karim, A.Mortreux, and F.Petit, J.Organomet.Chem., 1986, **312**, 375; C.R.Landis and J.Halpern, J.Am.Chem.Soc., 1987, **109**, 1746; H.Brunner, A.Knott, M.Kunz, and E.Thalhammer, J.Organomet.Chem., 1986, **308**, 55; W.Vocke, R.Haenel, and F.H.Flöther, Chem.Tech.(Leipzig), 1987, **39**, 123.
76 E.I.Klabunovskii, E.S.Levitina, L.N.Kaigorodova, D.D.Gogoladze, L.F.Godunova, E.I.Karpeiskaya, and G.O.Chivadze, Izv.Akad.Nauk S.S.S.R., Ser.Khim., 1987, 849; Zh.Org.Khim., 1987, **23**, 343.
77 Sh.G.Bitiev, G.V.Cheltsova, E.I.Karpeiskaya, and E.I.Klabunovskii, Izv.Akad.Nauk S.S.S.R., Ser.Khim., 1986, 2479.
78 J.E.Baldwin, R.L.Dyer, S.C.Ng, A.J.Pratt, and M.A.Russell, Tetrahedron Lett., 1987, **28**, 3743.
79 F.Bjorkling, J.Boutelje, M.Hjalmarsson, K.Hult, and T.Norin, J.Chem.Soc., Chem.Commun., 1987, 1041.
80 N.Makiguchi, Kagaku Kogyo, 1987, **38**, 439; D.M.Anderson, in "Biotechnology", Eds. C.P.Hohenberg and H.Sahm, Fischer, Stuttgart, 1987, Vol.1, p.41; K.Soda and H.Tanaka, Ibid., p.93; H.Enei, Hakko to Kogyo, 1986, **44**, 1070.
81 D.J.Rozzell, Methods Enzymol., 1987, **136**, 479.
82 K.Yokozeki, K.Sano, C.Eguchi, H.Iogami, and K.Mitsugi, Agric.Biol.Chem.,

1987, **51**, 729; K.Yokozeki, K.Sano, C.Eguchi, K.Yamada, and K.Mitsugi, Idem, p.363.
83 K.Yokozeki, S.Nakamori, C.Eguchi, K.Yamada, and K.Mitsugi, Agric.Biol.Chem., 1987, **51**, 355; K.Yokozeki, S.Nakamori, S.Yamanaka, C.Eguchi, K.Mitsugi, and F.Yoshinaga, Idem, p.713; K.Yokozeki and K.Kubota, Idem, p.721.
84 E.Schmidt, D.Vasic-Racki, and C.Wandrey, Appl.Microbiol.Biotechnol., 1987, **26**, 42.
85 K.Yokozeki, E.Majima, K.Izawa, and K.Kubota, Agric.Biol.Chem., 1987, **51**, 963.
86 M.Soukup, B.Wipf, E.Hochuli, and H.G.W.Leuenberger, Helv.Chim.Acta, 1987, **70**, 232.
87 K.M.Lee and R.W.Woodard, Misaengmul Kwa Sanop, 1987, **13**, 10.
88 Y.Kokusenya, S.Nakajima, and M.Matsuoka, Denki Kagaku Oyubi Kogyo Butsuri Kagaku, 1987, **55**, 235.
89 K.Tanaka, S.Yoshifuji, and Y.Nitta, Chem.Pharm.Bull., 1986, **34**, 3879.
90 A.J.Ozinskas and G.A.Rosenthal, Bioorg.Chem., 1986, **14**, 157.
91 H.Ripperger, J.Prakt.Chem., 1986, **328**, 719.
92 J.E.Baldwin, M.Otsuka, and P.M.Wallace, Tetrahedron, 1986, **42**, 3097.
93 B.P.Bashyal, H.F.Chow, L.E.Fellows, and G.W.J.Fleet, Tetrahedron, 1987, **43**, 415.
94 K.Hashimoto, K.Konno, H.Shirahama, and T.Matsumoto, Chem.Lett., 1986, 1399.
95 S.Takano, Y.Iwabuchi, and K.Ogasawara, J.Am.Chem.Soc., 1987, **109**, 5523.
96 J.Cooper, D.W.Knight, and P.T.Gallagher, J.Chem.Soc.,Chem.Commun., 1987, 1220.
97 J.E.Baldwin and C.-S.Li, J.Chem.Soc.,Chem.Commun., 1987, 166.
98 S.J.B.Tendler, M.D.Threadgill, and M.J.Tisdale, J.Chem.Soc.,Perkin Trans.I, 1987, 2617.
99 S.Wu, C.Zhou, and Y.Jiang, Synth.Colmmun., 1986, **16**, 1479.
100 A.Fadel and J.Salaun, Tetrahedron Lett., 1987, **28**, 2243.
101 Y.Jiang, Y.Ma, Z.Chen, and G.Li, Yingyong Huaxue, 1987, **4**, 44 (Chem.Abs., 1987, **107**, 198875).
102 M.Paventi and J.T.Edward, Can.J.Chem., 1987, **65**, 282.
103 M.Suzuki, G.F.Orr, and C.H.Stammer, Bioorg.Chem., 1987, **15**, 43.
104 D.Obrecht and H.Heimgartner, Helv.Chim.Acta, 1987, **70**, 102, 329.
105 G.Cipens, V.A.Slavinskaya, D.Sile, D.Kreile, A.Krikis, A.Strautina, and A.Gutmanis, Latv.P.S.R.Zinat.Akad.Vestis,Kim.Ser., 1986, 647.
106 M.Takasaki and K.Harada, Chem.Lett.,1987, 365.
107 D.W.McPherson, K.Rahman, I.Martinez, and P.B.Shevlin, Origins Life Evol. Biosphere, 1987, **17**, 275.
108 N.P.Kuzimina, Yu.A.Matveets, and E.V.Khoroshilova, Khim.Fiz., 1986, **5**, 1168.
109 J.Onoe and T.Kawai, J.Chem.Soc.,Chem.Commun., 1987, 1480.
110 C.Shen, L.Yang, S.L.Miller, and J.Oro, Origins Life Evol.Biosphere, 1987, **17**, 295.
111 J.H.McClendon, Origins Life Evol.Biosphere, 1987, **17**, 401.
112 S.L.Miller, Chem.Scr., 1986, **26B**, 5; A.Brack, Adv.Space Res., 1986, **6**, 17; Idem., Origins Life Evol.Biosphere, 1987, **17**, 367.
113 J.Pospisek and K.Blaha, Collect.Czech.Chem.Commun., 1987, **52**, 514.
114 M.P.Bueno, C.Cativiela, C.Fimol, J.A.Mayoral, and C.Jaime, Can.J.Chem., 1987, **65**, 2182.
115 N.Sakura, K.Hirose, and T.Hashimoto, Chem.Pharm.Bull., 1986, **34**, 3506.
116 H.L.Sham, H.Stein, and J.Cohen, J.Chem.Soc.,Chem.Commun., 1987, 1792.
117 H.L.Sham, C.A.Rempel, H.Stein, and J.Cohen, J.Chem.Soc., Chem.Commun., 1987, 683.
118 M.Thaniung and L.G.Wistrand, Helv.Chim.Acta, 1986, **69**, 1711.
119 Y.Vo-Quang, D.Marais, L.Vo-Quang, F.Le Goffic, A.Thiery, M.Maestracci, A.Arnaud, and P.Galzy, Tetrahedron Lett., 1987, **28**, 4057.
120 K.Kawada and T.Tsushima, Kagaku Kogyo, 1987, **38**, 164.
121 M.Akhtar, M.A.Cohen, and D.Gani, Tetrahedron Lett., 1987, **28**, 2413.
122 K.H.Hsieh, P.Needleman, and G.R.Marshall, J.Med.Chem., 1987, **30**, 1097.
123 S.Fujii, H.Kimoto, T.Okano, H.Muramatsu, and T.Ueda, Nagoya Kogyo Gijitsu Shikenso Hokoku, 1986, **35**, 107 (Chem.Abs., 1987, **106**, 176831).

124 Y.Kokusenya and M.Matsuoka, Denki Kagaku Oyobi Kogyo Butsuri Kagaku, 1987, 55, 174 (Chem.Abs., 1987, **107**, 134645).
125 Y.Kokusenya and M.Matsuoka, Denki Kagaku Oyobi Kogyo Butsuri Kagaku, 1987, 55, 257.
126 H.Kuzuhara, N.Watanabe, and M.Ando, J.Chem.Soc.,Chem.Commun., 1987, 95.
127 J.Wityak, S.J.Gould, S.J.Hein, and D.A.Keszler, J.Org.Chem., 1987, **52**, 2179.
128 M.Hasan, Stud.Org.Chem.(Amsterdam), 1986, **26**, 123.,
129 J.A.J.M.Vekemans, R.G.M.De Bruyn, R.C.H.M.Caris, A.J.P.M.Kokx, J.J.H.G. Konings, E.F.Godefroi, and G.J.F.Chittenden, J.Org.Chem., 1987, **52**, 1093.
130 K.Clinch, A.Vasella, and R.Schauer, Tetrahedron Lett., 1987, **28**, 6425.
131 B.P.Bashyal, H.F.Chow, and G.W.J.Fleet, Tetrahedron, 1987, **43**, 423; see also ref.93.
132 N.Passerat and J.Bolte, Tetrahedron Lett., 1987, **28**, 1277.
133 C.Shin, Y.Yonezawa, and T.Obara, Heterocycles, 1986, **24**, 1561.
134 C.Shin, T.Obara, S.Segami, and Y.Yonezawa, Tetrahedron Lett., 1987, **28**, 3827.
135 T.Weber, R.Aeschimann, T.Maetzke, and D.Seebach, Helv.Chim.Acta, 1986, **69**, 1365.
136 N.A.Thornberry, H.G.Bull, D.Taub, W.J.Greenlee, A.A.Patchett, and E.H.Cordes, J.Am.Chem.Soc., 1987, **109**, 7543.
137 R.G.Shea, J.N.Fitzner, J.E.Fankhauser, A.Spattenstein, P.A.Carpino, R.M.Peevey, D.V.Pratt, B.J.Tenge,and P.B.Hopkins, J.Org.Chem., 1986, **51**, 5243.
138 S.Bory, M.Gaudry, and A.Marquet, Nouv.J.Chem., 1986, **10**, 709.
139 J.E.Baldwin, R.M.Adlington, and N.G.Robinson, J.Chem.Soc.,Chem.Commun., 1987, 153.
140 A.Evidente, G.Marino, G.Randazzo, G.Sannia, C.Bertucci, and C.Rosini, Chem.Scr., 1986, **26**, 359.
141 D.G.Melillo, R.D.Larsen, D.J.Mathre, W.F.Shukis, A.W.Wood, and J.R.Colleluori, J.Org.Chem., 1987, **52**, 5143.
142 O.Kanamitsu, N.Kitajima, and I.Nagoya, J.Ferment.Technol., 1987, **65**, 395.
143 U.Madsen, K.Schaumburg, L.Brehm, D.R.Curtis, and P.Krogsgaard-Larsen, Acta Chem.Scand.,Ser.B, 1986, **B40**, 92.
144 J.C.Hodges, Synthesis., 1987, 20
145 G.Goto, K.Kawakita, T.Okutani, and T.Miki, Chem.Pharm.Bull., 1986, **34**, 3202.
146 T.Kolasa, S.Sharma, and M.J.Miller, Tetrahedron Lett., 1987, **28**, 4973.
147 T.Kolasa and M.J.Miller, J.Org.Chem., 1987, **52**, 4978.
148 R.W.Feenstra, E.H.M.Stokkingreef, R.J.F.Nivard, and H.C.J.Ottenheijm, Tetrahedron Lett., 1987, **28**, 1215.
149 S.Murahashi and T.Shiota, Tetrahedron Lett., 1987, **28**, 6469.
150 M.J.Milewska and A.Chimiak, Tetrahedron Lett., 1987, **28**, 1817.
151 J.Viret, J.Gabard, and A.Collet, Tetrahedron, 1987, **43**, 891.
152 W.Oppolzer and R.Moretti, Helv.Chim.Acta, 1986, **69**, 1923.
153 A.E.Miller and J.J.Bischoff, Synthesis, 1986, 777.
154 T.Hertzberg, T.Kuehler, and M.Nilsson, Acta Chem.Scand.,Ser B, 1986, **B40**, 387.
155 A.Oku, N.Horiie, and T.Harada, Bull.Chem.Soc.Jpn., 1987, **60**, 609.
156 H.T.Nagasawa, J.A.Elberling, and J.C.Roberts, J.Med.Chem., 1987, **30**, 1373.
157 J.Matos, F.M.Raushel, and C.H.Wong, Biotechnol.Appl.Biochem., 1987, **9**, 39.
158 H.J.Reich and C.P.Jasperse, J.Am.Chem.Soc., 1987, **109**, 5549.
159 D.H.R.Barton, D.Bridon, Y.Herve, P.Potier, J.Thierry, and S.Z.Zard, Tetrahedron, 1986, **42**, 4983.
160 A.D.Mikityuk, Yu.A.Strepikheev, B.A.Kashemirov, and P.S.Khokhov, Zh.Obshch. Khim., 1987, **57**, 305.
161 D.H.Kindler and M.M.Ames, J.Org.Chem., 1987, **52**, 2452.
162 P.Serafinowskowski, Nucleic Acids Res., 1987, **15**, 1121.
163 H.Yamada, K.Kuruyama, T.Eguchi, and M.Kajiwara, J.Labelled Compd. Radiopharm., 1987, **24**, 561.
164 P.J.Reider, R.S.E.Conn, P.Davis, V.J.Grenda, A.J.Zambito, and E.J.J.Grabowski, J.Org.Chem., 1987, **52**, 3326.
165 A.Haedener and C.Tamm, J.Labelled Compd.Radiopharm., 1987, **24**, 1291.
166 Q.Shen and H.Li, Hejishu, 1987, **10**, 48.

167 J.P.Beaucourt, J.Labelled Compd.Radiopharm., 1986, **23**, 553.
168 D.Ego, J.P.Beaucourt, and L.Pichat, J.Labelled Compd.Radiopharm., 1986, **23**, 229.
169 P.Johnstroem, S.Stone-Elander, K.Ericson, M.Mosskin, and M.Bergstroem, Appl.Radiat.Isot., 1987, **38**, 729.
170 C.Halldin, K.O.Schoeps, S.Stone-Elander, and F.A.Wiesel, Eur.J.Nucl.Med., 1987, **13**, 288.
171 R.Iwata, T.Ido, T.Takahashi, H.Nakanishi, and S.Iida, Appl.Radiat.Isot., 1987, **38**, 97.
172 J.R.Ropchan, A.R.Ricci, G.C.Low, M.E.Phelps, and J.R.Barrio, Appl.Radiat.Isot., 1986, **37**, 1013.
173 J.M.Bolster, W.Vaalburg, P.H.Elsinga, H.Wynberg, and M.G.Woldring, Appl.Radiat.Isot., 1986, **37**, 1069.
174 G.Antoni and B.Laengstroem, Acta Chem.Scand.,Ser.B, 1986, **B40**, 152.
175 G.Antoni and B.Laengstroem, Appl.Radiat.Isot., 1987, **38**, 655.
176 C.Halldin and B.Laengstroem, J.Labelled Compd.Radiopharm., 1986, **23**, 715.
177 J.E.Baldwin, S.C.Ng, A.J.Pratt, M.A.Russell, and R.L.Dyer, Tetrahedron Lett., 1987, **28**, 2303.
178 T.E.Walker and R.E.London, Appl.Environ.Microbiol., 1987, **53**, 92.
179 B.R.Branchini, F.G.Prendergast, G.A.Spencer, J.D.Hugdahl, B.D.Ray, and M.D.Kemple, J.Labelled Compd.Radiopharm., 1987, **24**, 637.
180 N.D.Ledford, C.R.Cutcliff, H.B.Wood, and M.Barfield, Org.Prep.Proced.Int., 1987, **19**, 209.
181 H.Parnes, E.J.Shelton, and G.T.Huang, Int.J.Pept.Prot.Res., 1986, **28**, 403.
182 Z.Yan, D.Feng, Y.Chen, and H.Sheng, Youji Huaxue, 1987, 216 (Chem.Abs., 1987, **108**, 22234).
183 T.Elbert, Isotopy, 1985, **26**, 153 (Chem.Abs., 1987, **107**, 198863).
184 R.H.D.Lambrecht, G.Slegers, G.Mannens, and A.Claeys, Enzyme Microb.Technol., 1987, **9**, 221.
185 E.Presecan, A.Ivanof, A.Mocanu, N.Palibroda, M.Bologa, V.Gorun, M.Oarga, and O,Barzu, Enzyme Microb.Technol., 1987, **9**, 663.
186 T.Furuta, Y.Kasuya, H.Takahashi, and S.Baba, J.Chem.Res.,Synop., 1987, 86.
187 T.Chaly and M.Diksic, J.Nucl.Med., 1986, **27**, 1896.
188 M.J.Adam, T.J.Ruth, J.R.Grierson, B.Abeysekera, and B.D.Palc, J.Nucl.Med., 1986, **27**, 1462.
189 P.H.Stern and R.M.Hoffman, Anal.Biochem., 1986, **158**, 408.
190 S.Chen, S.Bai, X.He, F.Wang, and Y.Ma, Yuanzineng Kexue Jishu, 1986, **20**, 136.
191 A.Plenevaux, R.Cantineau, M.Guillaume, L.Christaens, and G.Tihange, Appl.Radiat.Isot., 1987, **38**, 59.
192 J.D.Weinstein, S.M.Mayer, and S.I.Beale, Plant Physiol., 1987, **84**, 244.
193 A.Kah and D.Doernemann, Z.Naturforsch.,C: Biosci., 1987, **42**, 209.
194 A.Dureault, I.Tranchepain, and J.-C.Depezay, Synthesis, 1987, 491.
195 J.P.Wolf and H.Pfander, Helv.Chim.Acta, 1987, **70**, 116.
196 S.Takano, M.Yanase, Y.Sekiguchi, and K.Ogasawara, Tetrahedron Lett., 1987, **28**, 1783.
197 K.Barlos, P.Mamos, D.Papaioannou, and S.Patrianakou, J.Chem.Soc.,Chem.Commun., 1987, 1583.
198 D.Harris, K.L.Bhat, and M.M.Jouillie, Tetrahedron Lett., 1987, **28**, 2837.
199 R.G.Andrew, R.E.Conrow, J.D.Elliott, W.S.Johnson, and S.Ramezani, Tetrahedron Lett., 1987, **28**, 6535.
200 H.Kogen and T.Nishi, J.Chem.Soc.,Chem.Commun., 1987, 311.
201 M.Sakaitani and Y.Ohfune, Tetrahedron Lett., 1987, **28**, 3987.
202 P.Jouin, B.Castro, and D.Nisato, J.Chem.Soc.,Perkin Trans.I, 1987, 1177.
203 J.D.Aebi, M.K.Dhaon, and D.H.Rich, J.Org.Chem., 1987, **52**, 2881.
204 D.A.Evans and A.E.Weber, J.Am.Chem.Soc., 1986, **108**, 6757.
205 U.Schmidt and W.Siegel, Tetrahedron Lett., 1987, **28**, 2849.
206 R.D.Tung and D.H.Rich, Tetrahedron Lett., 1987, **28**, 1139.
207 N.Yamasaki, M.Murakami, and T.Mukaiyama, Chem.Lett., 1986, 1013.
208 J.M.Brown, A.P.James, and L.M.Prior, Tetrahedron Lett., 1987, **28**, 2179.
209 M.Seki, H.Kubota, T.Moriya, M.Yamagishi, S.Nishimoto, and K.Matsumoto, Chem.Pharm.Bull., 1986, **34**, 4516.
210 D.Seebach and H.Esterman, Tetrahedron Lett., 1987, **28**, 3103.

211 U.R.Joshi and P.A.Limaye, Indian J.Chem.,Sect.B, 1986, **25B**, 1176.
212 S.M.Brandstadter, I.Ojima, and K.Hirai, Tetrahedron Lett., 1987, **28**, 613.
213 R.Yoshioka, M.Tohyama, O.Ohtsuki, S.Yamada, and I.Chibata, Bull.Chem.Soc. Jpn., 1987. **60**, 649.
214 W.H.Pirkle and P.S.Reno, J.Am.Chem.Soc., 1987, **109**, 7189.
215 T.Shiraiwa, H.Miyazaki, T.Imai, M.Sunami, and H.Kurokawa, Bull.Chem.Soc. Jpn., 1987, **60**, 661.
216 T.Shiraiwa, H.Miyazaki, A.Uramoto, M.Sunami, and H.Kurokawa, Bull.Soc.Chem. Jpn., 1987, **60**, 1465.
217 T.Shiraiwa, H.Miyazaki, Y.Sakamoto, and H.Kurokawa, Bull.Chem.Soc.Jpn., 1986, **59**, 2331.
218 T.Shiraiwa, H.Yoshida, K.Mashima, and H.Kurokawa, Nippon Kagaku Kaishi, 1986, **6**, 177.
219 T.Shiraiwa, Y.Sado, M.Komure, and H.Kurokawa, Chem.Lett., 1987, 621.
220 T.Fukuhara, M.Isoyama, A.Shimada, M.Itoh, and S.Yuasa, J.Chromatogr., 1987, **387**, 562.
221 E.Alvira, L.Vega, and C.Girardet, Chem.Phys., 1987, **118**, 233.
222 F.Tsvetkov and U.Mingelgrin, Clays Clay Miner., 1987, **35**, 391.
223 Y.Dobashi and S.Hara, J.Org.Chem., 1987, **52**, 2490.
224 W.F.Lindner and I.Hirschboek, J.Liq.Chromatogr., 1986, **9**, 551.
225 W.H.Pirkle and T.C.Pochapsky, J.Am.Chem.Soc., 1987, **109**, 5975.
226 W.H.Pirkle, T.C.Pochapsky, G.S.Mahler, D.E.Corey, D.S.Reno, and D.M.Alessi, J.Org.Chem., 1986, **51**, 4991.
227 N.Oi and H.Kitahara, J.Liq.Chromatogr., 1986, **9**, 511.
228 I.Cho, K.M.Whang, and J.G.Park, J.Polym.Sci.,Part C:Polym.Lett., 1987, **25**, 19.
229 M.T.Aubel and L.B.Rogers, J.Chromatogr., 1987, **408**, 99.
230 K.Ravichandran and L.B.Rogers, J.Chromatogr., 1987, **402**, 49.
231 T.Shinbo, T.Yamaguchi, K.Nishimura, and M.Segiura, J.Chromatogr., 1987, **405**, 145.
232 Y.Yoichi, K.Saigo, K.Tachibana, and M.Hasegawa, Chem.Lett., 1986, 1347.
233 R.Chenevert and M.Letourneau, Chem.Lett., 1986, 1151.
234 B.I.Glaenzev, K.Faber, and H.Griengl, Tetrahedron, 1987, **43**, 771.,
235 I.Minematsu, Y.Shimohigashi, M.Waki, and N.Izumiya, Mem.Fac.Sci.,Kyushi Univ.,Ser.C, 1986, **15**, 269 (Chem.Abs., 1987, **107**, 7545).
236 S.F.Mason, Biochem.Pharmacol., 1988, **37**, 1.
237 G.E.Tranter, Nachr.Chem.Tech.Lab., 1986, **34**, 866, 871, 874, 876.
238 D.K.Kondepudi, Biosystems, 1987, **20**, 75.
239 E.Conte, Nuovo Cimento Soc.Ital.Fis.D, 1987, **9D**, 497.
240 W.J.Meiring, Nature, 1987, **329**, 712.
241 E.Conte and M.Pieralice, Nuovo Cimento Soc.Ital.Fis.D, 1987, **9D**, 283.
242 F.C.Frank, Biochim.Biophys.Acta, 1953, **11**, 459.
243 V.Babovic, I.Gutman, and S.Jokic, Z.Naturforsch.A. Phys.Sci., 1987, **42**, 1024.
244 E.Paparinasam, S.Natarajan, and N.C.Shivaprakash, Int.J.Pept.Prot.Res., 1986, **28**, 525.
245 P.Toffoli, P.Khodadad, N.Rodier, and J.Astoin, Acta Crystallogr.,Sect.C: Cryst.Struct.Commun., 1987, **C43**, 945.
246 B.Chevrier, D.Moras, V.L.R.Jeannoda, E.E.Creppy, and G.Dirheimer, Acta Crystallogr.,Sect.C: Cryst.Struct.Commun., 1986, **C42**, 1632.
247 A.H.J.Wang and S.K.Burley, Acta Crystallogr.,Sect.C: Cryst.Struct.Commun., 1987, **C43**, 1011.
248 V.Nastopoulos, G.Germain, P.Cordopatis, and S.Voliotis, Acta Crystallogr., Sect.C: Cryst.Struct.Commun., 1987, **C43**, 375.
249 C.P.Huber, Acta Crystallogr.,Sect.C: Cryst.Struct.Commun., 1987, **C43**, 902.
250 S.K.Burley and A.H.J.Wang, Acta Crystallogr.,Sect.C: Cryst.Struct.Commun., 1987, **C43**, 797.
251 M.Coll, J.A.Subirama, X.Solans, M.Font-Altaba, and R.Mayer, Int.J.Pept. Prot.Res., 1987, **29**, 708.
252 V.Busetti, H.C.J.Ottenheijm, H.J.M.Zeegers, D.Ajo, and M.Casavin, Recl.Trav.Chim.Pays-Bas, 1987, **106**, 151.

253 P.Gordopatis, S.Voliotis, V.Nastopoulos, G.Germain, and P.Main, Acta Crystallogr., Sect.C: Cryst.Struct.Commun., 1987, **C43**, 344.
254 Y.Yokomori, D.W.Deerfield, R.G.Hiskey, and D.J.Hodgson, Acta Crystallogr., Sect.C: Cryst.Struct.Commun., 1987, **C43**, 1567.
255 S.Talluri, G.T.Montelione, G.Van Duyne, L.Piela, J.Clardy, and H.A.Scheraga, J.Am.Chem.Soc., 1987, **109**, 4473.
256 K.Kabato and Y.Sasaki, J.Chem.Soc.,Chem.Commun., 1987, 670.
257 F.Mitte, R.Mensinger, and R.Borsdorf, Z.Chem., 1986, **26**, 107.
258 N.Koda, S.Yamaguchi, and T.Mori, Tokyo Jikeikai Ika Daigaku Zasshi, 1987, **102**, 1263, 1269 (Chem.Abs., 1988, **108**, 2889).
259 E.Pederoso, A.Grandas, and M.Dolors-Ludevid, J.Heterocycl.Chem., 1986, **23**, 921.
260 V.I.Dubrovin, S.V.Tarabakin, and T.A.Smetanina, Khim.-Farm.Zh., 1987, **21**, 877.
261 M.S.Morales-Rios and P.Joseph-Nathan, J.Heterocycl.Chem., 1986, **23**, 1617.
262 C.A.McDowell, A.Naito, D.L.Sastry, and K.Takegoshi, J.Magn.Reson., 1986, **69**, 283.
263 B.V.R.R.K.Murty, G.K.Kishore, D.K.Rao, and G.Satanandam, Pramana, 1987, **28**, 45.
264 J.Dorie, J.P.Gouesnard, B.Mechin, and P.Mellet, J.Mol.Struct., 1986, **144**, 185.
265 D.Krois and H.Lerner, Monatsh.Chem., 1986, **117**, 1205.
266 S.Higuchi, K.Tanaka, and S.Tanaka, Nippon Kagaku Kaishi, 1986, 1632.
267 T.Wakamiya, T.Teshima, and T.Shiba, Iyo Masu Kenkyukai Koenshu, 1986, **11**, 35.
268 S.Okude, F.Matsushima, H.Kuze, and T.Shimizu, Jpn.J.Appl.Phys., Part I, 1987, **26**, 627.
269 D.Holtkamp, H.Kempken, P.Kluesener, and A.Benninghaven, Springer Proc.Phys., 1986, **9**, 62 (Chem.Abs., 1987, **107**, 97073).
270 G.R.Pettit, G.M.Cragg, C.W.Holzapfel, A.A.Tuinman, and D.P.Gieschen, Anal. Biochem., 1987, **162**, 236.
271 G.Bojesen, J.Am.Chem.Soc., 1987, **109**, 5557.
272 A.Kolbe and A.Kolbe, Z.Chem., 1987, **27**, 74.
273 C.I.Jose, A.A.Belhakar, and M.S.Agashe, Biopolymers, 1987, **26**, 1315.
274 H.Matsuura, K.Hasegawa, amd T.Miyazawa, Spectrochim.Acta, Part A, 1986, **42A**, 1181.
275 F.Lopez-Arbeloa, R.Goedewieck, F.Ruttens, F.C.De Schryver and M.Sisido, J.Am.Chem.Soc., 1987, **109**, 3068.
276 T.A.Hatton, A.C.S. Symp.Ser., 1987, **342**, 170.
277 M.Housana, Kemikaru Enjiniyaringu, 1986, **31**, 965 (Chem.Abs., 1987, **107**, 35805).
278 K.E.Joergensen and M.I.Sheikh, Biochem.J., 1987, **248**, 533.
279 S.Inokuma, T.Hayase, and T.Kuwamura, Yukagaku, 1987, **36**, 487 (Chem.Abs., 1988, **108**, 33666).
280 P.M.Hardy and A.E.Langran-Goldsmith, Int.J.Pept.Prot.Res., 1986, **28**, 230.
281 L.R.Aleksandrova, V.N.Andreev, V.A.Bogdanovskaya, and A.Yu.Safronov, Elektrokhimiya, 1987, **23**, 276 (Chem.Abs., 1987, **106**, 145926).
282 G.Horanyi and E.M.Rizmayer, Electrochim.Acta, 1987, **32**, 433 (Chem.Abs., 1987, **106**, 145941).
283 S.Miyagishi, S.Matsumura, T.Asakawa, and M.Nishida, Bull.Chem.Soc.Jpn., 1986, **59**, 557.
284 G.Barone, G.Castronuovo, V.Elia, and C.Giancola, J.Therm.Anal., 1985, **30**, 1367.
285 W.W.Wendlandt, Thermochim.Acta, 1987, **114**, 381.
286 J.D.Ewbank, V.J.Klimkowski, K.Siam, and L.Schafer, J.Mol.Struct., 1987, **160**, 275.
287 A.-M.Sapse, L.Mallah-Levy, S.B.Daniels, and B.W.Erickson, J.Am.Chem.Soc., 1987, **109**, 3526.
288 U.C.Singh, F.K.Brown, P.A.Bash, and P.A.Kollman, J.Am.Chem.Soc., 1987, **109**, 1607.
289 J.A.Sordo, M.Robst, G.Corongiu, S.Chin, and E.Clementi, J.Am.Chem.Soc., 1987, **109**, 1702.
290 R.W.L.Kimber and C.V.Griffin, Geochim.Cosmochim.Acta, 1987, **51**, 839.

291 G.A.Goodfriend, Quat.Res., 1987, **28**, 374.
292 A.Julg, R.Lafont, and G.Perinet, Quat.Sci.Rev., 1987, **6**, 25.
293 L.Van Meervelt, V.C.V.Tijd., 1986, **13**, 4 (Chem.Abs., 1987, **106**, 175320).
294 Yu.N.Belokon, V.I.Tavarov, T.F.Saveleva, M.B.Saporovskaya, and V.M.Belikov, Makromol.Chem., 1986, **187**, 1065.
295 I.A.Yamskov, T.V.Tikhonova, V.E.Tikhonov, and V.A.Davankov, Izv.Akad.Nauk S.S.S.R.,Ser.Khim., 1986, 1593.
296 X.Ding and Q.Jiang, Huaxue Shijie, 1986, **27**, 545 (Chem.Abs., 1987, **107**, 78220).
297 I.Tabushi, Y.Kuroda, and M.Yamada, Tetrahedron Lett., 1987, **28**, 5095.
298 W.D.Lubell and H.Rapoport, J.Am.Chem.Soc., 1987, **109**, 236.
299 L.A.Carpino, Acc.Chem.Res., 1987, **20**, 401.
300 R.C.de L.Milton, E.Becker, S.C.F.Milton, J.E.J.Baxter, and J.F.Elsworth, Int.J.Pept.Prot.Res., 1987, **30**, 431.
301 L.Kisfaludy and L.Otvos, Synthesis, 1987, 510.
302 W.J.Paleveda, F.W.Holly, and D.F.Veber, Org.Synth., 1985, **63**, 171.
303 P.I.Svirskaya, C.C.Leznoff, and M.Steinnan, J.Org.Chem., 1987, **52**, 1362.
304 E.Wuensch, W.Graf, O.Keller, W.Keller, and G.Wersin, Synthesis, 1986, 958.
305 J.Metzger and G.Jung, Liebigs Ann.Chem., 1987, 895.
306 S.Kirkeley, P.Jakobsen and D.Moe, Anal.Lett., 1987, **20**, 303.
307 W.Greenaway and F.R.Whatley, J.Chromatogr., 1987, **409**, 383.
308 H.O.Kim, R.K.Olsen, and O.S.Choi, J.Org.Chem., 1987, **52**, 4531.
309 T.Chiba, J.Sakaki, and C.Kaneko, Yakugaki Zasshi, 1986, **106**, 154.
310 T.Munegumi, M.Fujita, T.Maruyama, S.Shiono, M.Takasaki, and K.Harada, Bull.Chem.Soc.Jpn., 1987, **60**, 249.
311 H.Kunz and H.-G.Lerchen, Tetrahedron Lett., 1987, **28**, 1873.
312 A.Castro, E.Iglesias, J.R.Leis, J.V.Tato, F.Meijide, and M.E.Pena, J.Chem. Soc.,Perkin Trans.II, 1987, 651.
313 S.Ram and L.D.Spicer, Tetrahedron Lett., 1987, **28**, 515.
314 A.Aramendia, V.Borau, C.Jimenet, J.M.Marinas, and M.E.Sempere, Can.J.Chem., 1987, **65**, 2791.
315 A.Ballio, Gazz.Chim.Ital., 1986, **116**, 519.
316 M.Tamura, J.Jacyno, and C.H.Stammer, Tetrahedron Lett., 1986, **27**, 5435.
317 I.Ruser and D.Chochkova, Kim.Ind.(Sofia), 1986, **58**, 445.
318 J.H.Jones and M.E.Wood, Synth.Commun., 1986, **16**, 1515.
319 D.Cantacazune, F.Pascal, and C.Guerreiro, Tetrahedron, 1987, **43**, 1823.
320 D.Cantacazune and C.Guerreiro, Tetrahedron Lett., 1987, **28**, 5153.
321 S.Plaue and D.Heissler, Tetrahedron Lett., 1987, **28**, 1401.
322 A.A.Mazurov, S.V.Antonenko, and S.A.Andronati, Khim.Prir.Soedin., 1986, 224.
323 M.Jaonadi, J.Martinez, B.Castro, G.Barcelo, G.Sennyey, and J.-P.Senet, J.Org.Chem., 1987, **52**, 2364.
324 P.Jouin, B.Castro, C.Zeggaf, A.Pantaloni, J.-P.Senet, S.Lecoller, and G.Sennyey, Tetrahedron Lett., 1987, **28**, 1661, 1665.
325 F.M.F.Chen and N.L.Benoiton, Can.J.Chem., 1987, **65**, 619.
326 F.M.F.Chen and N.L.Benoiton, Can.J.Chem., 1987, **65**, 1228.
327 F.M.F.Chen, Y.Lee, R.Steinauer, and N.L.Benoiton, Can.J.Chem., 613.
328 J.Cabre Castellvi and A.Palomo Coll, Afinidad, 1986, **43**, 401.
329 W.Gruszecki, M.Gruszecka, and H.Bradaczek, Liebigs Ann.Chem., 1987, 483.
330 A.Ookawa and K.Soai, J.Chem.Soc., Perkin I, 1987, 1465.
331 P.Renaud and D.Seebach, Helv.Chim.Acta, 1986, **69**, 1704.
332 K.Kamiyama, Y.Urano, S.Kobayashi, and M.Ohno, Tetrahedron Lett., 1987, **28**, 3123.
333 R.H.Schlessinger and E.J.Iwanowicz, Tetrahedron Lett., 1987, **28**, 2083.
334 R.B.Bates, F.A.Fletcher, K.D.Janda, and W.A.Miller, J.Org.Chem., 1984, **49**, 3038.
335 M.Muneer, R.K.Tikare, P.V.Kamat, and M.V.George, Can.J.Chem., 1987, **65**, 1624.
336 N.Kuramoto, T.Saki, K.Kato, K.Nagai, and S.Hayama, J.Colloid Interface Sci., 1987, **15**, 595.
337 R.Ueoka, Y.Matsumoto, T.Yoshino, N.Watanabe, K.Omura, and Y.Murakami, Chem. Lett., 1986, 1743.
338 R.Andriamanampisoa, B.Boyer, G.Lamaty, and J.P.Roque, Tetrahedron, 1987, **43**, 77.
339 Y.Ihara, E.Nakanishi, M.Nango, and J.Koga, Bull.Chem.Soc.Jpn., 1986, **59**,

1901.
340 D.S.Mahadevappa and A.S.Puttaswamy, Indian J.Chem.Sect.A, 1987, **26A**, 33.
341 D.Laloo and M.K.Mahanti, Afinidad, 1987, **44**, 324.
342 M.S.Ramachandran and T.S.Vivekamandam, Bull.Chem.Soc.Jpn., 1987, **60**, 3397.
343 B.T.Gowda and B.S.Sherigara, Oxid.Commun., 1986, **9**, 165.
344 L.M.Benzing-Purdic and J.A.Ripmeister, J.Carbohydr.Chem., 1987, **6**, 87.
345 D.H.R.Barton, J.Guilhem, Y.Herve, P.Potier, and J.Thierry, Tetrahedron Lett., 1987, **28**, 1413.
346 M.F.Aly, H.Ardill, R.Grigg, S.Leong-Ling, S.Rajviroongit, and S.S.Surendrakumar, Tetrahedron Lett., 1987, **28**, 6077.
347 E.Vedejs, J.W.Grisson, and J.K.Preston, J.Org.Chem., 1987, **52**, 3487.
348 H.Ardill, R.Grigg, V.Sridharan, and J.Malone, J.Chem.Soc.,Chem.Commun., 1987, 1296.
349 O.Yebdri and F.Texier, J.Heterocycl.Chem., 1986, **23**, 809.
350 S.Kim, S.B.Chang, and P.H.Lee, Tetrahedron Lett., 1987, **28**, 2735.
351 V.A.Burgess and C.J.Easton, Tetrahedron Lett., 1987, **28**, 2747.
352 T.Okamoto, M.Shimada, and S.Oka, Chem.Lett., 1987, 817.
353 S.Yoshifuji, K.Tanaka, F.Kawals, and Y.Nitta, Chem.Pharm.Bull., 1986, **34**, 3873.
354 M.Lee and J.W.Lown, J.Org.Chem., 1987, **52**, 5717.
355 J.E.Baldwin, M.North, and A.Flinn, Tetrahedron Lett., 1987, **28**, 3167.
356 J.E.Baldwin and A.Flinn, Tetrahedron Lett., 1987, **28**, 3605.
357 R.Bhatt, P.B.Gandhi, S.C.Armeta, and H.C.Chowdhry, Pak.J.Sci.Ind.Res., 1987, **30**, 347.
358 B.L.Milic, S.M.Djilas, and D.S.Vlaovic, Analyst, 1987, **112**, 779.
359 R.Albert, J.Danklmeier, H.Hoenig, and H.Kandolf, Synthesis, 1987, 635.
360 S.-T.Chen and K.-T.Wang, Synthesis, 1987, 581; S.-T.Chen, K.-T.Wang, and C.-H.Wong, J.Chem.Soc.,Chem.Commun., 1986, 1514.
361 B.D.Harris, K.L.Bhat, and M.M.Jouillie, Synth.Commun., 1986, **16**, 1815.
362 W.Dorsch and F.Effenberger, Chem.Ber., 1987, **120**, 55.
363 L.Gurarii and E.T.Mukmenev, Zh.Obshch.Khim., 1986, **56**, 2649.
364 N.A.Sasaki, C.Hashimoto, and P.Potier, Tetrahedron Lett., 1987, **28**, 6061.
365 F.Effenberger and T.Weber, Angew.Chem., 1987, **99**, 146.
366 J.Kohn and R.Langer, J.Am.Chem.Soc., 1987, **109**, 817.
367 Z.B.Alfassi, Radiat.Phys.Chem., 1987, **29**, 405.
368 D.L.Boger and D.Yohannes, J.Org.Chem., 1987, **52**, 5283.
369 D.W.Hobbs and W.C.Still, Tetrahedron Lett., 1987, **28**, 2805.
370 S.Ranganathan, D.Ranganathan, S.Singh, and D.Bhattacharyya, J.Chem.Soc., Chem.Commun., 1987, 1887.
371 S.Ranganathan, D.Ranganathan, and D.Bhattacharyya, J.Chem.Soc.,Chem. Commun., 1987, 1085.
372 L.D.Keys and G.A.Hamilton, J.Am.Chem.Soc., 1987, **109**, 2156.
373 D.M.Law, Physiol.Plant., 1987, **70**, 626.
374 J.R.Matos and C.H.Wong, Bioorg.Chem., 1987, **15**, 71.
375 C.O.Meese, Arch.Pharm.(Weinheim), 1987, **320**, 473.
376 D.K.Liu and S.G.Chang, Can.J.Chem., 1987, **65**, 770.
377 R.H.White, J.Labelled Compd.Radiopharm., 1987, **24**, 323.
378 C.N.Hsaio, L.Liu, and M.J.Miller, J.Org.Chem., 1987, **52**, 2201.
379 S.A.Sheldon and T.Shibamoto, Agric.Biol.Chem., 1987, **51**, 2473.
380 H.J.Reich and C.P.Jasperse, J.Am.Chem.Soc., 1987, **109**, 5549.
381 K.Kaleem, F.Chertok, and S.Erhan, J.Biol.Phys., 1987, **15**, 63.
382 M.Ruiz-Gayo, F.Albericio, E.Pedroso, and E.Giralt, J.Chem.Soc.,Chem. Commun., 1986, 1501.
383 F.Albericio, A.Grandas, A.Porta, E.Pedroso, and E.Giralt, Synthesis, 1987, 271.
384 R.Ramage and J.Green, Tetrahedron Lett., 1987, **28**, 2287.
385 C.J.Chivikas and J.C.Hodges, J.Org.Chem., 1987, **52**, 3591.
386 H.J.Keller, K.Q.Do, M.Zollinger, K.H.Winterhalter, and M.Cuenod, Anal. Biochem., 1987, **166**, 431
387 M.Shimizu, J.Phys.Soc.Jpn., 1987, **56**, 43.
388 A.Andreoni, Med.,Biol.,Environ., 1987, **15**, 57.
389 H.Droste and T.Wieland, Liebigs Ann.Chem., 1987, 901.
390 J.D.Kanner and O.Fennema, J.Agric.Food Chem., 1987, **35**, 71.

391 M.A.Krogull and O.Fennema, J.Agric.Food Chem., 1987, **35**, 66.
392 J.R.Wagner, H.Ali, R.Langlois, N.Brasseur, and J.E.Van Lier, Photochem. Photobiol., 1987, **45**, 507.
393 E.Reddi, C.R.Lambert, G.Jori, and M.A.J.Rodgers, Photochem.Photobiol., 1987, **45**, 345.
394 J.Butler, E.J.Land, A.J.Swallow, and W.A.Prutz, J.Phys.Chem., 1987, **91**, 3113.
395 K.Goda, M.Hisaoka, and T.Ueda, Biochem.Int., 1987, **15**, 635.
396 N.L.Vekshin, Stud.Biophys., 1987, **118**, 173.
397 S.G.Berlotti, M.Del V.Bohorquez, J.J.Cosa, N.A.Garcia, and C.M.Previtali, Photochem.Photobiol., 1987, **46**, 331.
398 A.Nishimura, Okayama Igakkai Zasshi, 1986, **98**, 827 (Chem.Abs., 1987, **107**, 35786).
399 E.J.Land, A.Thompson, T.G.Truscott, K.V.Subbarao, and M.R.Chedekel, Photochem.Photobiol., 1986, **44**, 697.
400 A.L.Simonyan, G.E.Khachatryan, S.Sh.Tatikyan, and T.S.M.Arakyan, Biol.Zh. Arm., 1986, **39**, 555.
401 "Amino Acid Analysis by Gas Chromatography", Eds. R.W.Zumwalt, K.C.Kuo, and C.W.Gehrke, 1987.
402 M.Aparici, V.Bori, J.Gonzalez, E.Iturgoyen, V.Liceras, S.Matoses, J.Perez de los Lobos, Laboratorio (Granada,Spain), 1986, **482**, 111.
403 R.W.Zumwalt, J.S.Absheer, F.E.Kaiser, and C.W.Gehrke, J.Assoc.Off.Anal. Chem., 1987, **70**, 147.
404 C.W.Gehrke, K.C.T.Kuo, F.E.Kaiser, and R.W.Zumwalt, J.Assoc.Off.Anal.Chem., 1987, **70**, 160.
405 R.W.Zumwalt, J.Desgres, K.C.T.Kuo, J.E.Pautz, and C.W.Gehrke, J.Assoc.Off. Anal.Chem., 1987, **70**, 253.
406 T.Furuta, Y.Kasuya, H.Shibasaki, and S.Baba, J.Chromatogr., 1987, **413**, 1.
407 S.L.Mackenzie, J.Assoc.Off.Anal.Chem., 1987, **70**, 151.
408 T.Miyata, Y.Okano, J.Nagata-Tanoue, S.Ijima-Miyamura, H.Iwamura, K.Takahama, and T.Hitoshi, Anal.Biochem., 1987, **163**, 303.
409 R.J.Early, J.R.Thompson, G.W.Sedgwick, J.M.Kelly, and R.J.Christopherson, J.Chromatogr., 1987, **416**, 15.
410 S.P.Stabler, P.D.Marcell, E.R.Podell, and R.H.Allen, Anal.Biochem., 1987, **162**, 185.
411 H.J.Chaves das Neves amd A.M.P.Vasconcelos, J.Chromatogr., 1987, **392**, 249.
412 S.L.Mackenzie, D.Tenaschuk, and G.Fortier, J.Chromatogr., 1987, **387**, 241.
413 H.Brueckner, I.Bosch, T.Graser, and P.Fuerst, J.Chromatogr., 1987, **395**, 569.
414 J.Velisek, T.Davidek, J.Davidek, P.Brezina, and C.Curda, Sb.UVTIZ Potravin. Vedy, 1986, **4**, 161 (Chem.Abs., 1987, **106**, 115923).
415 C.R.Krishnamurti and A.L.Schaefer, Nutr.Rep.Int., 1987, **35**, 683.
416 D.Yu, H.T.Stuewe and W.A.Koenig, Angew.Chem., 1987, **99**, 362.
417 M.Jemal and A.I.Cohen, J.Chromatogr., 1987, **392**, 442.
418 E.Bayer, H.Frank, J.Gerhardt, and G.Nicholson, J.Assoc.Off.Anal.Chem., 1987, **70**, 234.
419 I.Abe, H.Tsujioka, and T.Waso, Chem.Express, 1986, **1**, 335.
420 G.Wang, L.Zhou, S.Chen, H.Luo, and J.Wang, Fenxi Huaxue, 1987, **15**, 783 (Chem.Abs., 1988, **108**, 55064).
421 M.K.Park, J.S.Kang, J.H.Ryn, J.H.Park, and D.W.Jeon, Yakhak Hoechi, 1986 **30**, 47 (Chem.Abs., 1987, **106**, 176826).
422 R.Wettengel, ZfI-Mitt., 1986, **115**, 191.
423 R.Wettengel, J.Poerschmann, R.Herzschuh, G.Mehlhorn, R.Kliche, and L.Liebetrau, Nahrung, 1987, **31**, 39.
424 D.Liu and J.WQang, Sepu, 1987. **5**, 105.
425 P.J.M.Van der Oetelaar, J.R.C.M.Van Beckhoven, and H.J.Hoenders, J.Chromatogr., 1987, **388**, 441.
426 Y.Takata, Y.Fujii, H.Takahashi, and S.Ganno, Hitachi Hyoron, 1987, **69**, 333 (Chem.Abs., 1987, **107**, 55024).
427 L.B.James, J.Chromatogr., 1987, **408**, 291.
428 L.B.James, J.Chromatogr., 1987, **391**, 269.
429 L.B.James, J.Chromatogr., 1987, **404**, 321.
430 L.B.James, J.Chromatogr., 1987, **403**, 271.
431 L.B.James, J.Chromatogr., 1987, **410**, 176.

432 J.Wong and D.A.Applegarth, Clin.Chem.(Winston Salem), 1987, **33**, 1302.
433 W.Naumann, H.Noetzold, and G.Kempe, Z.Chem., 1987, **27**, 262.
434 A.M.Marx, H.Kronberg, and V.Neuhoff, J.Chromatogr., 1987, **393**, 407.
435 S.D.Mitrinovic, B.D.Medakovic, N.Djordjevic, S.M.Dragovic, and R.Kreacic, Iugoslav.Physiol.Pharmacol.Acta, 1986, **22**, 13.
436 R.Kraft, A.Otto, H.J.Zoepfl, and G.Etzold, Biomed.Environ.Mass Spectrom., 1987, **14**, 1.
437 R.Bhushan and I.Ali, J.Liq.Chromatogr., 1986, **9**, 3479.
438 R.Bhushan and G.P.Reddy, Anal.Biochem., 1987, **162**, 427.
439 R.Bhushan and I.Ali, J.Chromatogr., 1987, **392**, 460.
440 S.V.Kulikov and M.A.Samartsev, Khim.Prir.Soedin., 1987, 613.
441 K.Hirschberg, H.Faust, and A.Balint, Isotopenpraxis, 1987, **23**, 153.
442 G.Szepesi, Z.Pap-Sziklay, Z.Bertha-Somodi, and Z.Vegh, J.Pharm.Biomed. Anal., 1986, **4**, 799; E.Tyihak, Ibid., 1987, **5**, 191.
443 A.N.Boguslavskii and V.E.Egert, Khim.-Farm.Zh., 1987, **21**, 234.
444 T.M.Nogina, I.P.Stognii, O.R.Gvozdyak, O.A.Nesterenko, and E.I.Kvasnikov, Mikrobiol.Zh.(Kiev), 1987, **49**, 98.
445 N.S.Yang, S.G.Platt, and P.Christou, Anal.Biochem., 1987, **160**, 342.
446 M.H.Joseph and C.A.Marsden, in "H.P.L.C. of Small Molecules", Ed. C.K.Lim, IRL Press, Oxford, 1986, p.13.
447 R.F.Goldsmith, in "High Performance Liquid Chromatography in the Clinical Laboratory, Ed. D.C.Sampson, Australian Association for Clinical Biochemistry, Sydney, 1986, p.113.
448 R.Causon, D.Davies, and M.Brown, Life Sci., 1987, **41**, 909.
449 S.Hara, A.Dobashi, K.Kinoshita, T.Hondo, M.Saito, and M.Senda, J.Chromatogr., 1986, **371**, 153.
450 C.Hansson, B.Kaagedal, and M.Kaellberg, J.Chromatogr., 1987, **420**, 146.
451 J.L.Venthey, M.A.Bagnoud, and W.Haerdi, J.Chromatogr., 1987, **393**, 51.
452 T.A.Walker and D.J.Pietrzyk, J.Liq.Chromatogr., 1987, **10**, 161.
453 T.Nakagawa, A.Shibukawa, A.Kaihara, and H.Tanaka, Nippon Kagaku Kaishi, 1986, 1002 (Chem.Abs., 1987, **107**, 7541).
454 S.Levin and E.Grushka, J.Chromatogr., 1987, **384**, 249.
455 J.P.Richie and C.A.Lang, Anal.Biochem., 1987, **163**, 9.
456 A.Ichimura, M.Nakatsuka, K.Ogura, and T.Kiragawa, Nippon Kagaku Kaishi, 1986, 987.
457 T.Iwamoto, M.Yoshiura, and K.Iriyama, Bunseki Kagaku, 1987, **36**, 98.
458 J.Haginaka and J.Wakai, J.Chromatogr., 1987, **396**, 297.
459 Y.Hashimoto, S.Yamagata, and T.Hayakawa, Anal.Biochem., 1987, **160**, 362.
460 H.Gjerde, P.T.Normann, and J.Moerland, Biomed.Biochim.Acta, 1987, **46**, 53.
461 A.Csiba, L.Trezl, I.Rusznak,and T.Szarvas, Symp.Biol.Hung., 1986, **34**, 57.
462 M.J.Campello, E.Cortes, M.S.Aguilar, I.Ballester, and M.Moya, Tec.Lab., 1987, **11**, 21.
463 N.T.Soskel, Anal.Biochem., 1987, **160**, 98.
464 C.G.Zarkadas, J.A.Rochemont, G.C.Zarkadas, C.N.Karatzas, and A.D.Khalili, Anal.Biochem., 1987, **160**, 251.
465 Y.Yamaguchi, J.Haginaka, M.Kunitomo, H.Yasuda, and Y.Bando, J.Chromatogr., 1987, **422**, 53.
466 Y.Seyama, H.Inoue, M.Iwama, H.Watanabe, M.Irie, H.Orimo, and S.Yamashita, Rinsho Kagaku (Nippon Rinsho Kagakkai), 1987, **16**, 38 (Chem.Abs., 1988, **108**, 2830).
467 W.H.Mennicke, T.Kral, G.Krumbiegel, and N.Rittmann, J.Chromatogr., 1987, **414**, 19.
468 J.C.Rutledge and J.Riudy, Am.J.Chim.Pathol., 1987, **87**, 614.
469 J.T.Tyopponen, J.Chromatogr., 1987, **413**, 25.
470 A.Negro, S.Garbisa, L.Gotte, and M.Spina, Anal.Biochem., 1987, **160**, 39.
471 L.P.Pang, K.C.Ho, M.G.Jones, and C.K.Cheung, J.Chromatogr., 1987, **386**, 309.
472 G.J.Hughes, S.Frutiger, and C.Fonck, J.Chromatogr., 1987, **389**, 327.
473 E.Andersson. E.Haakanson, J.Larsson, and J.Maartensson, J.Chromatogr., 1987, **414**, 174.
474 P.E.Minkler, S.T.Ingalls, R.L.Griffin, and C.L.Hoppel, J.Chromatogr., 1987, **413**, 33.
475 D.F.Bushey, D.M.Law, and P.J.Davies, Anal.Biochem., 1987, **167**, 31.
476 L.J.Lehman, A.L.Olson, and C.J.Rebouche, Anal.Biochem., 1987, **162**, 137.

477 G.A.Qureshi, J.Chromatogr., 1987, **400**, 91.
478 J.Schmidt and C.J.McClair, J.Chromatogr., 1987, **419**, 1.
479 H.P.Fiedler and A.Playa, J.Chromatogr., 1987, **386**, 229.
480 M.Eslami, J.D.Smart, and K.A.Cohen, J.Chromatogr., 1987, **411**, 121.
481 D.E.Willis, J.Chromatogr., 1987, **408**, 217.
482 G.Blundell and W.G.Brydon, Clin.Chim.Acta, 1987, **170**, 79.
483 P.Fuerst, P.Stehle, and T.A.Graser, Infusionstherap.Klin.Ernaehr.-Forsch. Prax., 1987, **14**, 137 (Chem.Abs., 1987, **107**, 194398).
484 W.Rajendra, J.Liq.Chromatogr., 1987, **10**, 941.
485 G.A.Qureshi and P.Soedersten, J.Chromatogr., 1987, **400**, 247.
486 J.L.Tedesco and R.Schafer, J.Chromatogr., 1987, **403**, 299.
487 G.K.Svanberg, J.Periodontal Res., 1987, **22**, 133.
488 K.Kuwano, C.Sakamaki, and T.Mitamura, Nippon Nogei Kagaku Kaishi, 1987, **61**, 53 (Chem.Abs., 1987, **106**, 172203).
489 J.L.Rudy, J.C.Rutledge, and S.L.Lewis, Clin.Chem.(Winston Salem N.C.), 1987, **33**, 1152.
490 L.Montanarella, Chem.Oggi, 1987, 27.
491 H.E.Meyer, K.Swiderek, E.Hoffmann-Posorske, H.Korte, and L.M.G.Heimeyer, J.Chromatogr., 1987, **397**, 113.
492 M.M.J.O'Hare, O.Tortora, U.Gether, H.V.Nielsen, and T.W.Schwartz, J.Chromatogr., 1987, **389**, 379.
493 P.R.Young and F.Grynspan, J.Chromatogr., 1987, **421**, 130.
494 L.T.Ng, A.Pascaud, and M.Pascaud, Anal.Biochem., **167**, 47.
495 I.V.Nazimov and N.B.Levina, Chem.Pept.Proteins, 1986, **3**, 119 (Chem.Abs., 1987, **106**, 77987).
496 K.Matsumoto and S.Tsuge, Shitsuryo Bunseki, 1986, **34**, 243.
497 M.Okamoto, K.Jinno, M.Yamagami, K.Nobuhara, and K.Fukushima, J.Chromatogr., 1987, **396**, 345.
498 R.Murphy, J.B.Furness, and M.Costa, J.Chromatogr., 1987, **408**, 388.
499 H.Kotaniguchi, M.Kawakatsu, T.Toyooka, and K.Imai, J.Chromatogr., 1987, **420**, 141.
500 A.Araki and Y.Sako, J.Chromatogr., 1987, **422**, 43.
501 R.Cunico, A.G.Mayer, C.T.Wehr, and T.L.Sheehan, BioChromatogr., 1986, **1**, 8.
502 T.Naesholm, G.Sandberg, and A.Ericsson, J.Chromatogr., 1987, **396**, 225.
503 S.R.Spurlin and M.M.Cooper, Anal.Lett., 1986, **19**, 2277.
504 M.Lee, H.Nohta, K.Ohtsuto, B.Yoo, and Y.Ohkura, Chem.Pharm.Bull., 1987, **35**, 235.
505 S.Lam and G.Malikin, J.Chromatogr., 1986, **368**, 413.
506 M.Fujiwara, Y.Ishida, N.Nimura, A.Toyama, and T.Kinoshita, Anal.Biochem., 1987, **166**, 72.
507 J.Florance, A.Galdes, Z.Konteatis, Z.Kosarych, K.Langer, and C.Martucci, J.Chromatogr., 1987, **414**, 313.
508 R.H.Buck and K.Krimmen, J.Chromatogr., 1987, **387**, 255.
509 S.Einarsson, S.Folestad, and B.Josefsson, J.Liq.Chromatogr., 1987, **10**, 1509.
510 J.Wagner, C.Gaget, B.Heintzelmann, and E.Wolf, Anal.Biochem., 1987, **164**, 102.
511 J.Wagner, E.Wolf, B.Heintzelmann, and C.Gaget, J.Chromatogr., 1987, **392**, 211.
512 E.Armani, R.Marchelli, A.Dossena, G.Casnati, and F.Dallavalle, Helv.Chim.Acta, 1986, **69**, 1916.
513 S.Lam and A.Karmen, J.Liq.Chromatogr., 1986, **9**, 291.
514 G.Guebitz, J.Liq.Chromatogr., 1986, **9**, 519.
515 M.T.Aubel and L.B.Rogers, J.Chromatogr., 1987, **392**, 415.
516 D.S.Dunlop and A.Neidle, Anal.Biochem., 1987, **165**, 38.
517 K.Iwsaki, S.Yoshida, N.Nimura, T.Kinoshita, K.Takeda, and H.Ogura, J.Chromatogr., 1987, **404**, 117.
518 G.Krüger, J.Grötzinger, and H.Berndt, J.Chromatogr., 1987, **397**, 223.
519 P.Wenger and P.Javet, J.Biochem.Biophys.Methods, 1986, **13**, 275, 289.
520 M.Gleeson and R.J.Maugham, Clin.Chim.Acta, 1987, **166**, 163.
521 F.Weissbach, W.Schindler, J.Huber, and G.Kreibich, Acta Biotechnol., 1987, **7**, 377.
522 M.Hisatomi, T.Iida, T.Mitamura, K.Nagata, and K.Tomita, Denki Kagaku Oyobi Kogyo Butsuri Kagaku, 1987, **55**, 67.
523 J.L.Romette and C.L.Cooney, Anal.Lett., 1987, **20**, 1069.
524 T.Ageta. N.Mizobuchi, and H.Kodama, J.Chromatogr., 1987, **419**, 334.

525 B.Zarabian, F.Koushesh, and A.Vassef, Anal.Biochem., 1987, **166**, 113.
526 A.Csiba, Kiserl.Orvostud., 1987, **39**, 21 (Chem.Abs., 1987, **107**, 194414).
527 E.T.Backer, A.Van den Ende, and W.P.Geus, Clin.Chim.Acta, 1987, **165**, 351,
528 W.R.Frisell and D.J.Higginbotham-Willcox, Med.Sci.Res., 1987, **15**, 815.
529 S.Yamaguchi, T.Tsukamoto, and M.Senda, Bunseki Kagaku, 1987, **36**, 572.
530 J.Wu and Z.Onyang, Sepu, 1987, **5**, 236.

2
Peptide Synthesis

BY I. M. EGGLESTONE, D. T. ELMORE,
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1. Introduction

Ian Galpin's tragic death, at a time when he would have been planning his year's review and was poised to start writing, left us with a pressing task for which we were ill-prepared. Before we could begin, we had to face harvesting and winnowing the literature of 1987 for relevant references. Although one of us had done this before, that was a good many years ago, and his systematic collection of literature on a wide front had long since given way to more specialized reading and casual perusal. It was therefore necessary to stand back and consider how best to proceed. A rough survey of the 1986 literature showed that the 600 or so citations of that year had been distributed between about 120 journals: both the annual volume and the range of journals had doubled since 1970, when a similar analysis[1] had been made. The number of core journals, however, had not increased greatly. Now, as then, around a dozen account for over a third of the total output and well over half of the significant chemical papers. The key journals at the present time seem to be those listed in the Table.

Bull.Chem.Soc. Jpn.	J.Org.Chem.
Chem. Pharm. Bull.	Liebigs Ann. Chem.
Int. J. Pept. Protein Res.	Synthesis
J.Chem. Soc. Commun.	Tetrahedron
J.Chem.Soc. Perkin I	Tetrahedron Lett.
J. Med. Chem.	

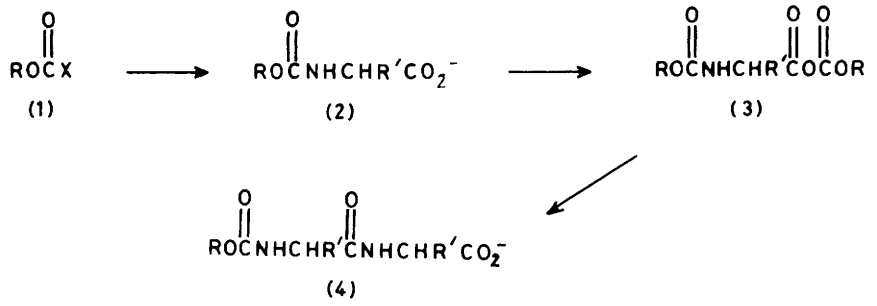
Table The principal primary journals for papers on peptide synthesis

The material reviewed here was gathered mainly from Chemical Abstracts (section 34 and cross-references therefrom) January 1987–April 1988, discarding papers already cited by Ian Galpin and adding papers found in the key journals up to the end of 1988 which had not yet been abstracted. We also sent out a large number of requests to leaders in the field, asking for reprints of publications within our remit. This must have been a nuisance to a large number of busy people, but they responded most helpfully, and enabled us to be more nearly exhaustive than we would otherwise have been. We thank them warmly for their cooperation.

In general, the structure of our report remains as before, as does our policy in matters such as symposium proceedings, which we cite as whole volumes without discussion of individual contributions. This is not to denigrate the importance of such proceedings, which are indeed a most valuable source of information and of comment which is more relaxed than the formal literature allows. But most of the substance of the symposia finds its way into the journals quite promptly, and blow-by-blow conference reporting would not only overwhelm us, but would also result in much duplication and repetition. We have mainly in mind, of course, the main series of international peptide symposia: the most recent volumes to be published are the Proceedings of the 19th European Symposium,[2] the Proceedings of the 10th American Symposium,[3] the Proceedings of the 25th Japanese Symposium,[4] and the Proceedings of the 5th USSR-FRG Symposium.[5]

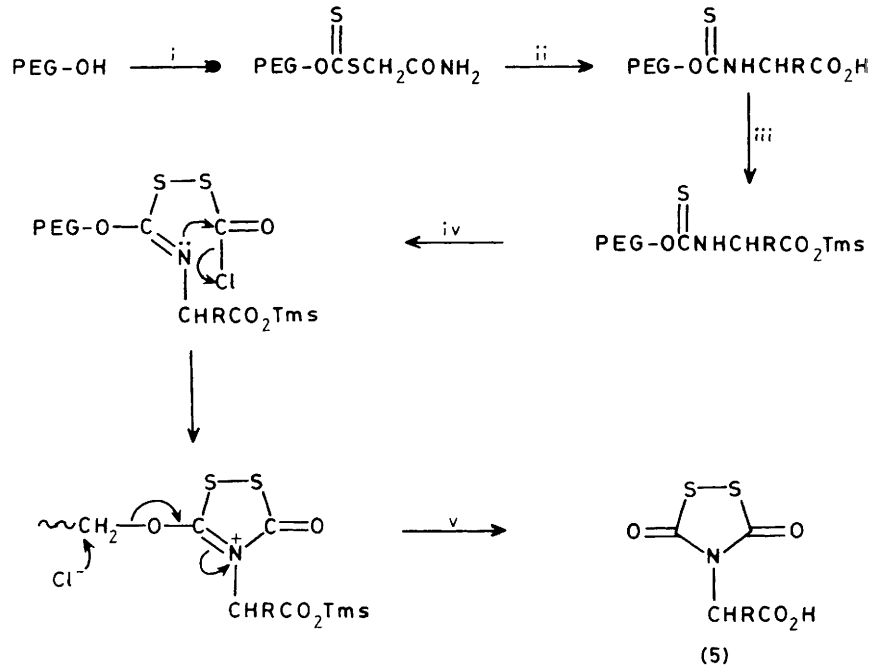
So far as the delineation of our subject matter is concerned, we deal here mainly with the chemical synthesis of "ordinary" peptides, their reasonably close analogues, and proteins. There is a little overlap with chapters 3 and 4, but by and large cyclic, conjugated and pseudopeptides are left alone for reporting there. Nor do we have anything beyond an occasional aside to say about the structure-activity studies which are the main raison d'être of the chemistry discussed. The use of enzymes as reagents we include, and partial synthesis employing peptides from natural sources as raw material is also covered. But techniques based on molecular genetics are excluded. On that subject, we note that the year under review saw the tenth anniversary of the first peptide preparation (somatostatin[6]) by genetic engineering. There has been massive growth in that area (readers may be interested in a recent monograph[7]) but this has not taken place at the expense of chemical peptide synthesis, which looks like remaining a going (nay, expanding) concern for the foreseeable future, if only because one of the main objectives in the field is to use natural peptides as springboards for the discovery of active but non-peptide analogues which have greater metabolic stability and other pharmacological advantages. This can in principle be done by systematically designing out of the natural peptide those features which allow its degradation by natural processes (e.g. the peptide bonds) whilst retaining the functional and topological characteristics required for biological action. Evolution produced morphine this way, and the approach is starting to have success in the hands of man.[8] It seems likely that this kind of trend, shifting the spotlight towards peptides with "unusual" features, either for their own sakes or as stepping stones to non-peptide analogues, will keep peptide chemistry vigorous for a long time yet.

A number of relevant surveys and monographs of a broad-ranging kind have appeared,[9-19] and there have also been several reviews devoted to the synthesis of particular classes of peptide: glycopeptides,[20] opioids,[21-22] gastrins and cholecystokinins,[23-24] ANF analogues,[25] neurohypophyseal hormones[26] (structure-activity studies on this class, which remain very active even after more than thirty years



Conditions: $NH_2CHR'CO_2H$/base

Scheme 1



Conditions: i, $KOBu^t$, then CS_2, then $ClCH_2CONH_2$; ii, NH_2CHRCO_2H/pH 9·5; iii, TmsNHCONHTms; iv, ClCOSCl; v, aqueous workup

Scheme 2

of synthetic endeavour, have been thoroughly reviewed: see refs. 27, 28 and 29), immunologically active peptides,[30] and peptide vaccines.[31] In addition, numerous specialised reviews on aspects of peptide chemistry or peptide synthesis methodology are cited in the sections which follow.

2. Methods

With minor adjustments in response to changes of emphasis and activity (most notably the abolition of the separate section on racemization and the introduction of one on disulphide bridge formation), we follow previous volumes of the series in the way our material is arranged. Assignments to one section as against another is sometimes arbitrary, but we have assumed that our expert readership will see this as obvious, enabling us to avoid burdening the text with an inordinate number of cross-references.

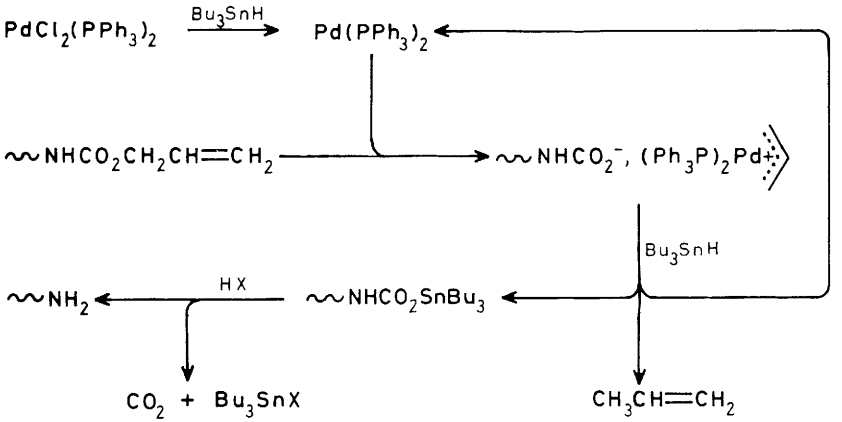
2.1 Protective Groups

The development and scope of enzyme-labile protecting groups for peptide synthesis have been reviewed.[32] Carboxyl groups, α-amino groups and side chains have been blocked in this way; so far, all the cleavage enzymes employed have been acylases.

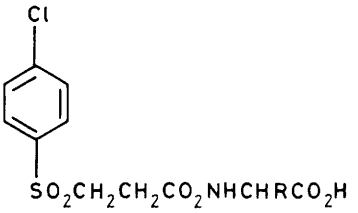
2.1.1 α-Amino Group Protection

Alkoxycarbonyl-type amino protection, in its several variations on the main theme, dominates the field as ever. Interest in the development of new alkoxycarbonylating reagents (1) for the introduction of familiar groups is thus sustained.[33, 34] One of the reasons for this is the fact that the most obvious reagents for the purpose - such as the chloroformates - often give small (but tiresome) amounts of dipeptide by-products (4) when amino acids are derivatised under Schotten-Baumann conditions, because the desired product (2) can react with unconsumed amino acid through a mixed carbonic anhydride (3), as shown in Scheme 1. For a recent example see ref. 35. The use of diisopropylethylamine instead of aqueous alkali is advantageous in this regard with some alkyl chloroformates, including FmocCl,[36] but a consensus seems to be emerging that the use of succinimido reagents is not significantly subject to the problem. Thus FmocONSu is recommended (for additional practical details see ref. 37), and of various reagents explored[38] for the introduction of 2-(trimethylsilyl)ethoxycarbonyl (Teoc) protection TeocONSu was found to be the best.

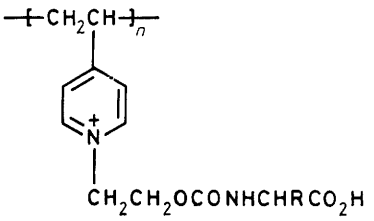
The electrolytic reductive cleavage of benzyl-oxygen bonds is possible, but requires very negative electrode potentials; the electrocatalytic hydrogenolysis of benzyloxycarbonyl groups, on the other hand, is facile and selective under mild conditions (a high surface area Pd on graphite cathode, divided cell, catholyte MeOH/2.5% AcOH + $NaClO_4$, cathode potential in the range -0.6 to -1.0V SCE).[39]


PdCl2(PPh3)2
Bu3SnH
Pd(PPh3)2
~NHCO2CH2CH=CH2
~NHCO2⁻, (Ph3P)2Pd+
Bu3SnH
~NH2
HX
~NHCO2SnBu3
CO2 + Bu3SnX
CH3CH=CH2

Scheme 3


Cl
SO2CH2CH2CO2NHCHRCO2H

(6)


CH2CH
n
N
CH2CH2OCONHCHRCO2H

(7)

Carpino has reviewed[40] the chemistry and applications of his Fmoc group (recently shown[41] to be cleavable with tetrabutylammonium fluoride as base in DMF) and its base-sensitive relatives. Atherton and Sheppard,[42] who have used the group to such effect in their solid-phase work, have also reviewed it exhaustively, providing in addition valuable tables of Fmoc derivatives.

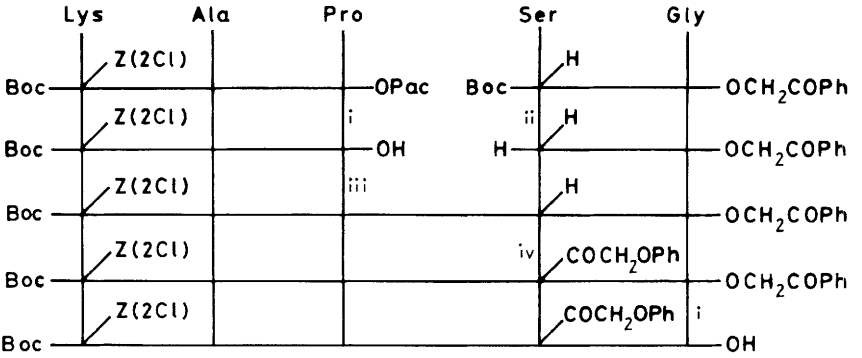
The complex chemistry associated with the synthesis of dithiasuccinoylamino acids (5) has been thoroughly explored, and a convenient general procedure has been defined in which the ring is closed on, and thereby released from, a polymeric carrier (the products of other modes of reaction remain conjugated to the polymer), as shown in Scheme 2.[43]

Allyloxycarbonyl amino-group protection was first explored and proposed for use many years ago, but was not seriously taken up through lack of satisfactory specific cleavage conditions. Details of the application of π-allyl palladium chemistry for this purpose have now been reported: allyloxycarbonylamino groups are cleaved rapidly on the treatment in methylene chloride with a slight excess of Bu_3SnH in the presence of a proton donor and a catalytic amount of $PdCl_2(PPh_3)_2$. The true catalytic species is probably $Pd(PPh_3)_2$, which reacts to give a π-allylpalladium complex, from which the catalyst is regenerated by Bu_3SnH, producing a tributyltin carbamate and thence an exposed amino group on protonolysis and decarboxylation: all this ("palladium-catalysed hydrostannolytic cleavage") is sketched in rough outline in Scheme 3.[44] The practicability of using allyloxycarbonyl amino acids (for the preparation of which the additional reagent diallyl dicarbonate[45] has recently been described) in an extended synthesis with this method of deprotection has been demonstrated by a solid-phase synthesis of substance P.[44] Neither benzyl- nor t-butyl-based protecting groups are affected by palladium-catalysed hydrostannolytic cleavage.

The potentialities of protected amino acids of type (6)[46] and (7)[348] - both types of protection are labile to base - have been investigated in simple exercises. N-Benzyl protection has been little used in peptide chemistry, in part because cleavage is sometimes difficult; two papers have appeared on the debenzylation of N-benzylamino derivatives by catalytic hydrogen transfer [10% Pd(C)/MeOH/donor/reflux: ammonium formate seems to be the best donor].[547]

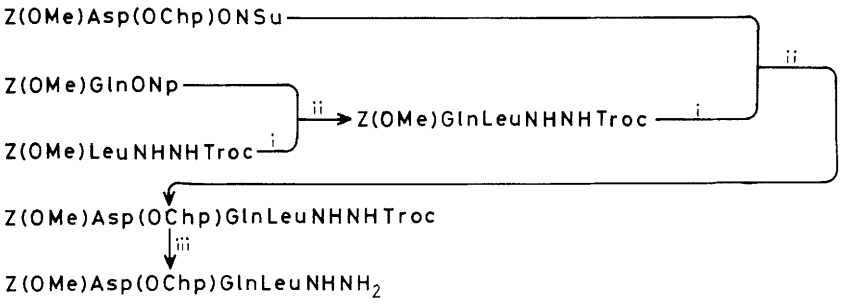
2.1.2 α-Carboxyl Group Protection

Three new methods for the esterification of N-acylamino acids have been reported: activation with isopropenyl chloroformate followed by alcohol-dimethylaminopyridine treatment;[48] reaction with 2,2'-carbonyl-bis(3,5-dioxo-4-methyl-1,2,4-oxadiazolidine) and the alcohol;[49] and reaction of the sodium salt plus 15-crown-5 with the appropriate alkyl halide in DMF.[50] Direct esterification by heating the amino acid with benzyl alcohol in the presence of tosic acid is a standard procedure for the preparation of amino acid benzyl ester tosic acid salts: a simple variation which works well for the histidine benzyl ester salt (previously problematic) has been described.[51]



Conditions: i, Zn/AcOH; ii, CF_3CO_2H; iii, $Me_2NCH_2CH_2CH_2N{=}C{=}NEt$/HOBt; iv, $(PhOCH_2CO{-})_2O$

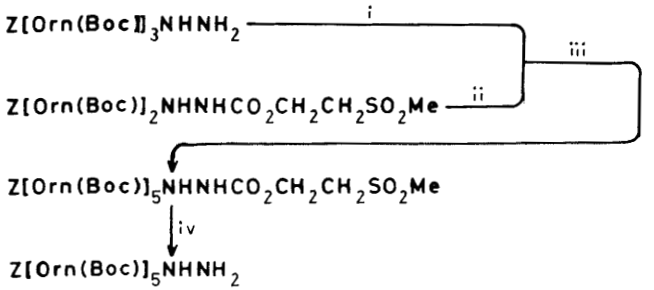
Scheme 4



Troc = 2,2,2-trichloroethyloxycarbonyl, Chp = cycloheptyl

Conditions: i, CF_3CO_2H; ii, base; iii, Cd/AcOH

Scheme 5



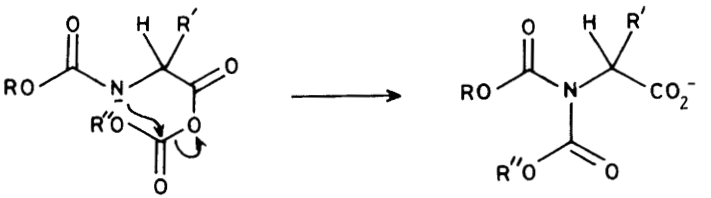
Conditions: i, $Bu^tONO/HCl/DMF$; ii, $H_2/Pd(C)/MeOH$; iii, Et_3N/DMF; iv, aq. NaOH/MeOH/DMF

Scheme 6

$Z(OMe)ProValN_3$ + H-Glu(OChp) HisProAsp(OBzl)Lys(Z)-
- Phe LeuLys(Z)PheGlyMet(O)Thr-
- ProSerLys(Z)GlyValLeuPhe-
- Tyr[Bzl(2,6-Cl_2)]-OBzl, CF_3CO_2H

Conditions: 3.5 equivalents of azide/Et_3N; DMF/DMSO (1:1), -15°C/48 h, then overnight at 4°C 81% yield

Scheme 7

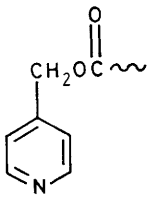


Scheme 8

H-Cys(SBu^t)Asp(OBu^t)Ser(Bu^t)-OH, HCl
i
TmsCys(SBu^t)Asp(OBu^t)Ser(Bu^t)OTms
iii
BocGlyGlu(OBu^t)PheSer(Bu^t)ValOH
ii
BocGlyGlu(OBu^t)PheSer(OBu^t)ValCys(SBu^t)Asp(OBu^t)Ser(Bu^t)OH

Conditions: i, *N*-Methylmorpholine/*N*,*O*-bis-trimethylsilylacetamide; ii, Bu^tOCOCl/*N*-methylmorpholine; iii, THF/DMF, then H_2O

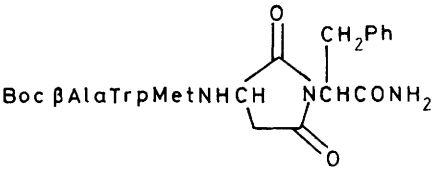
Scheme 9



(8)

Boc βAlaTrpMetAspPheNH$_2$

(9)



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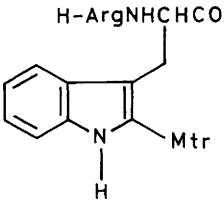

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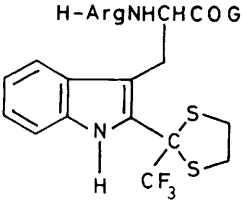

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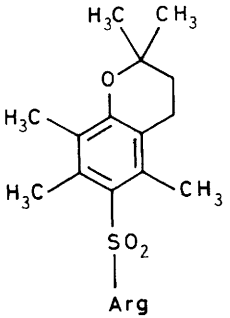


(13)

Amino acid diphenylmethyl (Dpm, = benzhydryl, Bzh) ester salts may be obtained directly by treatment of the amino acid tosic acid salt with tris(diphenylmethyl)phosphate[52] or by esterification ($Ph_2CHOH/Ph_3P/EtO_2CN{=}NCO_2Et$) of the N-trityl derivative followed by detritylation.[53] The relative stability of the diphenylmethyl ester group under the conditions required for detritylation (1-hydroxybenzotriazole in trifluoroethanol gives the best selectivity) enables the two groups to be used together for stepwise assembly, an approach which has been validated by a synthesis of leucine-enkephalin.[53] N(α)-trityl substituents seem generally to be more labile than ω-trityl groups, a fact which has been taken advantage of for the preparation of a number of ω-trityl derivatives by selective acidolysis ($1\%CF_3CO_2H/CH_2Cl_2$) of the corresponding α,ω-bistritylamino acids.[54]

α-Phenacyl ester protection has been used for the preparation of some of the fragments required for the synthesis of porcine cholecystokinin - pancreozymin (e.g. Scheme 4).[55]

The use of alkoxycarbonylhydrazide C-terminal substitution, as a device for α-carboxyl protection until a partially protected hydrazide is required for azide fragment conjunction, is increasing. Numerous instances of the use of trichloroethoxycarbonylhydrazide intermediates can be cited (e.g. refs. 56-60). The normal reagent for exposing the hydrazide function is Zn-AcOH, but Cd-AcOH is advantageous in some cases.[58-60] An example is shown in Scheme 5. Base-labile hydrazine protection is also feasible (e.g. Scheme 6).[61]

2.1.3 Side-chain Protection

For new permutations of differential protection, see Appendix II, as many do not receive comment here.

Lysine, etc.- An optimised procedure (97% yield) for the preparation of H-Lys(Z)-OH via the copper complex has been reported, and a number of papers have appeared on alternative reagents for the decomposition of ω-substituted lysine and other copper complexes.[62-64] Side-chain isonicotinyloxycarbonyl protection [(8); very acid-resistant, even survives HF; base-stable; reductively cleaved, e.g. by $H_2/Pd(C)$] is not novel but has not been employed very often: its capabilities have been demonstrated[65] in a synthesis of a cyclic somatostatin analogue.

Aspartic and Glutamic acids, etc.- One-pot procedures have been described for the (partially) selective mono-esterification of ZAsp(OH)OH and ZGlu(OH)OH via their respective cyclic anhydrides generated in situ.[66] The N-protected α-esters (which are of course valuable stepping-stones in indirect routes to selectively protected derivatives) generally predominate when such cyclic anhydrides are opened with alcohols, in contrast to the direct partial acid-catalysed esterification of the free amino acids, which gives side-chain esterification predominantly. The use of tetrafluoroboric acid has been

reported to be advantageous for the latter;[67] the ω-benzyl esters of aspartic and glutamic acid can also be prepared by alcalase-catalysed hydrolysis of the corresponding dibenzyl esters.[68] It has been reported that the activation of the diacid BocAsp(OH)OH with pivaloyl chloride/HOBt enables the preparation of α-aspartyl dipeptides without the need for side-chain protection.[69] The formation of aspartimidyl-containing peptides is a well-recognized and serious problem attending the synthesis of sequences including aspartic acid. A recent example (studied by FAB mass spectrometry) is provided by the derivative (9), which is converted into the amide (10) in 80% yield on triethylamine treatment in DMF at ambient temperature, although the cyclisation can also be induced by acid.[70] Various side-chain esters have been proposed for the suppression of this side reaction (see ref. 71 for leading references on the problem and attempts to solve it): to these must now be added 1- and 2-adamantyl, which are cleaved by mild and vigorous acidolysis respectively.[71] Recent instances of the use of these specially designed aspartic acid side-chain protecting groups in classical work include syntheses of porcine cholecystokinin-pancreozymin (cyclohexyl ester),[55] human pancreatic polypeptide (methyl ester),[58] porcine neuropeptide Y[72] and ovine corticotropin releasing factor (cycloheptyl ester).[59] In general glutamic acid is not as difficult to deal with as aspartic acid, but N-terminal pyrrolidone (i.e. pyroglutamyl) formation can occur with simple side-chain esters: cycloheptyl ester protection was employed[56] to suppress it in the sluggish fragment condensation shown in Scheme 7.

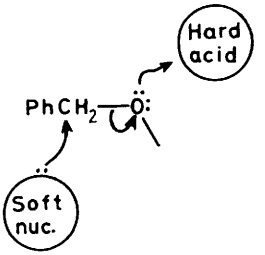
Serine etc. - O-Trityl serine protection has been used in a simple exercise starting from the N,O-bistrityl derivative (1% CF_3CO_2H/CH_2Cl_2 cleaves the N-trityl group selectively, as mentioned above in section 2.1.2), and FmocSer(Trt)OH has been described;[54] O-phenoxyacetyl protection (removed by O.1M ammonia in half an hour at room temperature, milder conditions than those required for acetyl or benzoyl cleavage) has been applied for the first time, in a synthesis of porcine cholecystokinin-pancreozymin.[55]

Histidine.- Two descriptions of FmocHis(Trt)OH (with significant discrepancies between the physical constants reported) have appeared.[54, 73] Because the trityl group is located at the tele position, it cannot prevent racemisation on carboxyl activation under extreme conditions altogether, but the extent to which it occurs is small. This is apparently because the trityl group depresses the basicity of the imidazole ring significantly. Under normal coupling conditions, the amount of racemisation is negligible and FmocHis(Trt)OH is probably the histidine intermediate of choice at the present time for strategies dependent on α-Fmoc and acid-labile side-chain protection. To the chagrin of one of the present reporters, FmocHis(Bum)OH, which might have taken this role, has not really fulfilled early promise because of difficulties with the introduction of the π-t-butoxymethyl (Bum) group reproducibly in good yield.

Cysteine.- A detailed one-pot procedure has been given for the preparation of FmocCys(Acm)-OH.[74] For disulphide bridge formation see section 2.7 below.

Arginine.- Z-Arg(Tos)OH is well known to be prone to cyclic lactam formation: a comparative study has shown that in the synthesis of two simple dipeptide derivatives, activation with diphenylphosphoryl azide gave cleaner coupling than DCCI-based or carbonic anhydride methods.[75] The 4-methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr) group was until recently (see below) the most acid-labile and most likely to become established of the many argylsulphonyl guanidine protecting groups which have been investigated during the last few years. Its acid-lability, however, is on a risky level - CF_3CO_2H cleavage requires more extended or vigorous treatment than, for example, t-butyl-based protection. In the presence of sensitive residues, this can be a source of difficulty. Thus H-Arg(Mtr)TrpGlyOMe gave about 10% of (11) in addition to the required tripeptide H-ArgTrpGlyOMe on treatment with 95% CF_3CO_2H at 50° for 80 minutes.[76] Of various scavengers investigated, ethanedithiol was the most effective in reducing the amount of (11) formed, but introduced a new problem in that some (12) was produced; indeed, it was found that H-ArgTrpGlyOMe is completely converted into (12) on treatment with 95% CF_3CO_2H containing 15 equivalents ethanedithiol at 50° for 20 hours.[76] A more acid-labile protecting group than Mtr was clearly desirable, but no rational basis for progress in that direction within the framework of the arylsulphonyl class was available until Ramage perceived the significance of the erratic structure-acid lability correlation which is observed. In general, since the cleavage must presumably involve the generation of $ArSO_2^+$, the more electron-donating the substitution pattern in the aryl moiety, the greater one would expect its stabilisation, and therefore its rate of formation, to be. In fact the situation is more complicated than this, and methoxy substitution is not always as acid-labilising as might have been expected from simple electronic considerations. This, as Ramage recognised, is due to the fact that in these highly substituted systems the methoxy group may be impeded from aligning itself for optimal conjugation with the ring. This observation was behind the design of the 2,2,5,7,8-pentamethylchroman-6-sulphonyl (Pmc) group (13), in which the oxygen para to the sulphonyl group is locked into the optimum orientation. The new protecting group is stable to hydrogenolysis conditions, base etc. but is cleanly cleaved by 50%CF_3CO_2H/CH_2Cl_2 at room temperature: its convenience and advantages have been demonstrated in several examples, and it promises to become the arginine side-chain protecting group of choice when acid-lability is required, as in Fmoc-based strategies, though its development is still in the early stages, and that application has yet to be reported.

Methionine.- The oxidation of Met side chains is frequently a nuisance, but methods for smooth selective reduction are available (see section 2.7 below and ref. 77); in several recent examples Yajima's school have opted to employ Met(O) en route as a protected form of methionine deliberately, with its reduction being a planned part of the end game (e.g. refs. 56, 58, 59, 77).



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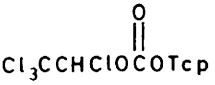

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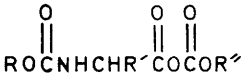

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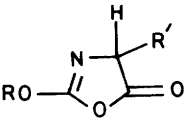

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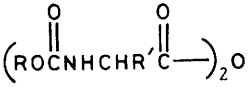


(19)

(20)

Tryptophan. - Further applications of in-formyl[55] and in-mesitylene sulphonyl[78] protection, in complex syntheses, have appeared.

2.1.4 General Deprotection

The rationale and practicalities of strong acid general procedures have been exhaustively reviewed.[79] The essential point of a great deal of sophisticated physical organic chemistry and empirical work has been to define conditions which will cleave the standard protecting groups smoothly without generating carbonium ions or other potent electrophiles which can engage in side reactions, i.e. by contriving maximal mechanistic bias in favour of fast S_N2 type cleavage under the mildest possible conditions. This can be done by employing media which have simultaneously sufficient (but no more than sufficient) hard acid acidity and high soft nucleophile activity, so that a cooperative 'push-pull' mechanism can operate: structure (14) illustrates the point for benzyl-oxygen cleavage. Combinations of HF or CF_3SO_3H/CF_3CO_2H with thioether soft nucleophiles and other additives have been used extensively in both classical and solid-phase work, the electrophilic catalysis (the 'pull' of the 'push-pull' mechanism) being provided by protonation. Protonation, however, is not the exclusive means of enabling nucleophilic attack. Potent trimethylsilylating reagents have the same effect, and $CF_3SO_3SiMe_3/CF_3CO_2H/PhSMe$ has been found to be a faster and cleaner cleavage system than $CF_3SO_3H/CF_3CO_2H/PhSMe$;[80, 81] it has already been used with success for final deprotection in several demanding cases. Acid-catalysed aspartimide formation remains a problem even with this method: preliminary results indicate that $Me_3SiBr/CF_3CO_2H/PhSMe$ is better in this respect.[82]

Palladium-sepiolite systems have been investigated as catalysts for benzyl-based protecting group hydrogenolysis with hydrogen[83] or by hydrogen transfer from a donor.[84]

A reversed-phase hplc technique with a volatile buffer system has been used for the conversion of peptide trifluoroacetate salts into acetate salts; the procedure is expected to be applicable to other salt conversions.[85]

2.2 The Formation of Peptide Bonds

Active esters. - 1,2,2,2-Tetrachloroethyl chloroformate [(15): readily prepared from chloral and phosgene] reacts with phenols and N-hydroxyimides to give crystalline mixed carbonates [e.g. (16)], which react with N-protected amino acids to give the corresponding active esters in a convenient and general synthesis.[86] It has been shown mathematically that if an optically labile active ester is to be coupled, then use of an excess should give product of higher optical purity than use of the stoichiometrically required amount.[87]

Carbonic anhydrides.- Traditional lore was that mixed carbonic anhydrides were fleeting creatures, to be made with delicacy and used without delay. This now seems a little overdone, as Benoiton has published details[88] of a wash procedure which enables those of

type (17) to be isolated in many cases - in one instance (Z-DL-AlaOCO$_2$Et) as a crystalline analytically pure material: they are in general stable for some hours at -5° or in dichloromethane solution at 25°. They eventually decompose, principally to the corresponding alkoxyoxazolones [(18): once thought incapable of formation, these now seem to crop up at every turn], symmetrical anhydrides (19) and esters (20). Benoiton has also made a thorough study of the side reactions associated with mixed carbonic anhydride couplings. The amount of urethane product formed by attack at the 'wrong' (carbonate) carbonyl group on coupling, a problem which is most serious when the amino component is sterically hindered, depends critically on the solvent-base combination employed: N-methylpiperidine/CH_2Cl_2 was best.[89] Benoiton has also identified a novel side reaction in aqueous DMF: intramolecular alkoxycarbonyl transfer as shown in Scheme 8.[90] After reaction of ZValOCO$_2$Me with H-PheO$^-$ in aqueous DMF, for example, a 6% yield of MeOCOPhe was isolated after hydrogenolysis of the acidic fraction. The coupling in Scheme 9[91] exemplifies a neat means of proceeding directly from a free peptide to a protected peptide acid via a mixed carbonic anhydride.

N-Carboxyanhydrides, etc. - NCA's and their thio analogues the NTAs are cheap and simple components in which CO_2 or CS_2 provides simultaneous carboxyl activation and N-protection. Their use in research-scale synthesis is limited by the fact that their rapid coupling reactions require optimisation for each new case, but their scope for industrial-scale synthesis is considerable: a monograph on their preparation and chemistry has appeared,[92] and their use for peptide bond formation has been reviewed.[93]

Miscellaneous.- The formation of peptides from Co(III)-activated amino esters has been reviewed.[94] Other relevant publications include reports on: the significance and control of disulphide interchange in peptide synthesis by prior thiol capture, and on the danger of racemisation attendant thereon;[95,546] the heterogeneous asymmetric hydrogenation of a chiral tripeptide containing two $\alpha\beta$-dehydroamino acid residues;[96] the synthesis of dipeptides via β-lactams obtained through asymmetric cycloaddition;[97] water-soluble active esters;[98, 99] use of CF_3CO_2NSu for the preparation of succinimido esters;[100] couplings with 4-nitrophenyl esters under phase-transfer conditions;[101-103] "gas-solid phase synthesis of peptides";[104] trimethylsilylcyanide-mediated coupling;[105-107] activation with propylphosphonic anhydride,[108] N,N'-bis(2-oxo-3-oxazolidinyl) phosphinic chloride,[109, 110] ethylmethylphosphinic anhydride,[108] and reagents of type $ROCS_2COCl$;[111] further examples[112, 113] of activation with diphenylphosphinic chloride (of particular value for coupling at or with N-methyl residues[112] - cf. ref. 109); polymer-bound mixed carboxylic dithiocarbamic anhydrides;[114] crown ethers as non-covalent blocking groups;[115] the optimisation of the mixed carbonic anhydride coupling of Boc-D-MetOH and H-GlyPheOMe;[116] activation with 1,2-dihydro-4,6-dimethyl-2-thioxo-3-pyridinecarbonitrile;[117] oxazolone aminolysis;[118] and coupling with 2,2'-carbonyl-bis(3,5-dioxo-4-methyl-1,2,4-oxadiazolidine).[119]

2.5 Solid-phase peptide synthesis

Several reviews, including a silver jubilee report, have appeared during 1987.[120-126] In addition, a useful bibliography on the Fmoc method has been published by Milligen, a division of Millipore (UK) Ltd., Harrow, Middlesex. In contrast to these reviews, an annual report of a wide field like solid-phase peptide synthesis tends to give a series of snapshots without a background and it is not easy to delineate relationships between several foreground subjects. In order to assist the reader, it is perhaps convenient to approach the choice of techniques on three levels.

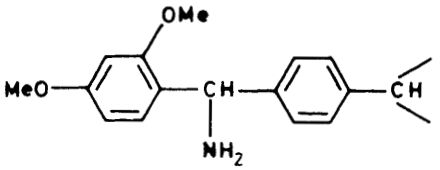
(1) Decisions based on the nature of the project:
   (a) requirement for C-terminal $-CO_2H$ or $-CONH_2$ group;
   (b) preferred groups for protection of α-amino and all other groups;
   (c) preferred method for detachment of completed peptide from matrix;
   (d) length of peptide(s) to be synthesized;
   (e) number of related peptides required (either structural analogues or building blocks for subsequent manual fragment synthesis);
   (f) amount(s) of peptide(s) required.

(2) Based on the responses to 1a-1f, decisions on the following dependent issues must be made:
   (a) choice of insoluble support;
   (b) choice of linker and its mode of attachment;
   (c) choice of method(s) for coupling N-protected amino acids for first and subsequent cycles;
   (d) choice of method for intermediate selective removal of protecting group from the N-terminus;
   (e) choice of degree of mechanization/automation that is desirable or justified.

   It should be noted that certain items under (1) and (2), e.g. 1b and 2d or 1c and 2b, can be interchanged.

(3) Application of measures to detect and deal with possible or actual experimental difficulties:
   (a) choice between manual interruption for monitoring or application of feedback control during synthesis;
   (b) choice of quality control techniques such as amino acid analysis, hplc, FAB mass spectrometry, sequence determination by the Edman method to be applied after completion of the synthesis.

The effort to be applied under (3) will depend on the accumulation of experience and its utilization in subsequent syntheses. For example, certain amino acids (e.g. Gln) or sequences of amino acids (e.g. hydrophobic runs) are prone to cause difficulties. The



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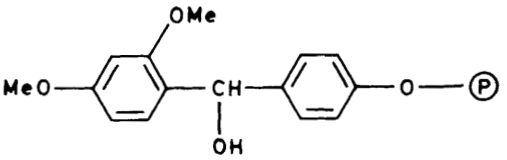

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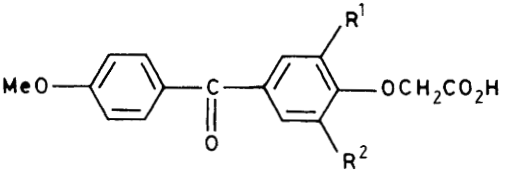
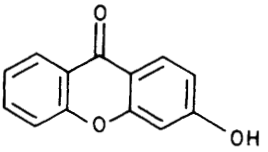


(23) R = H, alkyl, or aralkyl



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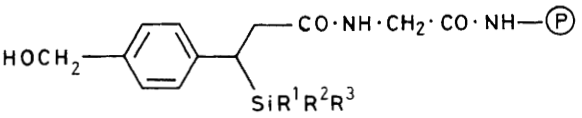

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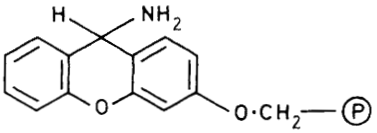
4 steps



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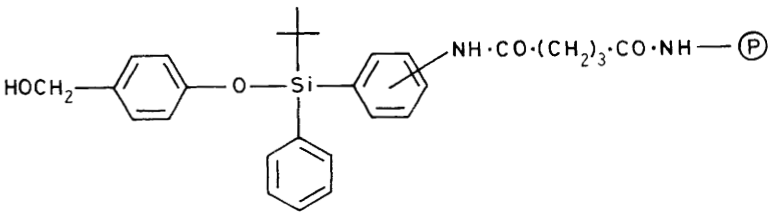
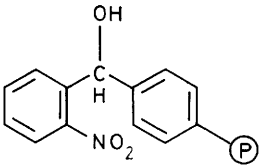


(27)

eventual availability of hardware with a computerized database for collecting details of experimental protocol and records of yield and purity of product should permit artificial intelligence methods to be used to optimize synthetic procedures and save consumables and time. With the above points in mind, it should be easier to relate the subject matter of individual research publications on solid-phase peptide synthesis in the past year to each other and to the wealth of knowledge already available.

A new cross-linked copolymer[127] of styrene and propylene has been prepared to facilitate multiple washing procedures during chain elongation. A Russian version of a macroporous silica matrix has been used for the synthesis of bradykinin.[128]

The incorporation of two methoxy groups in ortho or para positions to an aminomethyl group (21) that bears the peptide increases acid lability[129, 130] so that it can be detached with rather mild reagents such as a mixture of CF_3CO_2H and MeSPh (4:1).[129] This support was invaluable for the synthesis of the sleeper peptide isolated from the venom of the fish-hunting cone snail Conus geographicus, since the peptide contains five Gla residues which readily decarboxylate in acidic conditions.[131] A similiar idea has been advanced for the design of a linker to afford free peptides by a mild method of detachment.[183] The linker-resin conjugate (22) is made by reduction of the corresponding ketone with $LiBH_4$. The first amino acid, as its Fmoc derivative, is coupled using DCCI and 4-dimethylaminopyridine. Residual hydroxyl groups are blocked with benzoic anhydride. Subsequent amino acid residues are attached using the symmetrical anhydride approach. The final detachment is accomplished in a mixture of AcOH and CH_2Cl_2 (1:3), conditions so mild that HOBt can not be used during coupling unless it is buffered with di-isopropylethylamine. Apart from some reservations about the use of 4-dimethylaminopyridine in the first coupling step, this method looks very promising. For the synthesis of peptide amides on the same resin, the first residue, as the Fmoc-amino acid amide, is coupled in the presence of $PhSO_3H$ in dioxan at 50°C for 20h. After the remaining residues have been coupled by the symmetrical anhydride route, the peptide amide can be cleaved from the resin by acid. A similar philosophy led to the design of the ketoacids (23) (R^1, R^2=H, alkyl or aralkyl) which were coupled to an aminomethyl resin.[132] The keto group was reduced and an Fmoc-amino acid amide was coupled in the presence of H_2SO_4. For the special case where N-(aminoalkyl)amides of peptides are required because of their enhanced metabolic stability and biological activity, a linker has been designed[133] that contains an acid-labile urethane group and the support is used with Fmoc protection of the N-terminus throughout peptide assembly. The occasional use of the 9-xanthenyl group for the protection of the amide group of Asn and Gln and its removal by acid led to the design of another linker for the synthesis of peptide amides. 3-Hydroxyxanthone (24) can be coupled to chloromethyl polystyrene and converted into (25) in four steps.[134] After completion of the synthesis, the peptide can be rapidly cleaved from the resin by CF_3CO_2H in $ClCH_2CH_2Cl$. Two closely related linkers (26, 27) have been designed to be sensitive to cleavage by fluoride



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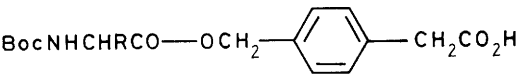

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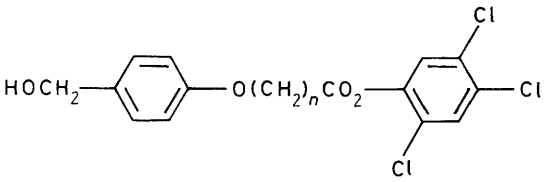

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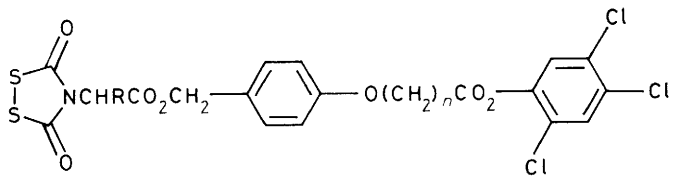

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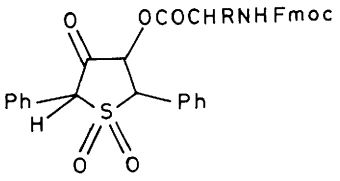

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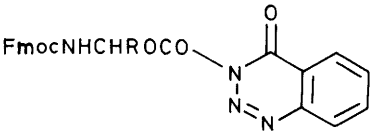

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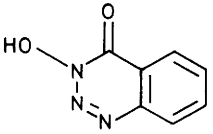

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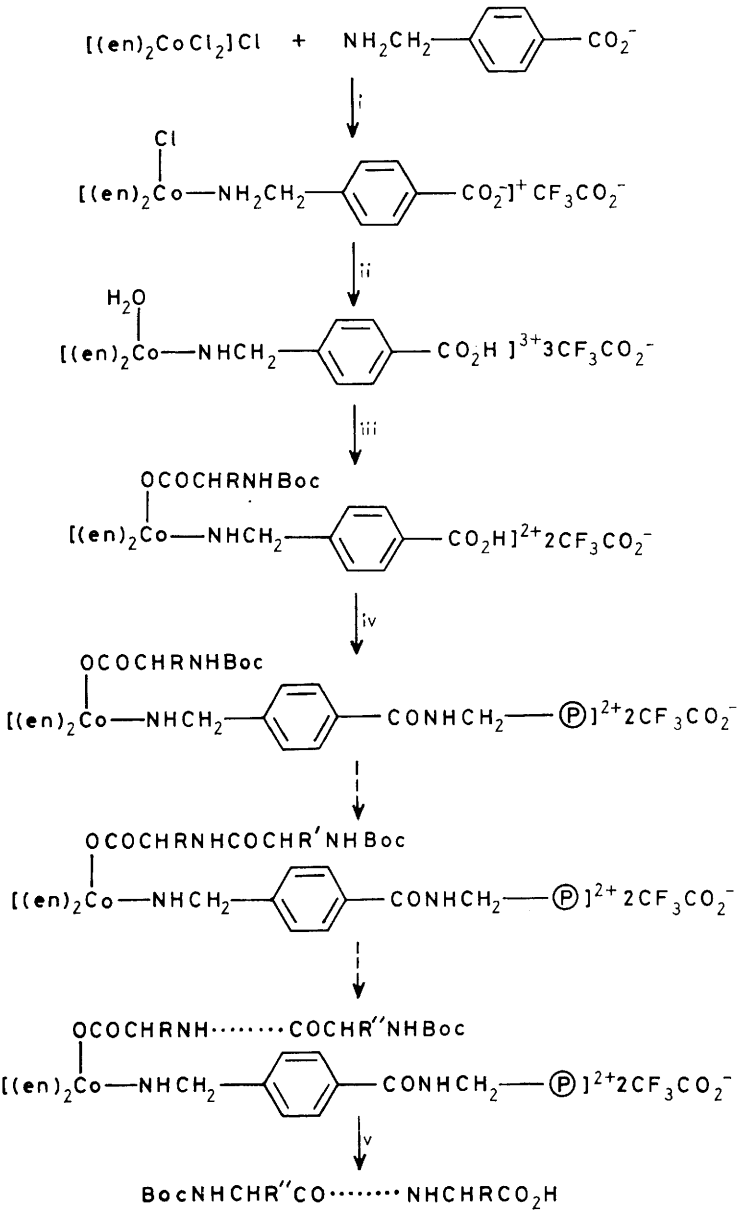


Conditions: i, Et_2NH/H_2O; ii, pH 11·5, then pH 3 with CF_3CO_2H;
iii, $BocNHCHRCO_2^-/Et_3N/DMF$; iv, DCCI/HOBt, NH_2CH_2— Ⓟ;
v, $HSCH_2CH_2OH$

Scheme 10

ion.[135, 136] The detachment of peptide from the resin at room temperature is a very attractive feature.

Whereas the presence of one or two ortho- or para-methoxy groups labilizes the peptide linker bond to acid, the incorporation of a nitro group (28)[137] permits the peptide to be detached photolytically. Assessment of this method should be deferred until it has been used successfully to synthesize peptides containing His, Met and Trp.

Usually, the linker is attached to the polymeric support before the first protected amino acid is coupled. There have been some instances where this sequence was reversed; one is cited above.[136] An improved route is now available to (4-Boc-aminoacyloxymethyl)phenylacetic acids (29)[138] for preparation of the well known PAM resins. In other example,[139] Dts-amino acids are esterified with (30) using DCCI or $Me_2N(CH_2)_3N{=}C{=}NEt$ to give (31). These active esters are coupled to aminomethyl-polystyrene. After completion of the assembly of the peptide, detachment from the resin is effected in CF_3CO_2H/CH_2Cl_2 (1:1). In a completely novel approach,[140] the carboxyl group of the C-terminal amino acid is a ligand in a Co(III) complex (Scheme 10). Although only one peptide, Leu-enkephalin, has apparently been synthesized by this route, the stability of the peptide-linker bond during peptide assembly and its easy cleavage by $HSCH_2CH_2OH$ in DMF afterwards makes it potentially very attractive and worthy of a thorough appraisal.

The search for improved methods for attaching the protected C-terminal amino acid to the resin continues. Sieber[141] recommends converting the Fmoc-amino acid into an unsymmetrical acid anhydride with 2,6-dichlorobenzoyl chloride because the coupling step does not require a base. This avoids the risk of racemization of this residue and the formation of a dipeptide by partial unblocking of the amino group. Sheppard's group[142] convert Fmoc-amino acids into active esters of 2,5-diphenyl-2,3-dihydro-3-oxo-4-hydroxythiophene dioxide (32). These undergo transesterification with the benzylic hydroxyl group of the linker moiety of the support in presence of di-isopropylethylamine. The esters (32) coupled faster than the corresponding pentafluorophenyl esters and no racemization was detected. N-Trityl and Fmoc-amino acids have been coupled to benzyl and benzhydryl hydroxyl groups as the respective esters using $Ph_3P/EtO_2CN{=}NCO_2Et/THF/0°$.[143] Although neither racemization nor steric hindrance caused problems, some nitrile formation was detected when TrtAsnOH and TrtGlnOH were attached to either type of linker.

There has been little activity in the design of new protecting groups for solid-phase peptide synthesis. Merrifield's group[144] recommend 4-methoxybenzyloxycarbonyl for protection of α-amino groups because it is rapidly cleaved in 5-10% CF_3CO_2H in CH_2Cl_2. Products were cleaner than when the Boc group was used and the procedure was well tested by a successful synthesis of thymosin α_1. The use of the Dts group has been mentioned above.[139] In this work, Pro was protected by the $Me_2CHSSCO$- group. For the protection of the thiol group of cysteine, the S-t-butylsulphenyl group is

recommended as an orthogonal partner to the Fmoc group.[145] The S-9-fluorenylmethyl group can be used in conjunction with the Boc group[146] but not with the Fmoc group, since both are labile to piperidine. Deprotection methodology has attracted even less attention. The use of trimethylsilyl trifluoromethanesulphonate,[80, 127] however, is a useful new tool. In the presence of thioanisole, the peptide benzyl ester link and other acid-labile groups are cleaved. A useful alternative to the use of secondary amines for the removal of Fmoc groups has been described.[147] Tetrabutylammonium fluoride in DMF effects cleavage in about 2 minutes.

The use of pentafluorophenyl esters of Boc-amino acids[148] complements the corresponding method for coupling Fmoc-amino acids. It is claimed that the rate of coupling is similar to that with symmetrical acid anhydrides and that the products are pure.

The growing importance of peptide-based drugs makes the synthesis of pseudopeptides by solid-phase methods highly desirable. Coy and his colleagues have developed a method for incorporating the $-CH_2NH-$ surrogate (reduced) peptide bond. A Boc-aminoaldehyde is allowed to condense with the terminal amino group of a peptide being assembled on a resin, and then the resultant Schiff base is reduced.[149, 150]

In spite of all the work that has gone into the design of linkers to ensure stability and freedom from side reactions during synthesis followed by detachment under mild conditions at the end of the synthesis, the perfect linker-matrix conjugate has not yet been produced. Little or no effort has been deployed to design linker-matrix conjugates that can be recycled, yet with syntheses being carried out on the kilo scale this must surely be an attractive goal. For those who can afford to work on this scale, there are chemical engineering problems that they should consider.[151] The small-scale synthesis of short peptides is a different world. Using the 'teabag' technique and the well-tried Merrifield methodology, Houghten has produced 250 new peptides in 3-4 weeks.[152, 153] The peptide productivity of the 'teabag' technique has already been considerably exceeded. By applying Merrifield's method with a polyacrylamide support grafted on small polyethylene rods, Geysen's group[154] are able to produce 2000 small peptides in 10 working days. The peptides are obtained in 30-50 nmol quantities and are not further purified. Spot checks on the quantitative amino acid composition of particular peptides confirm the validity of the method. For immunological purposes, the presence of impurities arising from racemization, deletions or specific side reactions is not important, since mutant peptides will not be recognized by an antibody raised against the protein from which the authentic peptide derived. Such a high productivity makes it more or less routine to identify linear epitopes of a protein of known amino acid sequence.

In the euphoric atmosphere that accompanies recent successes in peptide synthesis, it seems almost churlish to mention a few difficulties. A careful nmr examination has revealed that the accumulation of more than five consecutive Gly residues on the

Merrifield resin leads to chain aggregation.[155] Again, Gln, particularly when it is adjacent to the C-terminal residue, is prone to undergo cyclization to a pyroglutamyl residue.[156]

Until recently, monitoring of solid-phase peptide synthesis simply used the Kaiser adaptation of the ninhydrin reaction to test small samples of resin removed periodically from the reaction vessel. A positive result gives a qualitative assessment of the availability of a deprotected α-amino group before the next coupling step. On the other hand, a negative result after a coupling reaction indicates that the latter is complete. Post-synthetic quality control includes checks for heterogeneity by hplc, amino acid analysis, sequence determination of completed peptide by Edman degradation, and examination of the sensitivity of the peptide to exo- and endo-peptidases as a test of chiral purity. Recent developments in the chromatography and especially the hplc of peptides are summarized in section 6.

The Edman method of stepwise degradation has been applied to protected synthetic peptides while still attached to the matrix on which they were assembled.[157] Some acid-labile groups are cleaved under the conditions required to form 2-thiohydantoins, so that, for example, im-tosyl and in-formyl on His and Trp respectively cause no problems. The use of pyridine in the Edman procedure, however, causes racemization of H-Thr(Bzl)-OH which gives rise to two peaks. H-Ser(Bzl)-OH is also racemized but this causes no problems. Isotachophoretic analysis has been recommended[158] as a means for checking the purity of synthetic peptides; detection was achieved by measuring light absorption at 206nm and 254nm. Proton nmr spectroscopy at 360 MHz has been used[159] to characterize a synthetic peptide while still on the resin; side-chain protecting groups may be left on or removed. Spectral resolution was enhanced by using deconvolution techniques to separate the spectral contribution of the polyacrylamide resin and the peptide. The most significant step towards true automation has been taken by Sheppard's group.[160] 3,4-Dihydro-3-hydroxy-4-oxobenzotriazinyl esters of Fmoc-amino acids (33) are excellent acylating agents and the N-hydroxy derivative (34) liberated in the early stages of the coupling absorbs light strongly at 440nm in the presence of free amine. The active ester is used in excess, and when reaction is complete there is no amine to generate the anion of (34). This drop in light absorption indicating completion of reaction can be used to signal to the peptide synthesizer to proceed to the next stage. The same reagent has been added in the free state to act as indicator in a simple manually operated synthesizer.[161]

Some of the more significant syntheses are briefly discussed below and others are cited in the appendices. Atrial natriuretic peptides continue to receive attention. The (8-33) and (10-33) sequences from the rat hormone have been synthesized.[162, 163] Both syntheses involved fragment coupling of small peptides which had been produced by the solid-phase method and the (8-33) peptide was also made by total solid-phase synthesis.[163] In one of the syntheses,[162] coupling of the fragment (8-15) was

particularly troublesome. Various coupling procedures were used, impurities in the octapeptide fragment were identified and probable reasons for their formation were advanced. Careful reading of this paper should be compulsory for those who misguidedly think that peptide synthesis is a button-pushing exercise. In another study of fragment condensation,[164] peptides of the general formula Boc-$(Leu_3Pro_2Gly)_n$-OH (n=1-12) were coupled to peptides anchored to either soluble or insoluble cross-linked polystyrene. Not surprisingly, yields diminished as n increased; soluble polystyrene afforded better yields than the more usual insoluble form. The synthesis of a tetradecapeptide sequence from rat angiotensinogen[165] is notable for the remarkably successful use of benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate for peptide bond formation. The peptide was rigorously purified and the yield was 29% based on the amount of C-terminal serine attached to the resin. The synthesis[166] of an analogue of the pentadecapeptide gramicidin A, in which all Trp residues were replaced by Tyr or Tyr(Bzl), is notable for the use of the Bpoc protecting group and of DCCI for coupling. The slight sensitivity of the Bpoc group towards HOBt precluded its addition to catalyse coupling. In consequence, coupling steps had to be repeated three or four times. Medium-sized peptides synthesized by solid-phase methods include bombesin,[167] melittin,[168] and a 33-residue peptide analogue of bovine brain calmodulin calcium-binding site.[169]

Mouse myeloma M603 IgA binds phosphocholine at a site which is in a cleft between the hypervariable regions of its light (V_L) and heavy (V_H) chains. Merrifield's group have defined an ultimate objective to synthesize V_H (1-68) and V_H (69-120), to link these together either noncovalently or covalently and then to associate the product with the natural light chain or V_L derived from it to assemble the binding cavity of M603 protein. So far, the V_H (16-68) peptide has been assembled from fragments synthesized by conventional solid-phase methodology.[170] Although this peptide is less than half the size of the intended V_H (1-120) peptide, it associated with the M603 light chain and the resulting complex bound phosphocholine.

Human transforming growth factor α consists of a single polypeptide chain of 50 amino acids with three intramolecular disulphide bonds. This molecule has now been synthesized[171] using the Fmoc protecting group. This achievement not only puts the Fmoc group into the same league as the Boc group, but the synthesis was performed manually. The accolade for the longest peptide synthesized in 1987 must go to a group in Cincinatti for the synthesis of human apolipoprotein C-II.[172, 173] This polypeptide contains 79 amino acid residues, is present in very low-density lipoproteins, and enhances the activity of lipoprotein lipase towards triacylglycerols at the capillary endothelium. The synthesis employed Boc α-protection and assembly on a PAM resin. An interesting technical variation was employed. Most coupling steps were performed twice, once with the symmetrical anhydride in CH_2Cl_2 and then with the preformed ester with 1-hydroxybenzotriazole in DMF. The use of solvents with different polarities is known to enhance coupling efficiency.

It is significant that almost no attention has been given to the 'soluble-handle' method this year. Tuftsin has been synthesized by the picolyl ester method.[174] As mentioned above,[164] there is at least one example where a soluble polystyrene handle gave a better yield than insoluble cross-linked polymer. The 'soluble-handle' method should not be consigned to the history books without some additional examination of its difficulties and potential. To underline this point and to provide a lead into the next section, the value of carboxypeptidase Y for deblocking peptide esters, including those of polyethyleneglycol, has been reviewed[175]

2.6 Enzyme-mediated synthesis and semi-synthesis

These techniques are often used in conjunction, and it is sensible to bracket the reporting of progress in the two areas. A substantial fraction of the literature comprises reviews[32, 176-182] which perhaps reflects differences in opinions about fundamentals. For example, enzyme-mediated synthesis is sometimes carried out in wholly aqueous systems, despite the unfavourable equilibrium position,on the grounds that some enzymes will not withstand high concentrations of organic solvents.[184-191] One component for the synthetic reaction may be present in large excess in an attempt to shift the equilibrium position sufficiently to give more favourable yields. Since the concentration of water is about 55.5M, this may not be particularly effective. Moreover, the kinetic enhancement will not significantly improve by increasing the concentration above about five times its K_m value. In complete contrast, some syntheses are carried out in the presence of water-miscible solvents or even in almost anhydrous conditions to achieve a more favourable position of equilibrium.[192-201] It has been argued,[202] however, that the thermodynamic activity of water must be less than unity to achieve significant effect on the position of equilibrium. In the presence of a high concentration of solvent, the enzyme may be stabilized against denaturation by immobilization on an insoluble support. This may carry a kinetic penalty, since the system now has two phases. As a compromise between the two extreme conditions above, an immiscible solvent can be added so that the product may be preferentially extracted and thus removed from access to enzyme and the risk of hydrolysis without subjecting the enzyme to high concentrations of organic solvent in the same phase. A further interesting development aimed at kinetically favouring synthesis over hydrolysis uses either catalytically impaired proteinases or esterases that follow the Ping Pong mechanism.[203-206] Ester substrates, but not peptides, are able to form the acyl enzyme which can then undergo nucleophilic attack by an amino compound to give an amide product.

Some examples of these four main approaches will be given to illustrate further points. A mixture of tyrosine methyl ester and methionine methyl ester in citrate buffer (pH5) in presence of thermolysin gave 21% of H-$(TyrMet)_3$-OH, and most of the substrates were recovered unchanged. No product was isolated in a two-phase system.[185] Enzyme-catalysed synthesis of pressinoic acid was achieved using papain,

chymotrypsin or thermolysin.[190] The carboxyl group of the C-terminal fragment was protected as the phenylhydrazide, presumably to decrease the solubility of the product thereby increasing the yield; the carboxylic acid group was liberated by oxidation with iron (III) chloride.

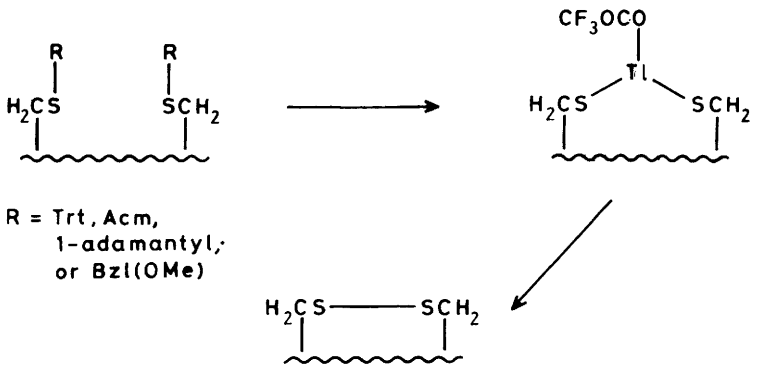
Alcohols are commonly added to the media used for enzyme-catalysed synthesis of peptide bonds. One advantage of this approach is the intermediate formation of the corresponding ester of the N-terminal fragment. The ester is a much superior acylating agent to the carboxylic acid or its anion. Indeed, esters of N-acetylated amino acids have been produced by α-chymotrypsin catalysis.[195] Preformed ester is also commonly used as substrate. Clostridiopeptidase B has been used to synthesize peptides from ZArgOMe and a range of amino acid amides in aqueous methanol. Yields ranged from over 90% with H-MetNH$_2$, H-PheNH$_2$, H-GlyNH$_2$, H-LeuNH$_2$ H-SerNH$_2$ and H-ValNH$_2$ to zero with H-GlnNH$_2$, H-AsnNH$_2$ and H-ThrNH$_2$.[192] It is difficult to explain these results. Interestingly, two groups have reported that subtilisin[194] and papain[193] relax their high stereospecificity in presence of high concentrations of organic solvents. For example, the ratio V_L/V_D for the hydrolysis of AcPheOCH$_2$CH$_2$Cl by subtilisin is 773 in aqueous solution but only 7.6 in t-amyl alcohol.[194] It must be stressed that the relaxation of stereoselectivity is not due to racemization. Thus, the subtilisin-catalysed reaction between Ac-D-PheOCH$_2$CH$_2$Cl and H-L-PheNH$_2$ gave only Ac-D-Phe-L-PheNH$_2$; no LL-diastereoisomer was detected. Again, papain catalysed the formation of ZGly-X-OMe (X = D-Ala, D-Leu, D-Phe, D-Val) in 40% methanol at an apparent pH of 9 giving yields of 55-92%. The addition of a competing nucleophile such as an amine or alcohol in the hydrolysis of substrates by proteinases has long been used as a kinetic tool to detect and quantify the rate of formation of an acyl enzyme with those enzymes that follow a Crypto Ping Pong Uni Bi mechanism. Similar evidence has been adduced for the wheat carboxypeptidase-catalysed synthesis of N-[3-(2-furyl)-acryloyl]dipeptide amides.[196]

As indicated above, esters of N-protected amino acids are better acylating agents than the corresponding free acids. Several papers describe the biphasic or triphasic esterification of N-protected amino acids. In addition to a general paper concerning enzyme-catalysed esterification,[207] papain[41, 208] and α-chymotrypsin[200] have been studied. The former enzyme functions well at pH 4.2 in presence of CH_2Cl_2 to esterify Boc-amino acids. α-Chymotrypsin, immobilized by adsorption on Sephadex LH-20, gave a good yield of AcTrpOEt in the presence of cyclohexane or heptane. The same enzyme in a one-phase system of EtOH-H_2O gave poorer yields which declined with increasing water content. Similar results were obtained in the synthesis of ZTyrLeuNH$_2$ in presence of α-chymotrypsin.[209] Yields were very small in aqueous systems but greatly enhanced by the addition of ethyl acetate due to extraction of the product into the organic phase.

The use of lipases,[203, 205, 206] or a suitably modified proteinase such as thiolsubtilisin,[204] provides a novel approach to the enzyme-catalysed synthesis of peptides. These enzymes are unable to catalyse the hydrolysis of peptide bonds at a significant rate, but they can use esters, especially the more reactive types, of N-protected amino acids as substrates. The intermediate acyl enzyme will react with the amino group to produce a peptide. For example, a 4-chlorophenyl ester of a protected amino acid with a 20-fold excess of an amino acid amide gives 80-95% of product after reaction with thiolsubtilisin in a mixture of DMF and phosphate buffer at pH 8.0.[204]

If enzymic peptide synthesis is to rival chemical synthesis, there will have to be a considerable investment in the design of suitable hardware and software. Carrying out syntheses under pressure has been shown to increase yields significantly,[210] but it is too soon to judge if the extra investment in hardware is adequately compensated for by increased yields. On the other hand, the design of an apparatus for continuous analysis of proteinase-catalysed peptide synthesis[211] is almost certainly a step in the right direction, since it provides a feedback for potential automation.

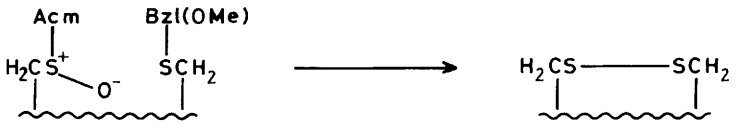
This year has witnessed a continued high level of activity in the semi-synthesis of insulin and some analogues. The appearance of a substantive review[182] with many experimental results backed by theoretical considerations is welcome. The semi-synthesis of human [Phe(T)^{B1}]-insulin of very high specific activity has been accomplished by purely chemical procedures.[212] The semi-synthesis of human [Phe(T)$^{\frac{1}{2}}$]-proinsulin has also been reported.[213] An instance of familial hyperproinsulinaemia has been shown to involve a single-point mutation leading to the production of [Asp^{10}]-insulin. This has been synthesized[214] using S-sulphonated A chain from porcine insulin and S-sulphonated [Asp^{10}]-B chain, which was made by fragment synthesis in a method adapted from that of Schwartz and Katsoyannis.[215] The mutant insulin was obtained by reductive coupling of the two S-sulphonated chains, in the presence of dithiothreitol at pH 10.6 and 4°C: it has a binding affinity to insulin receptors which is about five times that of natural insulin, and a similar activity was recorded in a lipogenesis assay. Reversed-phase hplc indicated that [Asp^{10}]-insulin is more hydrophobic than insulin, although Asp is less hydrophobic than the His residue it replaces.[216] A conformational change has been postulated to account for this surprising result. Trypsin was used to couple synthetic peptides to N-protected insulin from which the C-terminal octapeptide had been cleaved.[198] The purpose of this work was to provide a range of mutant insulins with changes in the (B^{24}-B^{26}) section, since this region is probably involved in binding to the receptor. In a related study, the same group[199] have prepared a series of insulin analogues with shortened B chains. The work stems from the observation that (B^{26}-B^{30}) can be dispensed with provided B^{25} is amidated.[217] Analogues were enzymically synthesized using trypsin as the catalyst and N$^{\alpha A1}$, N$^{\alpha B1}$-Boc_2-des-(B^{23}-B^{30})-insulin and seven tripeptide amides. Replacement of residue Phe^{B25} by His or Tyr increases biological activity significantly.



Conditions: $(CF_3CO_2)_3Tl/CF_3CO_2H$

The reaction can also be applied in an intermolecular manner.

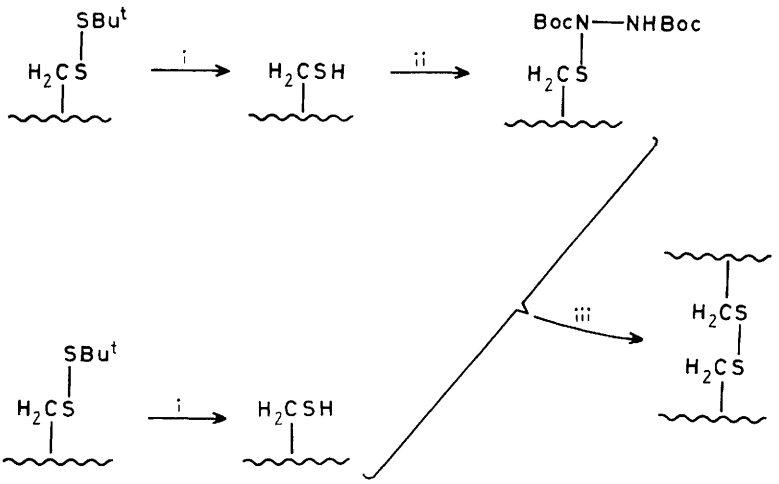
Scheme 11



Conditions: CF_3CO_2H/Me_2S

The reaction can also be applied in an intermolecular manner.

Scheme 12



Conditions: i, Bu_3P/CF_3CH_2OH; ii, BocN=NBoc/DMF; iii, DMF

Scheme 13

$$P\text{-}AA^1\text{-}OX + H\text{-}AA^2\text{-}Pro\text{-}OMe \longrightarrow P\text{-}AA^1\text{-}AA^2\text{-}Pro\text{-}OMe + \begin{bmatrix} AA^2 \\ \\ Pro \end{bmatrix}$$

P = a protecting group, AA = an α-amino acid, OX = a leaving group

Scheme 14

Teoc-Ala-OPfp + Z-AlaPro-OMe ⟶ Teoc-Ala Ala Pro-OMe

Teoc = $TmsCH_2CH_2OCO$—

Conditions: H_2/Pd(C)/dioxan, 79% yield

Scheme 15

cyclo-(MeAlaTyr-D-TrpLysValPhe), AcOH

(35)

2.7 Disulphide Bridge Formation

The synthesis of cystine peptides has been reviewed.[218]

Thallium (III) trifluoroacetate cleaves various S-protecting groups [Trt, Acm, Bzl(OMe), Bu^t, 1-adamantyl, etc., but not Bzl] with simultaneous oxidative disulphide bridge formation as outlined in Scheme 11, and the value of the procedure has been demonstrated in syntheses of oxytocin, urotensin II and human calcitonin gene-related peptides. Of the common trifunctional residues, only two are affected by the reagent: tryptophan gives unidentified products and methionine is partially oxidised to the sulphoxide. But in-mesitylenesulphonyl substitution protects tryptophan against the side reactions, and can be cleaved ($CF_3CO_2SiMe_3/CF_3CO_2H/Ph_2S$), and Met(O) reduced ($NH_4I/Me_2S/CF_3CO_2H$), without affecting the disulphide bridge.[77, 78, 219] Yajima and his co-workers have also reported the novel disulphide bond-forming reaction shown in Scheme 12, and exemplified its application impressively by conversion of the appropriate protected linear intermediate to oxytocin in 86% yield.[220]

The Wünsch-Romani method (Scheme 13), as its inventors have labelled it, has been used to very satisfactory effect in a synthesis of a complex unsymmetrical double-chain cystine peptide fragment of mouse nerve growth factor.[91, 221]

2.8 Miscellaneous

Couplings to dipeptide methyl (or Bzl etc., but not Bu^t) ester amino components are notorious for the problem of competing diketopiperazine formation (Scheme 14), particularly if Pro is present in the dipeptide. Indeed, as is well known in the thyroliberin field, even amides of X-Pro dipeptides cyclise with inconvenient ease. The simple expedient of generating the free dipeptide amino component by hydrogenolysis, in the presence of an N-protected amino acid active ester which is not affected by the hydrogenolysis conditions, enables the dipeptide ester to be trapped by peptide bond formation before it can cyclise. Trimethylsilylethoxycarbonylamino acid pentafluorophenyl esters are especially suited for use in this way, which is illustrated specifically in Scheme 15.[222] With two N-methyl residues in the dipeptide, however, this trick fails and diketopiperazine formation still dominates - for such a case a switch to t-butyl C-terminal protection is indicated.

Two interesting practical exercises introducing undergraduate students to peptide chemistry have been described.[223, 224] One of them,[224] however, will require tinkering with if it is to be used here in the UK: our safety officers are liable to attacks of the vapours at the very thought of anybody tasting anything in the laboratory, even if it is supposed to be a sweetener.

Couplings between large fragments are sometimes very difficult to achieve, for reasons which are not understood, and therefore not predictable. Solubility is of course an important aspect, but there are conformational influences at work as well, and in any case the solubility of synthetic intermediates is not predictable either. Any serious

studies[225, 226] on the solubility, solvation, and conformations of high molecular weight protected peptides, and the relationship of these factors to synthetic problems, are therefore to be welcomed.

The literature contains few detailed accounts and discussions of large-scale endeavours in peptide synthesis, which gives the synthesis[227] of the somatostatin analogue (35) special interest: the final product was prepared in 40-50g batches of better than 99% purity.

3. Synthesis: Selected Cases of Particular Interest

In section 2 above, we have done our best to pick out the salient points of general interest from the syntheses we have examined, all of which are listed in Appendix I below. Space does not allow us to give an overall review of every synthesis, and in any event some are such monumental undertakings and reported in the primary literature with such necessary terseness that they confound attempts at précis. There is really no adequate alternative to inspection of the original reports. On the classical side we recommend the following as especially instructive and illustrative of the sophistication the subject has now attained: thymopoietin II[57] mouse nerve growth factor trypsin unsymmetrical cystine peptide fragment (10-25)/(75-88),[91, 221] ovine corticotropin releasing factor,[59] perornithine-thymnine Z1,[61] porcine neuropeptide Y,[128] neuromedin U-25,[228] human calcitonin gene-related peptides,[77, 78] human pancreatic polypeptide,[58] valosin,[56] and porcine cholecystokinin-pancreozymin.[55] Of solid-phase and solid-phase/classical fragment condensation examples, particular interest attaches to: the atrial natriuretic peptides,[162, 163] melittin,[168] a 33-residue analogue of bovine brain calmodulin-binding site,[169] a 52-residue V_H peptide of mouse myeloma M603 IgA,[170] human transforming growth factor α (50 residues),[171, 229] and human apolipoprotein C-II (79 residues).[172, 173]

4. Appendix I: A List of Syntheses Reported in 1987

The syntheses are listed under the name of the peptide to which they relate, as in previous years, but no arrangement is attempted under the subheadings. We generally follow the nomenclature of the papers cited, eccentricities and all; we cannot forbear remarking that much obscurity is being introduced by the reckless invention of new trivial names and acronyms.

Peptide	Ref.
4.1 Natural Peptides, Proteins and Partial Sequences	
Alkaline phosphatase	
The signal sequence	230
Angiotensin	
Angiotensin II analogues containing β-alanine	231
Angiotensin II analogues containing α-methyldopa	232
Five cyclic analogues	233
Long-acting inhibitors containing hexafluorovaline at position 8	234
Antagonists with variations at position 5	235
[Aib^4, Leu^8] analogues	236
Angiotensinogen	
A tetradecapeptide derived from rat angiotensinogen	165
Antiarrhythmic peptide	237
Two potent derivatives	238
Apolipoprotein C-II	
Human plasma apoC-II	172, 173
Aspartame	239, 240
A series of esters of aspartyl-1-aminocyclopropane carboxylic acid	241
Various syntheses, analogues and derivatives	242-245
Atrial natriuretic factor (ANF) peptides (ANPs)	162
A series of α-ANP analogues	246
Atriopeptin III	163, 247, 248
Superactive analogues obtained by substitution of Cys^{105}	249
ANF (8-33) and analogues	250
Bacteriorhodopsin	
N-Retinylidenepeptides	251
Bombesin	167, 252
Bradykinin	
Numerous analogues	253, 254
Analogues of potentiating nonapeptide	255

4.2. Sequential Oligo-and Poly-peptides

4.3 Enzyme Substrates and Inhibitors

4.4 Glycopeptides

4.5 Miscellaneous Peptides

5. Appendix II: Useful Amino Acid Derivatives

Melting points are in °C; [∝] values are at the D line, at ambient temperature. The list of derivatives, which we must stress is selective (and in some respects arbitrary), is divided into two groups: those of the genetically coded and those of the non-coded amino acids. Most are new, but in a few cases a known compound is listed because of an improved preparation or amended physical constants. The abbreviations will mostly be familiar or obvious, but readers may need a reminder or help for the following:

Pac phenacyl
Dts dithiasuccinoyl
Tmse 2-(trimethylsilyl)ethyl
Teoc 2-(trimethylsilyl)ethoxycarbonyl
Dpm diphenylmethyl (previously sometimes Bzh, benzhydryl)
Pmc 2,2,5,7,8-pentamethylchroman-6-sulphonyl

When no position is indicated for a benzenoid ring substituent, it is para.

Compound	M.p.	[∝]	c	Solvent	Ref.
5.1 Coded Amino Acids					
Alanine					
Dts-Ala-OH	182-184	-	-	-	-
Dts-Ala, Dcha	105-108	-	-	-	-
Boc-Ala-OEt	-	-42	1	MeOH	208, 507
Teoc-Ala,Cha	125-131	-0.5	2	MeOH	38
H-Ala-ODpm, TosOH	179	-10.9	2	MeOH	53
$(Boc\text{-}Ala)_2O$	112-113	-25.2	2	CH_2Cl_2	508
Arginine					
Fmoc-Arg(Tos)-OH	103-106	-5.8	1	DMF	37
Dts-Arg(NO_2)-OH	158-160	-	-	-	-
Z-Arg($Adoc_2$), Dcha	137-138	+9.0	0.5	DMF	463
Z-Arg($Adoc_2$)-OH	121.5-122	+20.1	1	$CHCl_3$	463
Z-Arg(Pmc), Cha	150-152	-	-	-	113
Asparagine					
Dts-Asn-OH	157-159	-	-	-	43
Boc-Asn-OEt	105	-17.5	1	MeOH	507
Aspartic acid					
Dts-Asp(OBu^t)-OH	73-77	-	-	-	43
Dts-Asp(OBu^t), Dcha	140-141	-	-	-	43
Boc-Asp-OEt	107	-21.7	1	MeOH	507
$(Boc\text{-}Asp[OBzl])_2O$	85-86	-1.4	2	CH_2Cl_2	508

Cysteine					
Nps-Cys(SBu^t)-ONSu	100-102	-202.6	1	MeOH	221
Boc-Cys(SBzl)-OEt	50	-43.8	1	MeOH	507
(Boc-Cys[Bzl(OMe)])$_2$O	96-97	-15.3	2	CH_2Cl_2	508
Fmoc-Cys(Acm)-OH	147-148	-44	1	DMP	74
Cystine					
Boc-Cys-OTmse | Boc-Cys-OTmse	45-46	-64	0.2	EtOH	509
Glutamic acid					
Dts-Glu(OBu^t), Dcha	113	-	-	-	43
[Boc-Glu(OBzl)]$_2$O	96-98	-5.0	2	CH_2Cl_2	508
Nps-Glu(OEt) NCA	136	+40.5	1	THF	431
Boc-Glu(OH)-OEt	-	-28.9	1	MeOH	507
Glutamine					
Dts-Gln-OH	148-150	-	-	-	43
Boc-Gln-OEt	101	-25.5	1	MeOH	507
Glycine					
Dts-Gly-OH	140-142	-	-	-	43
Dts-Gly, Dcha	97-100	-	-	-	43
Histidine					
Fmoc-His(Tos)-OH	110-115	-13.1	1	DMF	37
Fmoc-His(Trt)-OH	133-134	+76.8	1	$CHCl_3$	54
Fmoc-His(Trt)-OH	150 (dec)	+86.6	5	$CHCl_3$	73
Isoleucine					
Dts-Ile-OH	118	-	-	-	43
Dts-Ile, Dcha	97-98	-	-	-	43
H-Ile-ODpm, TosOH	156-158	-21.7	2	MeOH	53
(Boc-Ile)$_2$O	90-91	+18.5	2	CH_2Cl_2	508
Leucine					
Dts-Leu-OH	69-71	-	-	-	43
Dts-Leu, Dcha	123-126	-	-	-	43
H-Leu-ODpm, TosOH	196	-14.7	2	MeOH	53
(Boc-Leu)$_2$O	82-83	-14.0	2	CH_2Cl_2	508
Lysine					
Boc-Lys(Z)-NH_2	141	+1.4	-	MeOH	336
Dts-Lys(Boc), Dcha	118-120	-	-	-	43
H-Lys(Trt)-OH, CF_3CO_2H	85-89	-	-	-	54
Fmoc-Lys(Trt)-OH	145	+5.9	2	$CHCl_3$	54
(Boc-Lys[Z(2Cl)])$_2$O	97-98	-2.7	2	CH_2Cl_2	508

Methionine					
Dts-Met-OH	114-116	-	-	-	43
Dts-Met, Dcha	98-100	-	-	-	43
Boc-Met-OEt	46	-35.8	1	MeOH	507
Teoc-Met-OH	97-98	+13.8	2.2	$CHCl_3$	507
H-Met-ODpm, TosOH	169-170	-8.4	2	MeOH	53
$(Boc\text{-}Met)_2O$	88-90	-11.6	2	CH_2	508
Phenylalanine					
Dts-Phe-OH	116-118	-	-	-	43
Dts-Phe, Dcha	138-139	-	-	-	43
$(Boc\text{-}Phe)_2)O$	97-101	+15.5	2	CH_2Cl_2	508
Proline					
Boc-Pro-OPac	80-80.5	-86.0	1	MeOH	432
Z-Pro-OPac	92	-84.0	1	$CHCl_3$	109
H-Pro-ODpm, hemioxalate	174 (dec)	-28.9	1	DMF	52
Serine					
Teoc-Ser, Cha	120-123	+12.8	1.9	MeOH	38
H-Ser(Dpm)-ODpm, hemioxalate	175 (dec)	-4.4	2	DMF	52
H-Ser(Dpm)-OH	216(dec)	+2.6	0.5	MeOH	52
Fmoc-Ser(Trt)-OH	195-197	+2.2	2	$CHCl_3$	54
$[Boc\text{-}Ser(Bzl)]_2O$	77-78	+5.9	2	CH_2Cl_2	508
Fmoc-Ser(Bzl)-OH	117-119	+13.8	1	MeOH	61
Dts-Ser(Bu^t), Dcha	137-140	-	-	-	43
Boc-Ser-OEt	60	-21.7	1	MeOH	507
Threonine					
$[Boc\text{-}Thr(Bzl)]_2O$	97-99	+6.6	2	CH_2Cl_2	508
Dts-Thr, Dcha	118-120	-	-	-	43
Boc-Thr-OEt	51	-24.5	1	MeOH	507
H-Thr(Dpm)-ODpm, hemioxalate	180(dec)	-4.6	2	DMF	52
H-Thr(Dpm)-OH	280(dec)	-24.6	1	MeOH	52
Tryptophan					
$[Boc\text{-}Trp(CHO)]_2O$	126-129	+15.1	2	CH_2Cl_2	508
Dts-Trp-OH	180-182	-	-	-	43
Boc-Trp-OEt	154	-9.0	1	MeOH	507
Tyrosine					
$(Boc\text{-}Tyr[Z(2Br)])_2O$	101-103	+9.0	2	CH_2Cl_2	508
$Fmoc\text{-}Tyr[Bzl(2,6Cl_2)])\text{-}OH$	170-172	-17.3	1	DMF	37

Dts-Tyr-OH	92-93	-	-	-	43
Dts-Tyr, Dcha	170-173	-	-	-	43
Dts-Tyr(Bu^t)-OH	132-135	-	-	-	43
Dts-Tyr(Bu^t), Dcha	143-145	-	-	-	43
Boc-Tyr-OEt	90	+2	1	MeOH	507
Valine					
$(Boc\text{-}Val)_2O$	91-93	+12.5	2	CH_2Cl_2	508
Dts-Val-OH	109	-	-	-	43
Dts-Val, Dcha	87-88	-	-	-	43
Boc-Val-OEt	-	-21.6	1	MeOH	507
Teoc-Val, Cha	127-133	-2.0	3.7	MeOH	38
H-Val-ODpm, TosOH	170-172	-20.6	2	MeOH	52

5.2 Uncoded Amino Acids

α-Aminoadipic acid (Aad) and α-Aminoadipamic acid (= homoglutamine)					
H-Aad(NHZ)-OH	165-167	+23	1	M HCl	509, 510
Z-Aad(NHZ)-OH	149-151	-5.8	1	Me_2CO	509, 510
Boc-Aad(NHZ)-OH	62-64	-7.0	1	MeOH	510
Numerous other useful derivatives					510
1-Aminocyclopropane carboxylic acid (Acc)					
H-Acc-OMe, HCl	180-182	-	-	-	241
β-(Benzo[b]thien-3-yl)-alanine (3Bal)					
Boc-D-3Bal	63-67	-16.0	1	$CHCl_3$	511
γ- Carboxyglutamic acid (Gla)					
Fmoc-Gla(OBu^t)$_2$-OH	121-123	-7.9	1	MeOH	131
β-(Cyclohexyl)-alanine (Cha)					
H-Cha-OMe, HCl	157-160	+17.4	1	DMF	478
Cysteines, β,β-dialkyl					
A series of N-Boc, S-Bzl(Me) derivatives					512
Dopa (Dihydroxyphenylalanine)					
Boc-Dopa(Bzl_2)-OH	105	+14.2	1	MeOH	430
Hexafluorovaline (Hfv)					
Boc-DL-Hfv-OH	131-132.5	-	-	-	234
Boc-DL-Hfv, Dcha	165.5-167	-	-	-	234
Homocysteine (Hcy)					
Boc-Hcy(Bzl)-ONp	98-99	-39.3	1	MeOH	386
Boc-Hcy(Bzl)-NH_2	134-135	-1.8	2	EtOH	386
Homoglutamic acid: see Aminoadipic acid					
Homoserine (Hse)					
Fmoc-Hse(Trt)-OH	108-110	+9.3	2	$CHCl_3$	54

Neopentylglycine (Npg)					
OHC-Npg-OH	175-177	-13	-	EtOH	505
Ornithine					
Z-Orn(Boc)-OBu^t	59-60	-12.7	1	DMF	61
H-Orn(Trt)-OH,CF_3CO_2H	85-88	-	-	-	54
Phenylalanines, substituted					
The Boc derivatives of various ring-substituted phenylalanines					476, 513
β-(Pyren-1-yl)-alanine (Pya)					
Boc-Pya-OH	133-135	-72.9	0.3	DMF	280
H-Pya-OMe, HCl	205-207	-	-	-	280
β-(Pyrid-3-yl)-alanine (3Pal)					
Boc-D-3Pal	139-140	-17.2	1	95% EtOH	511
Statine					
Various N-protected statine analogues and derivatives					514
4-Thiapipecolic acid (SPip)					
Boc-SPip-OH	95	-75.5	1.9	EtOH	109
H-SPip-OMe, HCl	173	-24.8	1.9	EtOH	109
Z-D-SPip-OH	89	+73.0	2.5	$CHCl_3$	109
Z-SPip-OH	-	-	-	-	109
H-SPip-OBu^t, AcOH	75	-14.5	1.9	$CHCl_3$	109
Tryptophans, substituted					
The Boc derivatives of a number of ring-substituted tryptophans					513

6. Appendix III: Purification Methods

Methods are cited for the separation of diastereoisomers of amino acids and peptides, and for the separation and purification of peptides.

	Ref.
Separation of enantiomeric and diastereoisomeric amino acids and peptides	515-519
Use of crown ethers in mobile phase during reversed-phase hplc of amino acids and peptides	520
Quantitative analysis of Fmoc peptides by reversed-phase hplc	521
Detection of side reactions in peptide synthesis by hplc	522
Effect of solvent composition and ion-pair reagents on hplc of protected peptides	523
Separation of derivatives of sulphidopeptide leukotrienes formed by reaction with phthalaldehyde	524
Purification of radio-iodinated ANF and vasopressin	525
Correlation of retention coefficient and sequence in peptide hplc	526

Corrigendum

Professor A.A. Pavia has asked for the allusion to the paper by B. Ferrari and A.A. Pavia (Tetrahedron, 1985, 41, 1939), which appeared in volume 18 (chapter 2, p.96) of this series, to be corrected. We cannot now explain how the late Ian Galpin came to get the facts of this work confused, but we must put the record straight: the work ('Synthese de glycopeptides T_N representant la partie N-terminale de la glycophorine humaine A^N et A^{Mc_1}) involved the first synthesis of the triglycosylated pentapeptides H-LeuSer*Thr*Glu-OH and H-SerSer*Thr*Glu-OH, in which the asterisk* indicates the attachment of 2-acetamido-2-deoxy-α-D-galactopyranosyl appendages. The peptides obtained were thus O-glycosides, and do not have N-glycosidic linkages as reported by Dr Galpin; his comment on the synthesis was therefore based on a false premise. So that the mistake is seen in proper perspective, however, it seems fair to point out that he reviewed about ten thousand papers for these Specialist Reports over the years, and as far as we know drew adverse comment in just two instances, which is an enviable record.

References

1. J.H. Jones, Specialist Periodical Reports on Amino-Acids, Peptides and Proteins, 1971, 3, 220.
2. D. Theodoropoulos (ed.), 'Peptides 1986', pub. Walter de Gruyter, Berlin and New York, 1987.
3. G.R. Marshall (ed.), 'Peptides: Chemistry and Biology', pub. ESCOM, Leiden, 1988.
4. T. Shiba and S. Sakakibara (eds.), 'Peptide Chemistry 1987', pub. Protein Research Foundation, Osaka, 1988.
5. W. Voelter, E. Bayer, Yu. A. Ovchinnikov and V.T. Ivanov (eds.), 'Chemistry of Peptides and Proteins', 3, pub. Walter de Gruyter, Berlin, 1986.
6. K. Itakura, T. Hirose, R. Crea, A.D. Riggs, H.L. Heyneker et al., Science, 1987, 198, 1056.
7. J.F. Habener (ed.), 'Molecular Cloning of Hormone Genes', pub. Humana Press, Clifton NJ, 1987.
8. B.E. Evans, M.G. Bock, K.E. Rittle, R.M. DiPardo, W.L. Whitter, D.F. Veber, P.S. Anderson, and R.M. Friedinger, Proc.Natl.Acad.Sci.USA, 1986, 83, 4918.
9. M. Narita, Kobunshi Kagaku, 1986, 35, 1026 ('Structure Control in Protein Synthesis').
10. G. Schmidt, Top.Curr.Chem., 1986, 136, 109 ('Recent Developments in the Field of Biologically Active Peptides').
11. E.Wünsch, Stud. Org. Chem. (Amsterdam) 1986, 26, 639 (peptide chemistry viewed as a branch of natural product chemistry).
12. J.D. Haug, Am. Biotechnol. Lab., 1987, 5, 40, 42, 44-47 ('Peptide Synthesis and Protecting Group Strategy').
13. P.K. Bhatnagar, Dev. Biol. Stand., 1986, 63, 69 ('Strategies for Peptide Synthesis').
14. A.A. Gershkovich and V.K. Kibirev, 'Sintez Peptidov. Reagenty i Metody', pub. Naukova Dumka, Kiev, 1987.
15. [R]. B. Merrifield, in 'Modern Biotechnology and Health: Perspectives for the year 2000', pub. Academic Press, 1987, p.25 ('The Chemical Synthesis of Peptides and Proteins for Studies on Disease').
16. A. Patchornik, CHEM TECH, 1987, 17, 58; S.M. Andreev, N.A. Samoilova; Yu. A. Davidovich, and S.V. Rogozhin, Usp.Khim, 1987, 56, 629 (polymeric reagents in peptide synthesis).
17. B. Gutte, R. Moser, S. Klauser, T. Leist, H. Langen, and T. Eprecht, in ibid, p.183 ('Applications of Synthetic Peptides').
18. J. Gante, Kontakte (Darmstadt), 1987, 38 (a review of "unusual" peptide syntheses, on "intramol. methods of peptide synthesis" according to Chem.Abs., 1988, 108, 112896b; original not seen).
19. J.W. Van Nispen, Pure Appl. Chem., 1987, 59, 331 ['Synthesis and analysis of (poly)peptides'].
20. H. Kunz, Angew.Chem.Int.Ed.Engl., 1987, 26, 297 ('Synthesis of Glycopeptides, Partial Structures of Biological Recognition Components').
21. J.M. Stewart, NIDA Res. Monogr., 1986, 69, 21.
22. J.M. Garcia-Anton, F. Reig, and G. Valencia, NATO ASI Ser., Ser. A, 1986, 125, 69.
23. L. Moroder and E. Wünsch, in 'Natural Product Chemistry', ed. Atta-ur-Rahman, pub. Springer-Verlag, Berlin, 1986, p.255.
24. L. Moroder and E. Wünsch, in 'Gastrin and Cholecystokinin. Chemistry Physiology and Pharmacology', ed. J.-P. Bali and J. Martinez, pub. Elsevier, 1987, p.21.
25. R.F. Nutt and D.F. Veber, Endocrinol. Metab. Clin. North Am., 1987, 16, 19 (structure-activity relationships are also reviewed).
26. M. Lebl., in 'CRC Handbook of Neurohypophyseal Hormone Analogs', 1, ed. K. Jost, M. Lebl and F. Brtnik, pub. CRC Press Inc. Florida, 1987, p.23 (see also the other chapters in this volume, and in its companion volume 2, for lists of analogues etc).
27. M. Manning, K. Bankowski, and W.H. Sawyer, in 'Vasopressin', ed. D.M. Gash and G.J. Boer, pub. Plenum Publishing Corp., 1987, p.335.

28. The Peptides, 1987, 8 ('Chemistry Biology and Medicine of Neurohypophyseal Hormones and their Analogues').
29. V.J. Hruby and C.W. Smith, The Peptides, 8, 1987, 77.
30. W. Voelter, H. Echner, H. Kalbacher, T.Q. Dinh, A. Kapurhiotu, M. Jahan, P. Link and M. Mihelic, Stud.Org.Chem. (Amsterdam), 1986, 26, 529.
31. C. Birr, in 'Chemical Synthesis in Molecular Biology', ed. H. Blöchker, R. Frank and H.-J. Fritz, pub. VCH 1987, p.217.
32. J.D. Glass, The Peptides, 1987, 9, 167.
33. P. Henklein, H.U. Heyne, W.R. Halatsch, and H. Niedrich, Synthesis, 1987, 166.
34. K. Takeda, K. Tsuboyama, K. Tsuboyama, M. Hashino, M. Kishino, and H. Ogura, Synthesis, 1987, 557.
35. S. Iguchi, K. Kawasaki, and Y. Okada, Int.J.Pept.Protein Res., 1987, 30, 695.
36. F.M.F. Chen and N.L. Benoiton Can.J.Chem., 1987, 65, 1224.
37. R.C. deL Milton, E. Becker, S.C.F. Milton, J.E.J. Baxter, and J.F. Elsworth, Int.J.Pept.Protein Res., 1987, 30, 431.
38. R.E. Shute and D.H. Rich, Synthesis, 1987, 346.
39. M.A. Casadei and D. Pletcher, Synthesis, 1987, 1118.
40. L.A. Carpino, Acc.Chem.Res., 1987, 20, 401.
41. M. Ueki and M. Amemiya, Tetrahedron Lett., 1987, 28, 6617.
42. E. Atherton and R.C. Sheppard, The Peptides, 1987, 9, 1.
43. S. Zalipsky, F. Albericio, U. Slomczynska, and G. Barany, Int.J.Pept.Protein Res., 1987, 30, 740.
44. O. Dangles, F. Guibe, G. Balavoine, S. Lavielle, and A. Marquet, J.Org.Chem., 1987, 52, 4984.
45. G. Sennyey, G. Barcelo and J.P. Senet, Tetrahedron Lett., 1987, 28, 5809.
46. A.N. Sabirov, V.I. Ofitserov, A.F. Shval'e, and V.V. Samukov, Bioorg.Khim., 1987, 13, 754.
47. N. Nishino, Y. Sugita, T. Yamamoto, and T. Fujimoto, Kenkyu Hokoku-Asahi Garasu Kogyo Gijutsu Shoreikai, 1986, 48, 149.
48. P. Jouin, B. Castro, C. Zeggaf, A. Pantaloni, J.-P. Senet, S. Lecolier, and G. Sennyey, Tetrahedron Lett., 1987, 28, 1661.
49. D. Grenouillat, J.-P. Senet and G. Sennyey, Tetrahedron Lett., 1987, 28, 5827.
50. A.A. Mazurov, S.V. Antonenko, and S.A. Andronati, Khim.Prir.Soedin., 1986, 224.
51. J.H. Jones and M.E. Wood, Synth. Commun., 1986, 16, 1515.
52. C. Froussios and M. Kolovos, Synthesis, 1987, 1106.
53. K. Barlos, J. Kallitsis, P. Mamos, S. Patriankou, and G. Stavropoulos, Liebigs Ann.Chem. 1987, 633.
54. K. Barlos, P. Mamos, D. Papaioannou, S. Patrianakou, C. Sanida, and W. Schaefer, Liebigs Ann. Chem., 1987, 1025.
55. Y. Kurano, T. Kimura, and S. Sakakibara, J.Chem.Soc., Chem.Commun., 1987, 323.
56. K. Akaji, M. Tanaka, S. Sumi, M. Kogire, K. Takaori, R. Doi, K. Inoue, T. Tobe, M. Moriga, M. Aono and H. Yajima, Chem.Pharm.Bull, 1987, 35, 535.
57. T. Abiko and H. Sekino, Chem.Pharm.Bull., 1987, 35, 2016.
58. N. Sugiyama, N. Fujii, S. Funakoshi, K. Miyasaka, M. Aono, M. Moriga, K. Inoue, M. Kogire, S. Sumi, R. Doi, T. Tobe, and H. Yajima, Chem.Pharm.Bull., 1987, 35, 3585.
59. K. Akaji, M. Nomizu, K. Watanabe, S. Unakoshi, H. Imura, T. Tsukada, J. Fukata, N. Nakamuta, M. Koida, N. Fujii and H. Yajima, Chem.Pharm.Bull., 1987, 35, 3859.
60. S. Kiyama, Y. Ooi, K. Kitagawa, T. Nakamura, T. Akita, M. Kogire, R. Hosotani, K. Inoue, T. Tobe, and H. Yajima, Int.J.Peptide Protein Res., 1987, 29, 533.
61. F. Marchiori, G. Borin, A. Calderan, and G. Chessa, Int.J.Pept.Protein Res., 1987, 30, 822.

62. M.S. Breslav, I.F. Sakhno, and U. Kalejis Latv.PSR Zinat. Akad. Vestris, Kim. Ser., 1986, 752.
63. Y. Ye, G. Fu, N. Yu, C. Li, Y. Lin, and Q. Xing, Kexue Tongbao (Foreign Lang.Ed)., 1986, 31, 1320.
64. K.H. Koenig, L. Kaul, M. Kuge and M. Schuster, Liebigs.Ann.Chem., 1987, 1115.
65. T.A. Lyle, R.M. Freidinger, R.F. Nutt, C.F. Homnick, R. Saperstein and D.F. Veber, Int.J.Pept.Protein Res., 1987, 29, 244.
66. P. Jouin, B. Castro, C. Zeggaf, A. Pantaloni, J.-P. Senet, S. Lecolier, and G. Sennyey, Tetrahedron Lett., 1987, 28, 1665.
67. A. Rainer, J. Danklmaier, H. Hoenig, and H. Kandolf, Synthesis, 1987, 635.
68. S.T. Chen and K.T. Wang, Synthesis, 1987, 581.
69. V.M. Bairamov, G.A. Vasil'eva, and R.P. Evstigneeva, Zh.Obshch.Khim., 1986, 56, 1631.
70. K. Jankowski and D. Gaudin, Spectrosc.Lett., 1987, 20, 255.
71. Y. Okada, S. Iguchi and K. Kawasaki, J.Chem.Soc., Chem.Commun., 1987, 1532
72. A.W. Lipowski, A.M. Konecka, I. Sroczynska, R. Przewlocki, L. Stala, and S.W. Tam, Life Sci., 1987, 40, 2283.
73. P. Sieber and B. Riniker, Tetrahedron Lett., 1987, 28, 6031.
74. F. Albericio, A. Grandas, A. Porta, E. Pedroso, and E. Giralt, Synthesis, 1987, 271.
75. L. Juliano, M.A. Juliano, A. de Miranda, S. Tsuboi, and Y. Okada, Chem. Pharm. Bull., 1987, 35, 2550.
76. P. Sieber, Tetrahedron Lett., 1987, 28, 1637.
77. N. Fujii, A. Otaka, S. Funakoshi, K. Bessho, T. Watanabe, K. Akaji, and H. Yajima, Chem.Pharm.Bull., 1987, 35, 2339.
78. N. Fujii, T. Watanabe, A. Otaka, K. Bessho, I. Yamamoto, T. Noda, and H. Yajima, Chem.Pharm.Bull., 1987, 35, 4769.
79. J.P. Tam and R.B. Merrifield, The Peptides, 1987, 9, 185.
80. N. Fujii, A. Otaka, O. Ikemura, K. Akaji, S. Funakoshi, Y. Hayashi, Y. Kuroda, and H. Yajima, J.Chem.Soc., Chem.Commun., 1987, 274.
81. N. Fujii, A. Otaka, O. Ikemura, M. Hatano, A. Okamachi, S. Funakoshi, M. Sakurai, T. Shioiri, and H. Yajima, Chem.Pharm.Bull., 1987, 35, 3447.
82. N. Fujii, A. Otaka, N. Sugiyama, M. Hatano and H. Yajima, Chem. Pharm. Bull, 1987, 35, 3880.
83. M.A. Aramendia, V. Borau, C. Jimenez, J.M. Marinas, and M.E. Sempere, React.Kinet.Catal.Lett., 1986, 32, 525.
84. A. Aramendia, V. Borau, C. Jimenez, J.M. Marinas, and M.E. Sempere, Can.J.Chem., 1987, 65, 2791.
85. T.F. Gabriel, Int.J.Pept.Protein Res., 1987, 30, 40.
86. J. Mahmoud, J. Martinez, B. Castro, G. Barcelo, G. Sennyey and J.-P. Senet, J.Org.Chem, 1987, 52, 2364.
87. E.M Holleran and J. Kovacs, J.Org.Chem., 1986, 51, 5419.
88. F.M.F. Chen, and N.L. Benoiton, Can.J.Chem., 1987, 65, 619.
89. F.M.F. Chen, Y. Lee, R. Steinauer, and N.L. Benoiton, Can.J.Chem., 1987, 65, 613.
90. F.M.F. Chen and N.L. Benoiton, Can.J.Chem., 1987, 65, 1228.
91. S. Romani, L. Moroder, W. Göhring, R. Scharf, E. Wünsch, Y.A. Barde, and H. Thoenen, Int.J.Peptide Protein Res., 1987, 29, 107.
92. H.R. Kricheldorf, 'α-Aminoacid-N-Carboxyanhydrides and Related Heterocycles', Springer-Verlag, Berlin, 1987.
93. T.J. Blacklock, R. Hirschmann und D.F. Veber, The Peptides, 1987, 9, 39.
94. P.A. Sutton and D.A. Buckingham, Acc.Chem.Res., 1987, 20, 357.
95. D.S. Kemp and N. Fotouhi, Tetrahedron Lett., 1987, 28, 4637.
96. M. Takasaki and K. Harada, J.Chem.Soc., Chem.Commun. 1987, 571.
97. I. Ojima, H. Chen, and C. Jyun, J.Chem.Soc., Chem.Commun., 1987, 625.
98. K. Kawasaki, T. Tsuji, M. Maeda, T. Matsumoto, and K. Hirase, Chem.Pharm.Bull., 1987, 35, 1044.
99. K. Kouge, T. Koizumi, H. Okai, and T. Kato, Bull.Chem.Soc.Jpn., 1987, 60, 2409.

100. S.M. Andreev, M.V. Sidorova, O.A. Rakova, D.E. Tsvetkov and L.A. Fonina, Bioorg.Khim., 1987, 13, 696.
101. L.M. Litvinenko, V.V. Kosmynin, L.N. Kaida, V.A. Savelova, and I.P. Verbovaya, Zh.Org.Khim., 1986, 22, 877.
102. V.V. Kosmynin, L.N. Kaida, V.A. Savelova, V.A. Danilov, E.I. Kononenko, and L.M. Litrinenko, Zh.Org.Khim., 1987, 23, 1775.
103. V.V. Kosmynin, L.N. Kaida, V.A. Savelova, L.V. Kovalik, and L.M. Litvinenko, Zh.Org.Khim., 1987, 23, 1782.
104. V.A. Basyuk, V.I. Bogomaz, A.A. Chuiko, V.P. Shabel'nikov, V.G. Golavatyi, and K.B. Yatsimirskii, Dokl. Akad. Nauk SSSR., 1986, 288, 1115 [Chem.Abs. 1987, 106, 154841t; original not seen].
105. M.J.O. Anteunis and C. Becu, Bull.Soc.Chim.Belg., 1987, 96, 119.
106. M.J.O. Anteunis and S. Van Damme, Bull.Soc.Chem.Belg., 1987, 96, 131.
107. M.J.O. Anteunis, C. Becu, F. Becu and R. Callens, Bull.Soc.Chem.Belg., 1987, 96, 133.
108. H. Wissmann, Phosphorus Sulphur, 1987, 30, 645.
109. C. Van der Auwera and M.J.O. Anteunis, Int.J.Pept.Protein Res., 1987, 29, 574.
110. C. Van der Auwera, S. van Damme, and M.J.O. Anteunis, Int.J.Peptide Protein Res., 1987, 29, 464.
111. Sh. Kh. Khalikov, M.M. Sobirov, and S.V. Alieva, Dokl.Akad. Nauk Tadzh. SSR, 1985, 28, 651.
112. I.J. Galpin, A. Karim, A. Mohammed, and A. Patel, Tetrahedron Lett., 1987, 28, 6517.
113. R. Ramage and J. Green, Tetrahedron Lett., 1987, 28, 2287.
114. V.K. Haridasan, A. Ajayaghosh, V.N. Rajasekharan Pillai, J.Org.Chem., 1987, 52, 2662.
115. P. Mascagni, C.B. Hyde, M.A. Charalambous and K.J. Welham, J.Chem.Soc., Perkin Trans. 2, 1987, 323.
116. J.L. Torres, I. Haro, G. Valencia, J.M. Garcia Anton and F. Reig, Tetrahedron, 1987, 43, 4031.
117. U. Schmidt and B. Potzolli, Liebigs. Ann.Chem., 1987, 935.
118. Sh.G. Bitiev, G.V. Chel'tsova, M.R. Lutsenko, E.I. Karpeiskaya and E.I. Klabunovskii, Izv. Akad.Nauk. SSSR, Ser. Khim., 1987, 380.
119. M. Denarie, D. Grenouillat, T. Malfroot, J.-P. Senet, G. Sennyey and P. Wolf, Tetrahedron Lett., 1987, 28, 5823.
120. G. Barany, N. Kneib-Cordonier, and D.G. Mullen, Int.J.Pept.Protein Res., 1987, 30, 705.
121. G. Roenne, Dan.Kemi, 1986, 67, 289.
122. S. Ohashi, Kagaku Kogyo, 1987, 38, 236.
123. M. Narida, Kobunshi Kako., 1986, 35, 496.
124. R.P. Andrews and C. Summers, Am.Biotechnol. Lab., 1986, 4, 28.
125. A.D. Auffrett, W. Hirst, L.G. Meade and M.W. Thacker, Protides Biol.Fluids, 1986, 34, 15.
126. J. Van Rietschoten, J.-M. Sabatier, M. Tessier, C. Granier, E. Pedroso, A. Grandars, F. Albericio, R. Eritja, E. Nicholas, Protides Biol. Fluids. 1986, 34, 23.
127. N. Fujii, Y. Hayashi, K. Akaji, S. Funakoshi, M. Shimamura, S. Yuguchi, L.H. Lazarus, and H. Yajima Chem.Pharm.Bull., 1987, 35, 1266.
128. V.N. Postnov, N.G. Bogomolova, V.R. Aleskovskii, Dokl.Akad.Nauk S.S.S.R., 1986, 291, 119.
129. B. Penke and J. Rivier, J.Org.Chem., 1987, 52, 1197.
130. F. Albericio and G. Barany, Int.J.Pept. Protein Res., 1987, 30, 206.
131. J. Rivier, R. Galyean, L. Simon, L.J. Cruz, B.M. Olivera and W.R. Gray, Biochemistry, 1987, 26, 8508.
132. G. Breipohl, J. Knolle, and W. Stüber, Tetrahedron Lett., 1987, 28, 5651. 133.G. Breipohl, J. Knolle, and R. Geiger, Tetrahedron Lett., 1987, 28, 5647.
134. P. Sieber, Tetrahedron Lett., 1987, 28, 2107.

135. D.G. Mullen and G. Barany, Tetrahedron Lett., 1987, 28, 491.
136. R. Ramage, C.A. Barron, S. Bielecki, and D.W. Thomas, Tetrahedron Lett., 1987, 28, 4105.
137. A. Ajayaghosh and V.N. Rajasekharan Pillai, J.Org.Chem., 1987, 52, 5714. 138.S. Plane and D. Heisler, Tetrahedron Lett., 1987, 28, 1401.
139. F. Albericio and G. Barany, Int.J.Pept. Protein Res., 1987, 30, 177.
140. N. Mensi and S.S. Isied, J.Am.Chem.Soc., 1987, 109, 7882.
141. P. Sieber, Tetrahedron Lett., 1987, 28, 6147.
142. R. Kirstgen, R.C. Sheppard, and W. Steglich, J.Chem.Soc., Chem.Commun., 1987, 1870.
143. K. Barlos, D. Gatos, J. Kallitsis, D. Papaioannou, P. Sotiriou, and W. Schaefer, Liebigs Ann. Chem., 1987, 1031.
144. S.S. Wang, S.T. Chen, K.T. Wang, and R.B. Merrifield, Int.J.Pept.Protein Res., 1987, 30, 662.
145. R. Eritja, J.P. Ziehler-Martin, P.A. Walker, T.D. Lee, K. Legesse, F. Albericio, and B.E. Kaplan, Tetrahedron, 1987, 43, 2675.
146. M. Ruiz-Gayo, F. Albericio, E. Pedroso, and E. Giralt J.Chem.Soc., Chem.Commun., 1986, 1501.
147. M. Ueki and M. Amerriya, Tetrahedron Lett., 1987, 28, 6617.
148. L. Otvos, B. Dietzschold and L. Kisfaludy, Int.J.Peptide Protein Res., 1987, 30, 662.
149. S. Yuski and D.H. Coy, The Peptides, 1987, 8, 119.
150. Y. Sasaki, W.A. Murphy, M.L. Heiman, V.A. Lance, and D.H. Coy, J.Med.Chem., 1987, 30, 1162.
151. M. Edelstein, P.E. Scott, M. Sherlund, A.L. Hansen, J.L. Hughes, Chem.Eng.Sci., 1986, 41, 617.
152. R.A. Houghten, S.T. DeGraw, M.K. Bray, S.R. Hoffmann and N.D. Frizzell, BioTechniques, 1986, 4, 522.
153. R.A. Houghten, Protides Biol.Fluids, 1986, 34, 95.
154. H.M. Geysen, S.J. Rodda, T.J. Mason, G. Tribbick and P.G. Schoofs, J.Immunol.Methods, 1987, 102, 259.
155. A.G. Ludwick, L.W. Jelinski, D. Live, A. Kintanar and J.J. Dumais, J.Am.Chem.Soc., 1986, 108, 6493.
156. A. Orlowska, E. Witkowska and J. Izdebski, Int.J.Pept.Protein Res., 1987, 30, 141.
157. L. Gozzini, R. de Castiglione, M. Ballabio, and C. Di Bello, Int.J.Pept. Protein Res., 1987, 30, 533.
158. M. Pugniere, A. Skalli, M.A. Coletti-Previero and A. Previero, Proteins Struct.Funct.Genet., 1986, 1, 134.
159. M. Mazure, B. Calas, A. Cave and J. Parello, C.R. Acad.Sci., Ser. 2, 1986, 303, 553.
160. L. Cameron, M. Meldal, and R.C. Sheppard, J.Chem.Soc., Chem.Commun., 1987, 270.
161. V. Krchnak, J. Vagner, M. Flegel and O. Mach, Tetrahedron Lett., 1987, 28, 4469.
162. T.A. Lyle, S.F. Brady, T.M. Ciccarone, C.D. Colton, W.J. Palevda, D.F. Veber, and R.F. Nutt, J.Org.Chem., 1987, 52, 3752.
163. G. Breipohl, J. Knolle and W. König, Klin. Wochenschr. 1986, 64 (suppl.6), 16.
164. M. Narita, S. Isokawa, S. Nagasawa, and T. Ishijima, Macromolecules, 1987, 20, 2306.
165. D. Le Nguyen, A. Heitz and B. Castro, J.Chem.Soc., Perkin Trans 1, 1987, 1915.
166. Y. Trudelle and F. Heitz, Int.J.Peptide Protein Res., 1987, 30, 163.
167. C. Di Bello, A. Luchiari, O. Buso, R. De Castiglione and L. Gozzini, Gazz. Chim. Ital., 1986, 116, 221.
168. M.T. Tosteson, J.J. Levy, L.H. Caporale, M. Rosenblatt, and D.C. Tosteson, Biochemistry, 1987, 26, 6627.
169. R.E. Reid, Biochemistry, 1987, 26, 6070.
170. T. Kubiak, D.B. Whitney and R.B. Merrifield, Biochemistry, 1987, 26, 7849.

171. D.B. Scanlon, M.A. Eefting, C.J. Lloyd, A.W. Burgess, and R.J. Simpson, J.Chem.Soc., Chem.Commun., 1987, 516.
172. T. Fairwell, A.V. Hospattankar, B.H. Brewer Jr., and S.A. Khan, Proc.Natl.Acad.Sci.USA., 1987, 84, 4796.
173. A. Balasubramaniam, Y.M. Chopra, M.T. Yates, and R.L. Jackson, Bioorg.Chem., 1987, 15, 141.
174. V.S. Chauhan, Indian J.Chem., 1986, 25B, 1138.
175. G.P. Royer, Methods Enzymol., 1987, 136, 157.
176. F. Widmer, S. Bayne, G. Houen, P. Thorbek and J.T. Johansen, Protides Biol. Fluids, 1986, 34, 27.
177. I.M. Chaiken, S. Ando and G. Fassina, Adv.Biosci., 1987, 65, 141.
178. H.-D. Jakubke, The Peptides, 1987, 9, 103.
179. V. Kasche, U. Haufler, and L. Riechmann, Methods Enzymol., 1987, 136, 280.
180. H.-D. Jakubke, Methods Enzymol., 1987, 136, 178.
181. J. Markussen, 'Human Insulin by Tryptic Transpeptidation of Porcine Insulin and Biosynthetic Precursors', pub. MTP Press Ltd, Lancaster, 1987.
182. V. Kasche, Adv.Biosci., 1987, 65, 151.
183. H. Rink, Tetrahedron Lett., 1987, 3787.
184. T. Muro, Y. Tominaga, and S. Okada, Agric.Biol.Chem., 1987, 51, 1207.
185. C. Selvi, M. Baboulenc, V. Speziale and A. Lattes, J.Chem.Technol.Biotechnol., 1986, 36, 427.
186. L.A. Lublinskaya, S.E. Boitsova and V.M. Stepanov, Bioorg.Khim., 1986, 12, 1301.
187. M.T. Machini de Miranda, E. Cheng, J. Muradian, W.F. Seidal and M. Tominaga, Bioorg.Chem., 1986, 14, 182.
188. S. Kitabatake, R. Tsurutani, H. Nakajima, K. Tomita, Y. Yoshihara, and H. Ueda, Pharm.Res., 1987, 4, 154.
189. V. Cerovsky, T. Saks and K. Josi, Coll.Czech.Chem.Commun., 1987, 52, 2309.
190. V. Cerovsky, Coll.Czech.Chem.Commun., 1986, 51, 1352.
191. V. Cerovsky and K. Jost, Coll.Czech.Chem.Commun., 1987, 52, 1079.
192. G. Fortier and S.L. MacKenzie, Biotechnol.Lett., 1986, 8, 777.
193. C.F. Barbas and C.H. Wong, J. Chem. Soc., Chem. Commun., 1987, 533.
194. A.L. Margolin, D.F. Tai, and A.M. Klibanov J.Am.Chem.Soc., 1987, 109, 7885.
195. H. Kise, H. Shirato and H. Noritomi, Bull.Chem.Soc.Jpn., 1987, 60, 3613.
196. H. Shima, M. Fukuda, K. Tanabe, T. Ito, and S. Kunugi, Bull.Chem.Soc.Jpn., 1987, 60, 1403.
197. J.G. Davies, K. Rose, C.G. Bradshaw and R.E. Offord, Protein Eng., 1987, 1, 407.
198. H. Zahn, H.-G. Gattner, W. Fischer, A. Jonczyk, and A. Wiese, Adv.Biosci., 1987, 65, 167.
199. M. Casaretto, M. Spoden, C. Diaconescu, H.G. Gattner, H. Zahn, D. Brandenburg and A. Wollmer, Biol.Chem.Hoppe-Seyler, 1987, 368, 709.
200. T. Mori, K. Nilssen, P.O. Larssen and K. Mosbach, Biotechnol. Lett., 1987, 70, 110.
201. A.E.I. Proudfoot and C.J.A. Wallace, Biochem.J., 1987, 248, 965.
202. P.J. Halling, Biotechnol.Adv., 1987, 5, 47.
203. J.B. West and C.-H. Wong, Tetrahedron Lett., 1987, 28, 1629.
204. T. Nakatsuka, T. Sasaki, and E.T. Kaiser, J.Am.Chem.Soc., 1987, 109, 3808.
205. A.L. Margolin and A.M. Klibanov, J.Am.Chem.Soc., 1987, 109, 3802.
206. J.R. Matos, J.B. West and C.H. Wong, Biotechnol. Lett., 1987, 9, 233.
207. C.S. Chen, S.-H. Lou, G. Girdoukas and C.J. Sih, J.Am.Chem.Soc., 1987, 109, 2812.
208. D. Cantacuzene and C. Guerreiro, Tetrahedron Lett., 1987, 28, 5153.
209. P. Kuhl, R. Schaaf and H.-D. Jakubke, Monatsh. Chem., 1987, 118, 1279.
210. S. Kunugi, K. Tanabe, M. Fukuda, S. Makimoto and Y. Taniguchi, J.Chem.Soc., Chem.Commun., 1987, 1335.
211. V. Schillenberger, U. Schillenberger, Yu. V. Mitin, and H.-D. Jakubke, Anal.Biochem., 1987, 165, 327.
212. K.I. Grant and C. von Holt, Biol.Chem.Hoppe-Seyler, 1987, 368, 239.
213. R.M.L. Jones, K. Rose and R.E. Offord, Biochem.J., 1987, 247, 785.

214. G.P. Schwartz, G.T. Burke, and P.G. Katsoyannis, Proc.Natl.Acad.Sci. USA., 1987, 84, 4083.
215. G.O. Schwartz and P.G. Katsoyannis, J.Chem.Soc., Perkin Trans 1, 1973, 2894.
216. J.L. Fauchere, Adv.Drug Res., 1986, 15, 29.
217. W.H. Fischer, D. Saunders, D. Brandenburg, A. Wollmer, and H. Zahn, Biol.Chem.Hoppe-Seyler, 1985, 366, 521.
218. L. Moroder and E. Wünsch, Stud.Org.Chem (Amsterdam), 1986, 26, 325.
220. N. Fujii, A. Otaka, T. Watanabe, H. Arai, S. Funakoshi, K. Bessho, and H. Yajima, J.Chem.Soc.Chem.Commun., 1987, 1676.
221. S. Romani, L. Moroder, and E. Wünsch, Int.J.Pept.Protein Res., 1987, 29, 99.
222. R.E. Shute and D.H. Rich, J.Chem.Soc., Chem.Commun., 1987, 1155.
223. R.J. Simmonds, J.Chem.Educ., 1987, 64, 966.
224. G. Lindeberg, in ref. 2, p.199.
225. C. Toniolo, G.M. Bonora, E.P. Heimer, and A.M. Felix, Int.J.Pept. Protein Res., 1987, 30, 232.
226. C. Toniolo, G.M. Bonora and S. Sakakibara, Farmaco, Ed.Sci., 1987, 27.
227. S.F. Brady, R.M. Freidinger, W.J. Palevda, C.D. Colton, C.F. Homnick, W.L. Whitter, P. Curley, R.F. Nutt and D.F. Veber, J.Org.Chem., 1987, 52, 764.
228. N. Fujii, O. Ikemura, S. Funakoshi, H. Matsuo, T. Segawa, Y. Nakata, A. Inoue, and H. Yajima Chem.Pharm.Bull., 1987, 35, 1076.
229. J.P. Tam, Int.J.Peptide Protein Res., 1987, 29, 421.
230. G.L. Reddy and R. Nagaraj, Proc.Indian Acad. Sci., Chem. Sci., 1986, 97, 71.
231. R. Paruszewski, R. Matusiak, P. Jaworski, S. Gumulka, and I. Wisniewska, Pol.J.Chem., 1986, 60, 301.
232. M. Hirata, K. Watanabe, E. Hattori, T. Kato, and N. Izumiya, Mem.Fac.Sci, Kyushu Univ., Ser.C., 1986, 15, 259.
233. D. Biseniece, J. Ancans, N. Myshlyakova, G.G. Kublik, E.A. Porunkevich, M.P. Ratkevich, and G. Cipens, Bioorg.Khim., 1987, 13, 149.
234. K.H. Hsieh, P. Needleman, and G.R. Marshall, J.Med.Chem., 1987, 30, 1097.
235. O. Nyecki, K.S. Szalay, L. Kisfaludy, E. Karpati, L. Szporny, G.H. Makara, and B. Varga, J.Med.Chem., 1987, 30, 1719.
236. P. Cordopatia, M. Alain, J. Matsoukas and J. Zanen, Gazz. Chim. Ital., 1987, 117, 287.
237. B. Kundu and K.B. Mathur, Indian J.Chem., 1986, 25B, 930.
238. Y. Kohama, N. Okimoto, T. Mimura, C. Fukaya, M. Watanabe, and K. Yokoyama, Chem.Pharm.Bull., 1987, 35, 3928.
239. P. Duhamel, B. Goument, and C. Plaquevent, Tetrahedron Lett., 1987, 28, 2595.
240. C.P. Yang and C.S. Su, J.Org.Chem, 1986, 51, 5186.
241. C. Mapelli, M.G. Newton, C.E. Ringold, and C.H. Stammer, Int.J.Pept.Protein Res., 1987, 30, 498.
242. N. Albini, S. Auricchio, F. Minisci and A. Mangia, Chim.Ind.(Milan), 1986, 68, 61.
243. G. Lindeberg, J.Chem.Educ., 1987, 64, 1062.
244. J. Chen and G. Zeng, Shipin Kexue (Beijing), 1987, 87, 13.
245. K. Nakanishi, Y. Kimura, and R. Matsuno, Eur.J.Biochem., 1986, 161, 541.
246. Y. Kiso, M. Shimokura, S. Hosoi, T. Fujisaki, Y. Fujiwara, and M. Yoshida J.Protein Chem., 1987, 6, 147.
247. G. Breipohl, J. Knolle, and W. König, Klin. Wochenschr., 1986, 64, (suppl.6), 16.
248. F. Shi, J. Zheng, Y. Wang, S. Lin, and C. Qiu, Shanghai Yike Daxue Xuebao, 1986, 13, 161.
249. P.W. Schiller, L.A. Maziak, T.M.D. Nguyen, J. Godin, R. Garcia, A. DeLean and M. Cantin, Biochem.Biophys.Res.Commun., 1987, 143, 499.
250. R.F. Nutt, S.F. Brady, T.A. Lyle, T.M. Ciccarone, W.J. Paleveda, C.D. Colton, D.F. Veber, and R.J. Winquist, Protides Biol.Fluids, 1986, 34, 55.
251. M. Sakurai, S. Tomomasa, Y. Seiya, Y. Inoue, and R. Chujo, Bull.Chem.Soc.Jpn., 1986, 59, 2157.
252. F. Thuillier, A.M. Graulet, J. Gueris, F. Rousselet, and M.L. Girard, Ann.Pharm.Fr., 1986, 44, 31.

253. R.J. Vavrek and J.M. Stewart, Adv.Exp.Med.Biol., 1986, 198A, 543.
254. J.M. Stewart and R.J. Vavrek, Adv.Biosci., 1987, 65, 73.
255. S. Reissmann and M.P. Filatova, Z.Chem., 1987, 27, 147.
256. I.E. Zel'tser, I. Yu. Reshetnikova, S.Yu. Borokova, E.P. Krysin, and E.E. Lavut, Khim.Prir. Soedin., 1986, 480.
257. N. Fujii, A. Otaka, S. Funakoshi, K. Bessho and H. Yajima, J.Chem.Soc., Chem. Commun., 1987, 163.
258. K. Ando, K. Kitagawa, S. Kiyama, T. Kawamoto, T. Akita, I. Yamamoto, and K. Torizuka, Chem.Pharm.Bull., 1987, 35, 60.
259. J.W. Perich, N.H. Langford, and R.B. Johns, Aust.J.Chem., 1987, 40, 1389.
260. J.W. Perich, P.F. Alewood, and R.B. Johns, Aust.J.Chem., 1987, 40, 257.
261. L. Moroder, C. Tzougraki, W. Göhring, G. Mourier, H.-J. Musiol and E. Wünsch, Biol.Chem.Hoppe-Seyler, 1987, 368, 855.
262. T. Najdovski, J.J.H.H.M. De Pont, G.I. Tesser, B. Penke, J. Martinez, and M. Deschodt-Lanckman, Neurochem.Int., 1987, 10, 459.
263. K. Iuchi, M. Nitta, T. Tanaka, K. Ito, Y. Morimoto, and G. Tsukamoto, Chem.Pharm.Bull., 1987, 35, 4307.
264. B. Charpentier, C. Durieux, I. Menant, and B.P. Roques, J.Med.Chem., 1987, 30, 962.
265. D.C. Horwell, A. Beeby, C.R. Clark, and J. Hughes J.Med.Chem., 1987, 30, 729.
266. P.K. Lemcke, E. Sugg, M. Malarchik, G. Lui, M. Serra, H.I. Yamamura, V.J. Hruby, J.E. Shook and T.F. Burks, Proc.West.Pharmacol.Soc., 1987, 30, 223.
267. I. Kimura, T. Kondoh, and M. Kimura, Eur.J.Pharmacol., 1987, 134, 181.
268. M.-F. Lignon, M.-C. Galas, M. Rodriguez, J. Laur, A. Aumelas and J. Martinez, J.Biol.Chem., 1987, 262, 7226.
269. M. Rodriguez, M.F. Lignon, M.C. Galas, P. Fulcrand, C. Mendre, A. Aumelas, J. Laur, and J. Martinez, J.Med.Chem., 1987, 30, 1366.
270. P. Henklein, B. Penke, M. Georgi, W.R. Halatsch, H. Niedrich and T. Ott, Pharmazie, 1987, 42, 413.
271. J. Rivier, R. Galyean, W.R. Gray, A. Azimi-Zonooz, J.M. McIntosh, L.J. Cruz and B.M. Olivera, J.Biol.Chem., 1987, 262, 1194.
272. W.H. Jorenby, J.P. Riehm and K.R. Rao, Bioch.Biophys.Res.Commun., 1987, 143, 652.
273. H.G. Hwan, V.V. Tolstikov, E.N. Zvonkova, and R.O. Evstigneeva, Zh.Obshch.Khim., 1987, 57, 222.
274. V.V. Tolstikov, H.G. Hwan, E.N. Zvonkova, and R.P. Evstigneeva, Zh. Obshch.Khim, 1986, 56, 1647.
275. J. Pospisek, Z.D. Bespalova, E. Kovarikova, M.I. Titov, T. Barth, and K. Medzihradszky, Coll.Czech.Chem.Commun., 1987, 52, 1867; Zh. D. Bespalova, N.F. Sepetov, O.L. Isakova, M.I. Titov, B. Pekelis, A.S. Molokoedov and V.N. Bushuev, Bioorg.Khim., 1987, 13, 160.
276. C.-L. Ho and C.H. Li, Int.J.Pept.Protein Res., 1987, 29, 134.
277. A.K. Judd, J.A. Lawson, C.M. Olsen, L.R. Toll, W.E. Polgar, E.T. Uyeno, C.J. Keys, and G.H. Loew, Int.J.Pept.Protein Res., 1987, 30, 299.
278. P. Rigaudy, Ch. Garbay-Jaureguiberry, A. Jacquemin-Sablon, J.-B. Le Pecq, and B.P. Roques, Int.J.Pept.Protein Res., 1987, 30, 347.
279. L. Varga, S. Horvat, C. Lemieux, and P.W. Schiller, Int.J.Pept.Protein Res., 1987, 30, 371.
280. H. Mihara, S. Lee, Y. Shimohigashi, H. Aoyagi, T. Kato, N. Izumiya, and T. Costa, Int.J.Pept.Protein Res., 1987, 30, 605.
281. R. Paruszewski, E. Gmitrzuk-Zietarska, R. Matusiak, and S. Gumulka, Pol.J.Chem., 1986, 60, 495.
282. G.O. Vlasov, E.N. Krasnikova, N. Ya. Kozhevnikova, N.G. Illarionova and I. Denisov, Biopolymers, 1987, 26, 1489.

283. S. Funakoshi, M. Kubota, N. Kosuga, J.-P. Xu, H. Yajima, W. Li, S. Tamura, S. Kaneko, M. Satoh and H. Takagi, Chem.Pharm.Bull., 1987, 35, 2561.
284. J.A. Lovett and P.S. Portoghese, J.Med.Chem., 1987, 30, 1668.
285. C. Herdeis and R. Gebhard, Arch.Pharm.(Weinheim, Ger.), 1987, 320, 546.
286. K. Rolka, Z. Kleinrok, and G. Kupryszewski, Pol.J.Pharmacol.Pharm., 1986, 38, 391.
287. K. Naagren, U. Ragnarsson, and B. Laangstroem, Appl.Radiat.Isot. 1986, 37, 537.
288. D. Andreu and R.B. Merrifield, Eur.J.Biochem., 1987, 164, 585.
289. P.W. Schiller, L.A. Maziak, B.C. Wilkes, C. Lemieux, and T.M.D. Nguyen, J.Med.Chem., 1987, 30, 2094.
290. H. Oyamada and M. Ueki, Bull Chem.Soc.Jpn., 1987, 60, 267.
291. G. Toth, J. Hepp, F. Sirokman, and K. Medzihradszky, J.Labelled Compd. Radiopharm., 1987, 24, 859.
292. K. Okumura, Y. Hatanaka, H. Nakayama, and Y. Kanaoka, Chem.Pharm.Bull., 1987, 35, 256.
293. A.A. Carr, N.W. Dudley, E.W. Huber, J.M. Kane, and F.P. Miller, J.Heterocycl.Chem., 1987, 24, 239.
294. C. Choi, Saenghwahak Nyusu, 1986, 6, 34.
295. H.I. Mosberg, J.R. Omnaas, K. Ramalingam, and R.W. Woodard, J. Labelled Compd. Radiopharm., 1987, 24, 1265.
296. G.P. Vlasov, V.A. Gusel, N. Yu. Kozhevnikova, I.B. Ditkovskaya, M. Yu Dorosh, E.N. Krasnikova, Yu. B. Moskvicheva, O.P. Kulyba, A.P. Titov, and G.G. Kachurin, Zh. Obshch. Khim., 1986, 56, 1635.
297. M. Kondo, H. Uchida, H. Kodama, H. Kitajima, and Y. Shimohigashi, Chem.Lett., 1987, 997.
298. M. Marastoni, S. Salvadori, G. Balboni, P.A. Borea, G. Marzola, and R. Tomatis, J.Med.Chem., 1987, 30, 1538.
299. P.S. Portoghese, D.L. Larson, G. Ronsisvalle, P.W. Schiller, T.M.D. Nguyen, C. Lemieux and A.E. Takemori, J.Med.Chem., 1987, 30, 1991.
300. J.S. Davies, E.J. Tremeer and R.C. Treadgold, J.Chem.Soc., Perkins Trans.1., 1987, 1107.
301. V.I. Unkovskii, G.A. Vasil'eva, and R.P. Evstigneeva, Zh. Obshch.Khim., 1986, 56, 1641.
302. K. Kouge, H. Soma, Y. Katakai, H. Okai, M. Takemoto, and Y. Muneoka, Chem.Express, 1987, 2, 153.
303. K. Kouge, H. Soma, Y. Katakai, H. Okai, M. Takemoto, and Y. Muneoka, Bull.Chem.Soc.Jpn., 1987, 60, 4343.
304. K. Kouge, K. Katakai, H. Soma, M. Kondo, H. Okai, M. Takemoto, and Y. Muneoka, Bull.Chem.Soc.Jpn., 1987, 60, 4351.
305. K. Sakina, K. Kawazura and K. Morihara, Agric.Biol.Chem., 1987, 51, 1717.
306. A.J. Douglas, B. Walker, D.T. Elmore, and R.F. Murphy, Biochem.Soc.Trans., 1987, 15, 927.
307. M. Rodriguez, P. Dubreuil, J.P. Bali, and J. Martinez, J.Med.Chem., 1987, 30, 758.
308. G.-S. Lu, S. Mojsov and R.B. Merrifield, Int.J.Pept.Protein Res., 1987, 29, 545.
309. C.G. Unson, D. Andreu, E.M. Gurzenda, and R.B. Merrifield, Proc.Natl.Acad.Sci. USA, 1987, 84, 4083.
310. B. Gysin, D.G. Johnson, D. Trivedi, and V.J. Hruby, J.Med.Chem., 1987, 30, 1409.
311. J. Murphy, W.-J. Zhang, W. Macaulay, G. Fasman, and R.B. Merrifield, J.Biol.Chem., 1987, 262, 17304.
312. X. Ling and Y. Gong, Shengwu Huaxue Yu Shengwu Wuli Xuebao, 1987, 19, 157.
313. S. Minchev and N. Sofroniev, Liebigs Ann.Chem., 1987, 69.
314. W.R. Moore and A. Meister, Anal.Biochem., 1987, 161, 487.
315. E. Giralt, C. Celma, M.D. Ludevid, and E. Pedroso, Int.J.Pept.Protein Res., 1987, 29, 647.
316. L.S. Swart, and J.G. Van der Walt, S.Afr.J.Chem., 1986, 39, 108.
317. L. Sheh, M. Mokotoff and D.J. Abraham, Int.J.Pept.Protein Res., 1987, 29, 509.

318. H. Bodenmüller, E. Escher, B. Zachmann, and E. Schilling, Int.J.Pept.Protein Res., 1987, 29, 140.
319. N. Sakura, M. Nishijima, Y. Uchida, T. Tani, and T. Hashimoto, Chem.Pharm.Bull., 1987, 35, 2327.
320. Y. Shimonishi, Y. Hidaka, M. Koizumi, M. Hane, S. Aimoto, T. Takeda, T. Miwatani, and Y. Takeda, FEBS Lett., 1987, 215, 165.
321. Yu. A. Semiletov, M.G. Petrova, and V.D. Smirvov, Khim.Prir. Soedin., 1987, 152.
322. J.L. Krstenansky, T.J. Owen, and S.J.T. Mao, J.Med.Chem., 1987, 30, 1688.
323. N. Helbecque, J.L. Bernier, J.P. Henichart, A. Martinage, and P. Sautiere Int.J.Pept.Protein Res., 1987, 30, 689.
324. H. Ogawa, G.T. Burke, J.D. Chanley, and P.G. Katsoyannis, Int.J.Pept.Protein Res., 30, 460.
325. W.H. Fischer, D. Saunders, D. Brandenburg, C. Diaconsecu, A. Wollmer, G. Dodson, P. De Meyts, and H. Zahn, Biol.Chem.Hoppe-Seyler, 1986, 367, 999.
326. Y.P.Shvachkin, S.P.Krasnoshchekova, A.M. Nikitina, S.M. Funtova, T.M. Anokhina, V.P. Fedetov, and A.I. Ivanova, Khim.Prir.Soedin, 1987, 151.
327. Yu.P. Shvachkin, S.P. Krasnoshchekova, A.M. Nikitina, S.M. Funtova, T.M. Anokhina, V.P. Fedetov, and A.I. Ivanova, Zh.Obshch.Khim., 1987, 57, 962.
328. M.A. Zewail, M.F. Abdel-Wahhab, Y.M. Megahed, and A.M. Shalaby, Isotopenpraxis, 1986, 22, 218.
329. H. Zahn, H.G. Gattner, W. Fischer, A. Jonczyk, and A. Wiese, Adv. Biosci, 1987, 65, 167.
330. X. Chang, K. Lei, C. Li, Y. Ye, and Q. Xing, Shengwu Huaxue Zazhi., 1987, 3, 81.
331. G. Losse and H. Raddatz, J.Prakt.Chem., 1987, 329, 1.
332. C. Yanaihara, T. Mochizuki, T. Inoue, K. Iguchi, M. Toyoshige, M. Hoshino, T. Iwanaga, T. Fujita, S. Izumi, K. Nakane, and N. Yanaihara, Acta Histochem.Cytochem., 1987, 20, 245.
333. N. Teno, S. Tsuboi, Y. Okada, N. Itoh, and H. Okamoto, Chem.Pharm.Bull., 1987, 35, 3853.
334. T. Teshima, T. Nakai, M. Kitazawa, and T. Shiba, Tetrahedron Lett., 1987, 28, 4705.
335. J. Seprodi, J. Erchegyi, Z. Vadasz, I. Teplan, I. Mezo, B. Kanyicska, M. Kovacs, and S. Vigh, Biochem. Biophys.Res.Commun., 1987, 144, 1214.
336. J.M. Bernard, H. Gras-Masse, H. Drobeq, A. Tartar, P. Lefrancier, A. Hosmalin, C. Carelli, and L. Chedid, Int.J.Pept.Protein Res., 1987, 29, 455.
337. A. Horvath, D.H. Coy, M.V. Nekola, E.J. Coy, S.J. Hocart, G. Keri, and I. Teplan, Act.Chim.Acad.Sci.Hung., 1986, 121, 323.
338. S.V. Burov, S.V. Nikolaev, V.V. Korkhov, G.E. Lupanova, and V.P. Makusheva, Khim.Prir. Soedin., 1987, 590.
339. Z. Mackiewicz, S. Belisle, D. Bellabarba, N. Gallo-Payet, J.G. Lehoux, G. Lagace, and E. Escher Helv.Chim.Acta., 1987, 70, 423.
340. S.J. Hocart, M.V. Nekola, and D.H. Coy, J.Med.Chem., 1987, 30, 739.
341. S.J. Hocart, M.V. Nekola, and D.H. Coy, J.Med.Chem., 1987, 30, 1910.
342. G. Kupryszewski, C.L. Rivier, M. Perrin, W.W. Vale and J.E. Rivier, Polish J. Chem., 1987, 61, 627.
343. S.J. Hocart, M.V. Nekola, and D.H. Coy, J.Med.Chem., 1987, 30, 735.
344. K. Folkers, C. Bowers, P.F.L. Tang, M. Kobota, S.B. Xiso, W. Bender and Y.Z. Liu, Z.Naturforsch., C., 1986, 41, 1087.
345. M.A. Tallon, P. Shenbagamurthi, S. Marcus, J.M. Becker and F. Naider, Biochemistry, 1987, 26, 7767.
346. M.E. Hadley, C. Zechel, B.C. Wilker, A. M. de L. Castrucci, M.A. Visconti, M. Pozo-Alonso, and V.J. Hruby, Life Sci., 1987, 40, 1139.
347. A.M. de L. Castrucci, M.E. Hadley, B.C. Wilkes, C. Zechel and V.J. Hruby, Life Sci., 1987, 40, 1845.
348. H. Ritter and J. Thoene, Makromol.Chem., 1987, 188, 2047.

349. V.J. Hruby, B.C. Wilkes, MacE. Hadley, F. Al-Obeidi, T.K. Sawyer, D.J. Staples, A.E. deVaux, O. Dym, A.M. de L. Castrucci, M.F. Hintz, J.P. Riehm, and K.R. Rao, J.Med.Chem., 1987, 30, 2126.
350. T.P. Andersen and A. Sennig, Liebigs Ann.Chem., 1987, 59.
351. H. Zhang, Shengwu Huaxue Yu Shengwu Wuli Jinzhan, 1987, 48.
352. T. Hashimoto, K. Kurosawa, and Y. Uchida, Bull.Chem.Soc.Jpn., 1987, 60, 3071.
353. T. Hashimoto, Y. Uchida, K. Okimura, and K. Kurosawa, Chem.Pharm.Bull., 1987, 35, 3442.
354. Y. Uchida, K. Okimura, K. Kurosawa, N. Sakura, K. Hirose, and T. Hashimoto, Bull.Chem.Soc.Jpn., 1987, 60, 1561.
355. A. Balasubramaniam, I. Grupp, L. Srivastava, K. Tatemoto, R.F. Murphy, S.N. Joffe, and J.E. Fischer, Int.J.Peptide Protein Res,. 1987, 29, 78.
356. J.-M. Sabatier, M. Tessier-Rochat, C. Granier, J. Van Rietschoten, E. Pedroso, A. Grandas, F. Albericio and E. Giralt, Tetrahedron, 1987, 5973. 357.F. Albericio, E. Nicolas, J. Josa, A. Grandas, E. Pedroso, E. Giralt, C. Granier and J. Van Rietschoten, Tetrahedron, 1987, 43, 5961.
358. M. Lebl, E.E. Sugg, G. Van Binst, P. Vander Elst, D. Tourwe, J. Slaninova, and V.J. Hruby, Int.J.Pept.Protein Res., 1987, 30, 318.
359. W.Y. Chan, V.J. Hruby, T.W. Rockway, and J. Hlavacek, J.Pharmacol. Exp.Ther., 1986, 239, 84.
360. J. Elands, C. Barberis, S. Jard, E. Tribollet, J.-J. Driefuss, K. Bankowski, M. Manning, and W.H. Sawyer, Eur.J.Pharmacol., 1987, 147, 197.
361. J. Hlavacek, I. Fric, P. Malon, K. Jost and K. Blaha, Coll.Czech.Chem.Commun., 1987, 52, 1841.
362. J. Hlavacek, J. Pospisek, J. Slaninova, W.Y. Chan, and V.J. Hruby, Coll.Czech.Chem.Commun., 1987, 52, 2317.
363. V.N. Medvedkin and Yu. V. Mitin, Bioorg Khim., 1987, 13, 183.
364. V.N. Medvedkin, Yu.V.Mitin, and E.A. Permyakov, Bioorg.Khim., 1987, 13, 1019.
365. J.P. Riehm and K.R. Rao, Int.J.Pept.Protein Res., 1987, 29, 415.
366. T. Sasaki and S. Isoda, Chem.Pharm. Bull., 1987, 35, 2797.
367. T. Sasaki and S. Isoda, Chem.Pharm.Bull., 1987, 35, 2804.
368. T. Sasaki, N. Sugiyama, M. Iwamoto, and S. Isoda, Chem.Pharm.Bull., 1987, 35, 2810.
369. D. Konopinska, B. Kazanowska, W. Sobotka, and J. Boguslawaska-Jaworska, Bull.Pol.Acad.Sci.,Chem., 1986, 34, 327.
370. K.M. Sivanandaiah, and N.S. Rangaraju, Indian J.Chem., 1986, 25B, 787.
371. R.M.L. Jones, K. Rose, and R.E. Offord, Biochem. J., 1987, 247, 785.
372. R. Katakai, Biopolymers, 1987, 26, 5.
373. R.S. Pottorf, D.H. Rich, J. Engelke, and J.W. Suttie, J.Med.Chem., 1987, 30, 445.
374. J. Bouhnik, F.-X. Galen, J. Menard, P. Corvol, R. Seyer, J.A. Fehrentz, D. Le Nguyen, P. Fulgrand, and B. Castro, J.Biol.Chem., 1987, 262, 2913.
375. F.-X. Galen, G. Evin, C. Carelli, J. Bouhnik, J.-B. Michel, J.-A. Ferhentz, D. Le Nguyen, R. Seyer, W.D. Carlson, B. Castro, and P. Corvol, Nucl.Med.Biol. (Int.J.Radiat.Appl.Instrum.Part B), 1987, 14, 305.
376. C. Yanaihara, M. Kadowaki, M. Hoshino, S. Kishida, T. Suzuki and N. Yanaihara, Biomed.Res., 1987, 8, 95.
377. C. Mitchinson and R.L. Baldwin Proteins: Struct., Funct., Genet., 1986, 1, 23.
378. N. Teno, S. Tsuhoi, T. Shimamura, Y. Okada, Y. Yanagida, M. Yoshinaga, K. Ohgi, and M. Irie, Chem.Pharm.Bull., 1987, 35, 468.
379. I. Pavo, B. Penke, J.R. Varga, L. Vescei, and K. Kovacs, Acta.Chim.Hung., 1986, 122, 261.
380. R.Z. Cai, T. Karashima, J. Guoth, B. Szoke, D. Olson, and A.V. Schally, Proc.Natl.Acad.Sci. USA, 1987, 84, 2502.
381. B. Calas, R. Michelot, J.P. Lecaer, A. Cave, J. Parello, and P. Potier, Int.J.Pept.Protein Res., 1987, 29, 170.
382. T. Wasiak, A. Janecka, and W. Koziolkiewicz, Pol.J.Chem., 1986, 60, 477.
383. Y. Lu, Y. Zhu, D. Tan, and G. Zou, Shengwu Huaxue Zazhi, 1986, 2, 23.

384. S.V. Egorova, N.A. Sloboda, and A.A. Akhrem, Vestsi Akad. Navuk BSSR, Ser.Khim.Navuk, 1987, 72.
385. S.V. Egorova, Vestsi Akad. Navuk BSSR, Ser.Khim.Navuk, 1986, 87.
386. H.M. Franzen, U. Ragnarsson, K. Nagren and B. Langstrom, J.Chem.Soc., Perkin Trans. 1., 1987, 2241.
387. C. Sergheraert, P. Maes and A. Tartar, J.Chem.Soc., Perkin Trans.1, 1986, 1061.
388. C. Poulos, G. Stavropoulos, J.R. Brown, and C.C. Jordan, J.Med.Chem., 1987, 30, 1512.
389. T. Hashimoto, N. Sakura, K. Hirose, Y. Uchida, M. Nishijima, and T. Moro, Bull.Chem.Soc.Jpn., 1987, 60, 827.
390. T. Hashimoto, Y. Uchida, M. Nishijima, N. Sakura, and K. Hirose, Bull.Chem.Soc.Jpn., 1987, 60, 1207.
391. O. Ploux, G. Chassaing and A. Marquet, Int.J.Peptide Protein Res., 1987, 29, 162.
392. K.M. Sivanandaiah and N.S. Rangaraju, Indian J.Chem., 1986, 25B, 1045.
393. A.A. Amoscato, G.F. Babcock, R.M. Sramkoski, B.A. Hynd, and J.W. Alexander, Int.J.Pept.Protein Res., 1987, 29, 177; G.A. Heavner, D.J. Kroon, T. Audhya and G. Goldstein, Peptides, 1986, 7, 1015.
394. W. Voelter and W. Heinzel, Chem.-Ztg., 1987, 111, 82.
395. M. Cerny, E. Kasafirek, J. Rovensky, and J. Krepelka, Coll.Czech.Chem.Commun., 1986, 51, 2909.
396. M. Cerny, E. Kasafirek, J. Rovensky, and J. Pekarek, Coll.Czech.Chem.Commun., 1987, 52, 1857.
397. T. Abiko and H. Sekino, Chem.Pharm.Bull., 1987, 35, 3757.
398. T. Abiko and H. Sekino, Biotechnol.Appl.Biochem., 1987, 9, 20.
399. G. Auger, D. Blanot, M. Magnin, L.N. Gastinel, J.M. Pleau, M. Dardenne and J.F. Bach, Biol.Chem.Hoppe-Seyler, 1987, 368, 463.
400. M. Kruszynski, Pol.J.Chem., 1986, 60, 95.
401. Yu. P. Svachkin, Yu. A. Semiletov, V.P. Fedotov, I.S. Komolov, G.I. Bushueva, L.A. Batrameeva and A.I. Ivanova, Zh.Obshch.Khim., 1987, 57, 712.
402. A.A. Mazurov, S.A. Andronati, T.I. Korotenko, A.F. Galatin, A.N. Petrov and E.K. Georgianova, Khim.-Farm.Zh., 1987, 21, 1310.
403. S.A. Andronati, A.A. Mazurov, and T.I. Korotenko, Khim.Prir.Soedin., 1986, 222.
404. R.E. Reid, Int.J.Pept.Protein Res., 1987, 30, 613.
405. G. Perseo, M. Gigli and R. de Castiglione, Int.J.Peptide Protein Res., 1987, 29, 478.
406. D.N. Rao, R.R. Lyer, H.K. Prasad, and I. Nath, Med.Sci.Res., 1987, 15, 1199.
407. K. Akaji, M. Tanaka, S. Sumi, M. Kogire, K. Takaori, R. Doi, K. Inoue, T. Tobe, and M. Moriga, Chem.Pharm.Bull., 1987, 35, 535.
408. E. Wünsch and G. Wendlberger, Wien Tieraerztl. Monatsschr., 1986, 73, 164.
409. M. Manning, A. Misicka, A. Olma, W.A. Klis, K. Bankowski, E. Nawrocka, M. Kruszynski, A. Kolodziejczyk, L.L. Cheng, J. Seto, N.C. Wo, and W.H. Sawyer, J.Med.Chem., 1987, 30, 2245.
410. F.E.-F. Ali, H.L. Chang, W.F. Huffman, G. Heckman, L.B. Kinter, E.F. Weidley, R. Edwards, D. Schmidt, D. Ashton-Shue, and F.L. Stassen, J.Med.Chem., 1987, 30, 2291.
411. A. Buku, I.L. Schwartz, N. Yamin, H.R. Wyssbrod, and D. Gazis, J.Med.Chem., 1987, 30, 1509.
412. B. Lammek, P. Rekowski, G. Kupryszewski, P. Melin and U. Ragnarsson, Experimenta, 1987, 43, 1211.
413. M. Manning, J.P. Przybylski, A. Olma, W.A. Klis, M. Kruszynski, N.C. Wo, G.H. Pelton and W.H. Sawyer, Nature, 1987, 329, 839.
414. A. Buku, P. Eggena, H.R. Wyssbrod, I.L. Schwartz, D. Gazis, L.I. Somuza and J.D. Glass, J.Med.Chem., 1987, 30, 1526.
415. F. Fahrenholz, P. Eggena, E. Kojro, D. Gazis and I.L. Schwartz, Int.J.Pept.Protein Res., 1987, 30, 577.
416. R. Rocchi, L. Biondi, F. Filira, and B. Scolaro, Int.J.Pept. Protein Res., 1987, 30, 240.

417. V. Guantieri, A.M. Tamburro, D. Cabrol, H. Broch, and D. Vasilescu, Int.J.Pept.Protein Res., 1987, 29, 216.
418. L. Krzyzanowski and B. Rzestotarska, Int.J.Pept.Protein Res., 1987, 29, 672.
419. Y. Trudelle, A. Brack, A. Delmas, S. Pedoussant, and P. Rivaille, Int.J.Pept.Protein Res., 1987, 30, 54.
420. N. Nishi, K. Hagiwara and S. Tokura, Int.J.Pept. Protein Res., 1987, 30, 275.
421. I.Z. Siemion, A. Pedyczak, and M. Rospenk, Int.J.Pept.Protein Res., 1987, 30, 339.
422. H. Eckstein, Z. Hu, and H. Schott, Biopolymers, 1986, 25, 1055.
423. E. Panoy-Pomonis, C. Sakarellos, and M. Sakarellos-Daitsiotis, Biopolymers, 1986, 25, 655.
424. R. Katakai, Biopolymers, 1987, 26, 5.
425. H. Aoyaji, S. Lee, H. Mihara, T. Kato, and N. Izumiya, Mem.Fac.Sci.Kyushu Univ.,Ser.C, 1986, 15, 247.
426. S. Ando, T. Itaya, H. Nishikawa, and H. Takiguchi, Fukuoka Daigaku Rigaku Shuho, 1986, 16, 27.
427. M. Sisido, Polym.Prepr.(Am.Chem.Soc., Div.Polym.Chem.), 1986, 27, 324.
428. D.W. Urry, R.D. Harris, H. Sugano, M.M. Long, and K. Prasad Polm.Sci.Technol.(Plenum), 1987, 35, 335.
429. H. Yamamoto, A. Nishida, T. Hayakawa, N. Nishi, and R. Yamamoto, Colloid Polym.Sci., 1986, 264, 779.
430. H. Yamamoto, J.Chem.Soc., Perkin Trans. 1., 1987, 613.
431. R. Katakai, J.Chem.Soc., Perkin Trans. 1., 1987, 2249.
432. G.L. Reddy and R. Nagaraj, Int.J.Pept.Protein Res., 1987, 29, 497.
433. G. Maret, D. Blanot, M. Welti, and J.-L. Fauchere, Int.J.Pept.Protein Res., 1987, 29, 734.
434. J.R. Luly, J.J. Plattner, H. Stein, N. Yi, J. Soderquist, P.A. Marcotte, H.D. Kleinert, and T.J. Perun, Biochem.Biophys.Res.Commun., 1987, 143, 44.
435. K.Y. Hui, W.D. Carlson, M.S. Bernatowicz, and E. Haber, J.Med.Chem., 1987, 30, 1287.
436. M.G. Bock, R.M. DiPardo, B.E. Evans, K.E. Rittle, J. Boger, M. Poe, B.I. LaMont, R.J. Lynch, E.H. Ulm, G.P. Vlasuk, W.J. Greenlee, and D.F. Veber, J.Med.Chem., 1987, 30, 1853.
437. H. Paulsen and M. Schultz, Carbohydr. Res., 1987, 159, 37.
438. R. Paruszewski, P. Jaworski, and M. Strzalkowska, Pol.J.Chem., 1986, 60, 121.
439. S. Thaisrivongs, D.T. Pals, J.A. Lawson, S.R. Turner, and D.W. Harris, J.Med.Chem., 1987, 30, 536.
440. G. Bolis, A.K.L. Fung, J. Greer, H.D. Kleinert, P.A. Marcotte, T.J. Perun, J.J. Plattner, and H.H. Stein, J.Med.Chem., 1987, 30, 1729.
441. D.J. Kempf, E. De Lara, H.H. Stein, J. Cohen, and J.J. Plattner, J.Med.Chem., 1987, 30, 1978
442. S. Thaisrivongs, H.J. Schostarez, D.T. Pals, and S.R. Turner, J.Med.Chem., 1987, 30, 1837.
443. K. Fearon, A. Spaltenstein, P.B. Hopkins, and M.H. Gelb, J.Med.Chem., 1987, 30, 1617.
444. J.F. Dellaria, R.G. Maki, B.A. Bopp, J. Cohen, H.D. Kleinert, J.R. Luly, I. Merits, J.J. Plattner, and H.H. Stein, J.Med.Chem., 1987, 30, 2137.
445. J.R. Luly, N. Yi, J. Soderquist, H. Stein, J. Cohen, T.J. Perun, and J.J. Plattner, J.Med.Chem., 1987, 30, 1609.
446. S.H. Rosenberg, J.J. Plattner, K.W. Woods, H.H. Stein, P.A. Marcotte, J. Cohen, and T.J. Perun, J.Med.Chem., 1987, 30, 1224.
447. J.G.C. Van Amsterdam, K.J.H. Van Buuren, M.W.M. Blad, and W. Soudijn, Eur.J.Pharmacol., 1987, 135, 411.
448. V.B. Muratov and A.P. Kharchevnikov, Zh.Org.Khim., 1986, 22, 1730.
449. D. Zicane, I. Ravina, Z. Tetere, E. Gudriniece, and U. Kalejis, Latv.PSR. Zinat. Akad. Vestis, Kim. Ser., 1987, 348.

450. L.A. Lyublinskaya, I. Haidu, G.N. Balandina, I. Yu. Filippova, A.N. Markaryan, E.N. Lysogorskaya, E.S. Oksenoit, and V.M. Stepanov, Bioorg.Khim., 1987, 13, 748.
451. T.L. Voyushina, L.A. Lyublinskaya, E.A. Timokhina, and V.M. Stepanov, Bioorg.Khim., 1987, 13, 615.
452. H. Okunski, J. Spragg, and J. Burton, in 'Vasodepressor Hormones', pub. Birkhäuser Verlag, Basel, 1987.
453. A.S. Dutta, M.B. Giles, J.J. Gormley, J.C. Williams, and E.J. Kusner, J.Chem.Soc., Perkin Trans 1., 1987, 111.
454. A. Lentini, F. Farchione, B. Ternai, N. Kreus-Ongarjnukool and P. Fovivich, Biol.Chem.Hoppe-Seyler, 1987, 368, 369.
455. M. Tamai, C. Yokoo, M. Murata, K. Oguma, K. Sota, E. Sato, and Y. Kanaoka, Chem.Pharm.Bull., 1987, 35, 1098.
456. Y. Tsuda, Y. Okada, Y. Nagamatsu, and U. Okamoto, Chem.Pharm.Bull., 1987, 35, 3576.
457. F.G. Salituro, N. Agarwal, T. Hofmann and D.H. Rich, J.Med.Chem., 1987, 30, 286.
458. M.W. Holladay, F.G. Salituro, and D.H. Rich J.Med.Chem., 1987, 30, 374.
459. A.S. Dutta, M.B. Giles, and J.C. Williams, J.Chem.Soc.,Perkin Trans. 1., 1986, 1655.
460. A. Krantz, R.W. Spencer, T.F. Tam, E. Thomas, and L.J. Copp, J.Med.Chem., 1987, 30, 591.
461. H.L. Sham, C.A. Rempel, H. Stein, and J. Cohen, J.Chem. Soc., Chem. Commun., 1987, 683.
462. N. Teno, S. Tsuboi, Y. Okada, N. Itoh, and H. Okamoto, Int.J.Peptide Protein Res., 1987, 30, 93.
463. L. Grehn, B. Fransson, and U. Ragnarsson, J.Chem.Soc., Perkin Trans. 1, 1987, 529.
464. B. Voigt and G. Wagner, Pharmazie, 1986, 41, 378.
465. F. Cumin, D. Le Nguyen, B. Castro, J. Menard and P. Corvol, Biochim. Biophys. Acta, 1987, 913, 10.
466. K. Dill, R.D. Carter, J.M. Lacombe and A.A. Pavia, Carbohydr. Res., 1986, 152, 217.
467. H.G. Garg, T. Hasenkamp, and J. Paulsen Carbohydr. Res., 1986, 151, 225.
468. N.J. Maeji, Y. Inoue and R. Chujo, Int.J.Pept.Protein Res., 1987, 29, 699.
469. Y. Hioki, E. Yamamoto, M. Kiso, and I. Azuma, J. Carbohydr.Chem., 1986, 5, 359.
470. A. Calvo-Mateo and F.G. De Las Heras, Carbohydr.Res., 1986, 155, 266.
471. A. Calvo-Mateo and F.G. De la Heras, Liebigs Ann.Chem., 1987, 1017.
472. J. Jezek, R. Straka, V. Krchnak, M. Ryba, J. Rotta, P. Mayer and M. Zaoral, Coll.Czech.Chem.Comm., 1987, 52, 1609.
473. A. Claesson, A.M. Jansson, B.G. Pring, S.M. Hammond, and B. Ekstroem, J.Med.Chem., 1987, 30, 2309.
474. R. Rocchi, L. Biondi, F. Cavaggio, F. Filira, M. Gobbo, S. Dagan and M. Fridkin, Int.J.Peptide Protein Res., 1987, 29, 262.
475. R. Rocchi, L. Biondi, F. Filira, M. Gobbo, S. Dagan, and M. Fridkin, Int.J.Peptide Protein Res., 1987, 29, 250.
476. M. Fukuda, C.M. Beechan, M. Verlander, M. Goodman, M.M. Khan, and K.L. Melmon, Int.J.Pept.Protein Res., 1987, 29, 300.
477. R. Kishore, S. Raghothama and P. Balaram, Int.J.Pept.Protein Res., 1987, 29, 381.
478. G. Vertuani, S. Spisani, M. Boggian, S. Traniello, and A. Scatturin, Int.J.Pept.Protein Res., 1987, 29, 525.
479. M.T. Garcia-Lopez, R. Gonzalez-Muniz, R. Herranz, M.T. Molinero, and J. Del Rio, Int.J.Pept.Protein Res., 1987, 29, 613.
480. D.H. Schlesinger, A. Buku, H.R. Wyssbrod, and D.I. Hay, Int.J.Pept.Protein Res., 1987, 30, 257.
481. J.W. Perich, R.M. Valerio, and R.B. Johns, Tetrahedron Lett., 1986, 27, 1377.
482. J.W. Perich, P.F. Alewood, and R.B. Johns, Tetrahedron Lett., 1986, 27, 1373.
483. W. Lindner and F.A. Robey, Int.J.Pept.Protein Res., 1987, 30, 794.

484. P.O.L. Mack, D.P. Kelly, R.F. Martin, and L.P.G. Wakelin, Aust.J.Chem., 1987, 40, 97.
485. R. Kishore, S. Ragothama, and P. Balaram, Biopolymers, 1987, 26, 873.
486. M.A. Ponomareva-Stepnaya, V.N.Nezavibat'ko, L.V. Antonova, L.A. Andreeva, V.N. Potman, A.A. Kamenskii, and I.P. Ashmarin, Khim.-Farm.Zh., 1986, 20, 1195.
487. Y. Du, J. Shen, K. Wang, W. Wu, C. Wu, and B. Gu, Shengwu Huaxue Yu Shengwu Wuli Xuebao, 1986, 18, 60.
488. I. Bruzgo, K. Midura-Nowaczek, J. Popalawski, W. Roszkowaka-Jakimiec, and K. Worowoski, Acta.Pol.Fharm., 1986, 43, 36.
489. D. Eisenberg, W. Wilcox, S.M. Eshita, P.M. Pryciak, S.P. Ho, and W.F. DeGrado, Proteins: Struct., Funct., Genet., 1986, 1, 16.
490. S.P. Ho and W.F. DeGrado, J.Am.Chem.Soc., 1987, 109, 6751.
491. T.B. Johnson and J.K. Coward, J.Org.Chem., 1987, 52, 1771.
492. E.P. Krysin, V.N. Karel'skii, A.K. Rabinovich, and S. Yu. Borovkova, Khim.Prir.Soedin, 1986, 759.
493. A.M. El-Naggar, M.R. Zaher, and F.A. Kora, J.Indian Chem.Soc., 1986, 63, 525.
494. H. Mihara, T. Kanmera, M. Yoshida, S. Lee, H. Aoyagi, T. Kato, and N. Izumiya, Bull.Chem.Soc.Jpn., 1987, 60, 697.
495. K. Ienaga, K. Higashiura and H. Kimura Chem.Pharm.Bull., 1987, 35, 1249.
496. H. Suli-Vargha, A. Jeney, K. Lapis and K. Medzihradszky, J.Med.Chem., 1987, 30, 583.
497. K.A. Jacobson, A.W. Lipkowski, T.W. Moody, W. Padgett, E. Pijl, K.L. Kirk and J.W. Daly, J.Med.Chem., 1987, 30, 1529.
498. R. Andruszkiewicz, H. Chmra, S. Milewski and E. Borowski, J.Med.Chem., 1987, 30, 1715.
499. G.M. Anantharamaiah, A. Gawish, M. Iqbal, S.A. Kahn, C.G. Brouillette and J. Segrest, Protides Biol.Fluids, 1986, 34, 63.
500. G. Valle, C. Toniolo and G. Jura, Gazz.Chim.Ital., 1987, 117, 549.
501. E. Hamsikova, H. Zavadova, M. Zaoral, J. Jezek, K. Blaha and V. Vonka J.Gen.Virol., 1987, 68, 2249.
502. R.E Shute, B. Dunlap, and D.H. Rich, J.Med.Chem., 1987, 30, 71.
503. J. Metzger, A. Olma, M.T. Leplawy and G. Jung, Z.Naturforsch.,B., 1987, 42, 1195.
504. W. Prass, H. Ringsdorf, W. Bessler, K.H. Wiesmueller, and G. Jung, Biochim. Biophys. Acta, 1987, 900, 116.
505. J. Pospisek and K. Blaha, Coll.Czech.Chem.Commun., 1987, 52, 514.
506. I. Shinoda, Y. Nosho, K. Kouge, N. Ishibashi, H. Okai, K. Tatsumi and E. Kikuchi, Agric.Biol.Chem., 1987, 51, 2103.
507. D. Cantacuzene, F. Pascal, and C. Guerreiro, Tetrahedron, 1987, 43, 1823.
508. D. Yamashiro, Int.J.Peptide Protein Res., 1987, 30, 9.
509. B. Liberek and R. Kasprzykowska, Int.J.Pept. Protein Res., 1987, 30, 222.
510. B. Liberek and R. Kasprzykowska, Int.J.Pept.Protein Res., 1987, 30, 522.
511. P.N. Rao, J.E. Burdett, Jr., J.W. Cessac, C.M. DiNunno, D.M. Peterson, and H.K. Kim, Int.J.Pept.Protein Res., 1987, 29, 118.
512. C.F. Stanfield, W.L. Cody, and V.J. Hruby, J.Org.Chem., 1986, 51, 5153.
513. J. Porter, J. Dykert, and J. Rivier, Int.J.Pept.Protein Res. 1987, 30, 13.
514. P. Jouin, B. Castro, and D. Nisato, J.Chem.Soc., Perkin Trans. 1., 1987, 1177.
515. K. Iwaki, S. Yoshida, N. Nimura, T. Kinoshita, K. Takeda and H. Ogura, J.Chromatogr., 1987, 404, 117.
516. R.H. Buck and K. Krummen, J.Chromatogr., 1987, 387, 255.
517. W.H. Pirkle, D.M. Alessi, M.H. Hyun and T.C. Pochapsky, J.Chromatogr., 1987, 398, 203.
518. K. Ravichandran and L.B. Rogers, J.Chromatogr., 1987, 402, 49.
519. G. Krüger, J. Grötzinger and H. Berndt, J.Chromatogr., 1987, 397, 223.

520. T. Nakagawa, A. Shibukawa, A. Kaihara, and H. Tanaka, Nippon Kagaku Kaishi., 1986, 1002.
521. W. Vogt, E. Egeler, W. Sommer, F. Eisenbeiss, and H.D. Meyer, J. Chromatogr., 1987, 400, 83.
522. G. Szokan, A. Torok, and B. Penke, J.Chromatogr., 1987, 387, 267.
523. E.V. Titova, M.A. Chlenov and L.I. Kudryashov, J.Chromatogr., 1986, 364, 209.
524. R.T. Dobrowsky, G. O'Sullivan, L.M. Ballas, L.N. Fleisher and N.C. Olson, J.Liq.Chromatogr., 1987, 10, 137.
525. H. Ong, S. Meloche, A. De Lean and P. Larose, J.Liq.Chromatogr., 1987, 10, 3085.
526. R.A. Houghten and S.T. De Graw, J. Chromatogr., 1987, 386, 223.
527. G.B. Irvine, J.Chromatogr., 1987, 404, 215.
528. C.T. Mant, J.M.R. Parker and R.S. Hodges, J.Chromatogr., 1987, 397, 99.
529. F. Perin, R. Presentini and G. Antoni, J.Chromatogr., 1987, 397, 365.
530. M. Nakano, M. Kai, M. Ohno and Y. Ohkura, J.Chromatogr., 1987, 411, 305.
531. M.T.W. Hearn and M.I. Aguilar, J.Chromatogr., 1987, 397, 47.
532. M.T.W. Hearn and M.I. Aguilar, J.Chromatogr., 1987, 392, 33.
533. D. Guo, C.T. Mant and R.S. Hodges, J.Chromatogr., 1987, 386, 205.
534. J.L. Glajch, J.J. Kirkland and J. Köhler, J.Chromatogr., 1987, 384, 81.
535. E.J. Draviam, G.H. Greeley, R.D. Beauchamp and J.C. Thompson, J.Liq.Chromatogr., 1987, 10, 1431.
536. T. Cserhati, B. Bordas, and G. Osapay, Chromatographia, 1987, 23, 184.
537. G. Folena-Wasserman, R.D. Sitrin, F. Chapin and K.M. Snader, J.Chromatogr., 1987, 392, 225.
538. E. Hochuli, H. Döbeli and A. Schacher, J.Chromatogr., 1987, 411, 177.
539. S.M. Cramer, Z. el Rassi and C. Horvath, J.Chromatogr., 1987, 394, 305.
540. P. Stehle, H.P. Bahsitta and P. Fuerst, J.Chromatogr., 1986, 370, 131.
541. C. Hoeger, R. Galyean, J. Boublik, R. McClintock and J. Rivier, BioChromatography, 1987, 2, 134.
542. M.E. Younes, S. Abdel Rahman and A. Hattaba, Coll.Czech.Chem.Commun., 1987, 52, 1352.
543. D. Konopinska, G. Rosinski, A. Lesicki, W. Sobotka, P. Sujak, and A. Kasprzyk Bull.Pol.Acad.Sci., Chem., 1987, 35, 125.
544. S. Kumar, M.V.R. Rao, and M. Atreyi, Indian J.Chem., 1986, 25B, 926.
545. T. Komori, T. Nonaka, T. Fuchigami, and N. Zhang, Bull.Chem.Soc.Jpn., 1987, 60, 3315.
546. B.J. McBride and D.S. Kemp, Tetrahedron Lett., 1987, 28, 3435.
547. S. Ram and L.D. Spicer, Tetrahedron Lett., 1987, 515; B.M. Adger, C. O'Farrell, N.J. Lewis and M.B. Mitchell, Synthesis, 1987, 53.

3
Analogue and Conformational Studies on Peptide Hormones and Other Biologically Active Peptides

BY J. S. DAVIES

1. Introduction

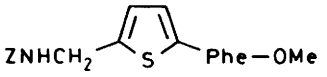
Productivity in this area is just a little down on the number of papers covering this subject matter in Vol. 19. This Chapter is based on a scan of the relevant journals for the calendar year 1987, scanning the journals themselves and relevant sections of Chemical Abstracts (up to its May 1988 issues). Fifty per cent of the papers accessed were distributed equally between the Int. Journal of Peptide and Protein Research and the Journal of Medicinal Chemistry, with the remaining papers being retrieved from a plethora of organic chemical/biochemical journals.

In what is an active area of research for the pharmaceutical industry it is always debatable whether the reviewer could produce a more balanced review, especially on biologically active analogues, if the patents literature was also scanned. However, time, and limited access to the patents, preclude such a comprehensive coverage, but the hope is that our industrial colleagues do publish their observations in the journals, once the commercial aspects of the work have been taken care of.

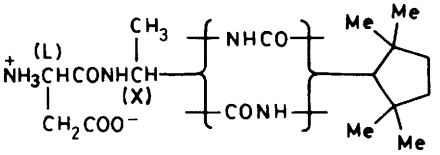
Many of the papers[1] from the 19th European Peptide Symposium at Porto Carras, are very relevant to this Chapter, but have not been reviewed in detail as more often than not, full papers covering the same work usually appear, albeit sometime later.

2. Peptide-backbone Modifications

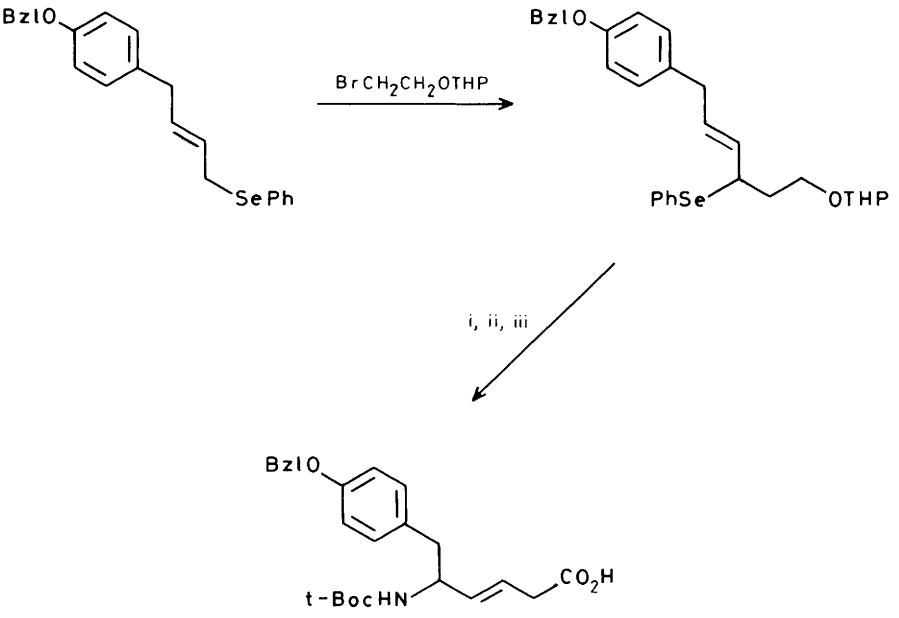
2.1 ψ[CSNH]-Analogues. - Endothionated analogues of the C-terminal region of gastrin have been synthesised[2] either by the use of P_4S_{10} or of Lawesson's Reagent. Thionation of Boc-Phe-NH-R(R=H or Me) with P_4S_{10} to give Boc-Pheψ[CSNH]R provided the starting point for the synthesis of Ac-Trp-Leu-Asp-Pheψ[CSNH]R, and treatment of Boc-Leu-Asp(OBzl)-OMe with Lawesson's reagent



(1)



(2) X = L, D, *R* or *S*



(3)

(THP = tetrahydropyranyl)

i, t-BocNH$_2$; ii, H$^+$; iii, Jones Reagent

Scheme 1

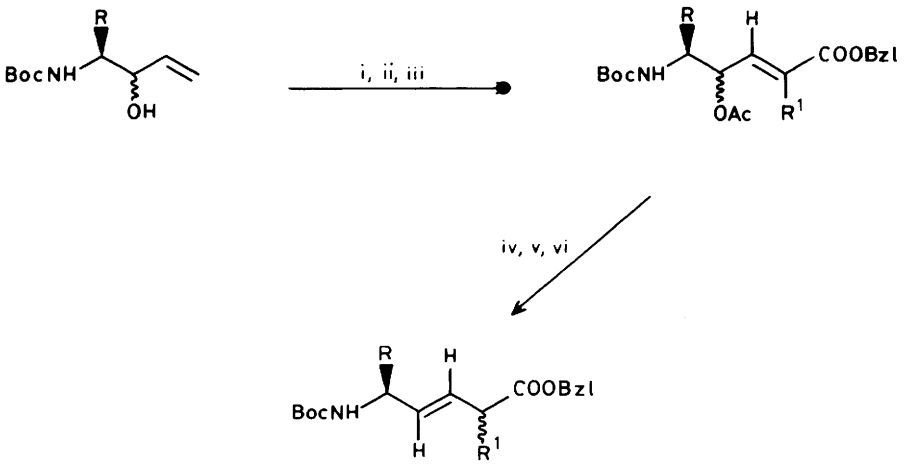
provided the key stage to Ac-Trp-Leuψ[CSNH]-Asp-OMe. Use of the latter reagent with Boc-Pro-NH_2 provided the C-terminal unit in pyroGlu-His-Proψ[CSNH]H a TRH analogue which exhibits[3] TRH receptor binding affinity. Its TSH and α-MSH releasing activity was equal to natural TRH. The monothiono analogue of the enkephalins H-Tyr-Glyψ[CSNH]-Gly-Phe-LeuOH has been synthesised[4], again using Lawesson's reagent on the unhindered Gly-Gly bond. In this study when Z-Glyψ[CSNH]-Glyψ[CSNH]-Phe-OMe was treated with a peracid oxidative transformation into the thiazole (1) was observed. The stereochemical consequence of introducing thioamide groups into peptides has been the subject[5] of theoretical analysis using a Ramachandran hard sphere model to calculate possible φ and ψ dihedral angles.

2.2 ψ[NHCO]-Retro-inverso Analogues. - ^{1}H N.m.r. and energy minimisation analysis on the sweet-tasting aspartame analogues (2)[6] lend support to a conformational model very similar to the previously published X-ray crystallographic structure of aspartame (L-Asp-L-Phe-OMe). The three sweet analogues (the two retro-inverso isomers L,(R) and L,(S) forms and the L,D-peptide) all have this conformation, while the bitter tasting L,L-peptide is strikingly different. Retro-inverso pseudopeptides related[7] to the C-terminal gastrin tetrapeptide exhibited little or no activity in acid secretion in rats but were able to antagonise gastrin action. In the series investigated, Boc-Trp-Leu-gAsp-CO-CH_2CH_2Ph, Boc-Trp-Leu-gAsp-m(RS)PheNH_2 and Boc-Trp-gLeu-D-Asp-m(RS)PheNH_2 were the most potent, the gem-diamino residues being formed in the synthesis by reaction of RS $NH_2COCH(CH_2Ph)CO_2H$ with bis(trifluoroacetoxy)iodobenzene. A retro-inverso cyclic somatostatin analogue cyclo-(mAla-Phe-D-Trp-Lys-Thr-gPhe) has been the subject[8] of a 500 MHz n.m.r. analysis. Two intramolecular H-bonds involving the NH of threonine and one of the NH's of gPhe were detected. The former H-bond is compatible with a type II' β-turn whereas the latter is only compatible with a C_8 ring including the CO of mAla.

2.3 ψ[CONR]-N-Alkylated analogues. - In order to investigate the possible involvement of the peptide bonds of the A^2-A^8 helical

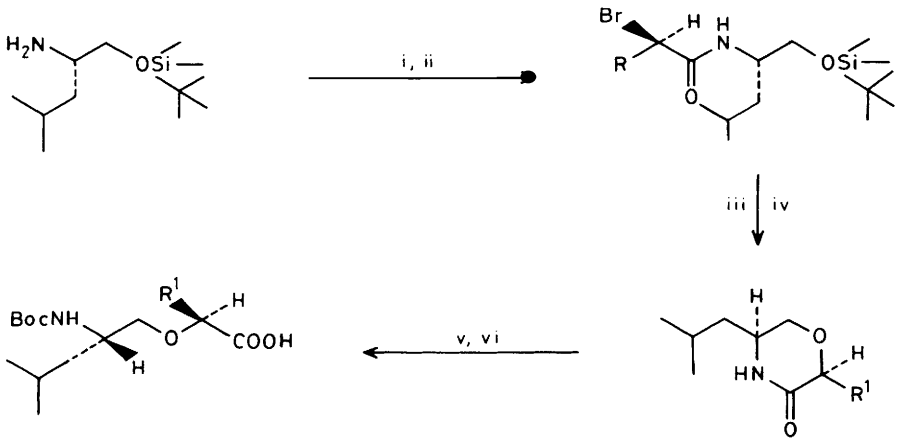
segment of insulin in the insulin-receptor interaction [MeIle^{2}-A]-insulin and [MeVal^{3}-A]-insulin have been synthesised[9]. CD spectra imply that these analogues remained monomeric at concentrations at which insulin is predominantly dimeric and that their A^{2}-A^{8} helical segments are distorted. Both analogues were weak full agonists in receptor binding assays but only exhibited about 12% of the potency of the natural hormone in radioimmunoassays. It is possible that loss of H-bonding capacity of A^{2}-A^{8} could account for the loss in potency.

2.4 ψ[CH_2NH] Amino Methylene Analogues. - A newly developed solid phase method for direct introduction[10] of [CH_2NH] utilising a sodium cyanoborohydride reductive alkylation of a resin-bound peptide amine with a t-Boc-amino-aldehyde has enabled a series of ψ[CH_2NH] pseudopeptide analogues of the highly potent somatostatin octapeptide, H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 to be synthesised. In terms of their ability to inhibit Growth Hormone release in vivo the analogues with modified CO groups of Cys^{2} and Lys^{5} showed the highest activities, but considerably lower potency was achieved through other [CH_2NH] substitutions within the cyclic ring. The results lend support to the proposed type II β-turn solution conformation centred on the D-Trp-Lys portion of the molecule. Replacement of each peptide bond in turn in the C-terminal gastrin tetrapeptide Boc-Trp-Leu-Asp-PheNH_2 has been reported before and now a ^{1}H and ^{13}C n.m.r. study[11] reveals that the conformational effect of each modification is localised around each of the [CH_2NH] substitutions. Each of the analogues recognise the gastrin receptor but are devoid of agonist activity. Each peptide bond one at a time has been replaced[12] by [CH_2NH] in the C-terminal heptapeptide of cholecystokinin(CCK), Z-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-Asp-PheNH_2. All the pseudopeptides stimulated amylase secretion with the same efficacy as CCK-8 (Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-Asp-PheNH_2), but with varying potencies. It appears therefore that peptide bonds are not crucial for the pancreozymin activity or for the binding to CCK receptors. Selective reduction of the peptide bond in N-protected dipeptide t-butyl esters has provided[13] a convenient method for synthesising [L-Pheψ[CH_2NH]-L-Leu$^{4-5}$]Leu-enkephalin. However, insertion[14] of a CH_2NH moiety between the positions 8 and 9 in deamino-oxytocin and



i, Ac_2O/Pyr; ii, $NaIO_4$/OsO_4; iii, $Ph_3P{=}CR^1CO_2Bzl$; iv, Na_2CO_3; v, Ph_3P/CBr_4/THF; vi, Zn/HOAc

Scheme 2



i, (*R*)-R^1CH(Br)COOH; ii, DCCI/HOBt; iii, $BF_3 \cdot Et_2O$; iv, NaH/THF; v, 6N HCl; vi, Boc_2O/Bu^tOH/OH^-

Scheme 3

deamino-oxypressin gives analogues with much lowered biological activities.

2.5 ψ[CH=CH]-Ethylenic Isosteres. - New approaches have been developed for the synthesis of protected trans alkene isosteres of the dipeptides Tyr-Ala, Phe-Phe, Leu-Phe and Leu-Leu using the 'Julian' olefine synthesis as a key step[15]. In the same work a procedure based on Scheme 1 also proved successful for making the Tyr-Gly isostere (3). A general methodology for a wide range of isosteres summarised in Scheme 2 has yielded[16] the three pseudodipeptides Aspψ[ECH=CH]Phe, Leuψ[ECH=CH]Asp and Glyψ[ECH=CH]Trp.

2.6 ψ[CH_2O]-Methyleneoxy Analogues. - A similar synthetic strategy to that reported by Nicolaides et al. (J. Med. Chem., 1986, 29, 959) has been developed independently[17] using silyl ethers of L-prolinol, L-leucinol, D-leucine or D-valine as key synthons, for the preparation of stereochemically defined ψ[CH_2O] pseudodipeptides. Key steps are summarised in Scheme 3.

2.7 ψ[NHCONH] - Ureylene Analogues. - A preliminary report of a new isostere ψ[NHCONH] insertion into the enkephalin sequence has been reported[18]. The ureylene bond is formed by the coupling of an isocyanate with an amino component. Studies on the enkephalin analogues H-Tyr-Gly-Gly-Pheψ[NHCONH]Leu-OH, H-Tyr-Glyψ[NHCONH]Gly-Phe-Leu-OH, and H-Tyrψ[NHCONH]Gly-Gly-Phe-Leu-OH revealed good stability to aminopeptidase, but the analogues had low opioid activity.

2.8 ψ[CH_2N(OH)], ψ[CONHNH] and ψ[NHOH] - Isosteres. - Reduction of dipeptides containing the nitrono isostere e.g. BocNH(R)CH=N(O)CH(R^1)CO_2Me with $NaBH_4$ has yielded[19] a series of methylene hydroxyamino [CH_2N(OH)] analogues, while silylation of hydroxylamines RNHOH(R=Et, or PhCHMe) followed by in situ acylation with mixed anhydrides of N-protected amino-acids or peptides yield[20] N-hydroxypeptides R^1CON(OH)R. If aza peptides are required then it is useful to note that hydrazino acids $NH_2NHCH(R)CO_2H$ can be obtained[21] from hydantoic acids $NH_2CONHCH(R)CO_2H$ by the Shestakov rearrangement promoted by KOCl.

2.9 [CH_2S] - Thiomethylene Analogues. - The (R)- and (S)- leucine configurational isomers of the cyclic enkephalin pseudopeptide H-Tyr-D-Lys-Gly-Pheψ[CH_2S]Leu- have been synthesised[22], as well as the analoguous linear analogues H-Tyr-D-Ala-Gly-Pheψ[CH_2S]Leu-OH. Two approaches to the cyclisation step were used; cyclisation of the linear pentapeptide precursor or a solution phase coupling of the N-terminal amino-acid to a previously produced cyclic tetrapeptide. Although not strictly an isostere, the leucinethiol dimer [$Me_2CHCH_2CH(NHBoc)CH_2S]_2$ has been used[23] to make a dimeric enkephalin which on Zn/AcOH reduction yielded H-Tyr-D-Ala-Gly-Phe-$NHCH(CH_2CHMe_2)CH_2SH$ which is a probe for the essential thiol group in opiate receptors.

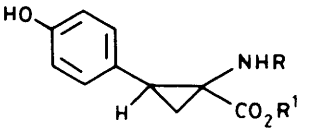
2.10 Phosphono-Peptides. - Acylaminoacids degraded using the Hunsdiecker reaction, provide[24] bromo derivatives which can be reacted with $NaPO(OEt)_2$ to give aminophosphonic esters, suitable as intermediates for Alafosfalin synthesis. Boc-L-Pro-DL-NHCH[P(O)$(OEt)_2$]-L-Phe-OMe has been synthesised[25] by coupling Boc-L-Pro-OH to DL$(EtO)_2P(O)CH(NH_2)CO$-Phe-OMe, a phosphonoglycyl peptide obtained by reacting $(EtO)_2P(O)CH_2NC$ with $KOCMe_3$ and then with $OCN(CH_2Ph)CO_2Me$.

2.11 Replacement of L- by D-Residues. - Comparison[26] of the analgesic activity of [D-Phe^4]-Met-enkephalin with that of [D-Ala^2, D-Phe^4]-enkephalin confirmed the increased potency through having D-Ala at position 2, while for [D-His^2]Leu-enkephalin only the analogue H-Tyr-D-Arg-Gly-Phe-Leu-Arg-OH exhibited[27] higher activities than Leu-enkephalin in guinea pig ileum preparations. Previous work had shown that substitutions in the N-terminal 1-29 sequence of growth hormone factor (GRF) boosted the biological activity. D-Amino acid substitutions have now been made[28] in sequences shorter than the 29 residues to study potencies. None of the D-substitutions in positions 2,3 and 8 (made singularly and in combination) were effective in increasing the 1-22 peptide sequence to potencies of detectable level. The highest in vivo potency in the 1-27 series was for [D-Asp^3, D-Asn^8, Leu^{27}]-GRF(1-27)NH_2 but this was far less than in its 1-29 counterpart. Potencies of the GRF sequences seem to be very dependent on a minimal chain length of 29 residues. Solid phase synthesis of a

number of D-residue analogues of neurokinin B (NKB) H-Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2 have provided analogues[29] for study on the isolated guinea pig ileum and rat duodenum. None of them possessed agonist activity but in the ileum bioassay, [Arg^3, Gly^6, D-Ala^8]- and [Arg^3, Gly^6, D-Trp^8]-NKB were found to be fairly potent antagonists. [Arg^3, D-Ala^6, D-Trp^8]- and [Arg^3, D-$Trp^{6,8}$, Gly^7]-NKB showed antagonist activities against NKB and substance P in the ileum bioassay. A NKB(3-10) analogue with D-Ala at position 6 also acts[30] as an antagonist of neurokinin B in these assays. D-Homoglutamine (D-Hgn) and D-pyroglutamic acid (D-pyroHgu) residues have been inserted[31] into substance P (SP) and tested for smooth muscle contractile activities. Potency of [D-Hgn^5, Hgn^6]-SP was as high as SP itself but the [Hgn^5, D-Hgn^6]-SP was much less active. The D-pyroHgu-Hgn-Phe-Phe-Gly-Leu-Met-NH_2 analogue possessed the highest potency amongst the analogues. It is suggested that an L-residue at position 6 is more important than in position 5. In a conformational analysis using infra-red spectroscopy[32] it is suggested that β-turn structures can be kept stable by D-amino acid residues in positions i + 1 and i + 2 of the β-turn structure.

Incorporation of D-residues using enzyme catalysis can now be carried out[33] by papain as well as the previously reported α-chymotrypsin using a high concentration of the D-amino acid ester. However, what promises to be a more versatile approach for incorporation of D-residue containing peptides as well as amino acids comes from the observation[34] that the stereoselectivity of the protease subtilisin is drastically relaxed upon transition from aqueous solution to anhydrous solvent.

2.12 α,α,-Di-Alkylated Glycine Analogues. - Substitution of α-aminoisobutyric acid (Aib) in the 1,1′-positions of gramicidin S and the 1-position of semi-gramicidin S gave analogues[35] which are inactive against Gram positive bacteria, and proof that the conformations of the analogues were different from that of the parent systems was obtained from the CD spectra. A series of esters of L-Asp-1-aminocyclopropane acid have been investigated[36] as aspartame analogues. In their synthesis, although the β-carboxyl group of aspartic acid had been protected by the t-butyl group, succinimide formation was a major problem. The n-propyl



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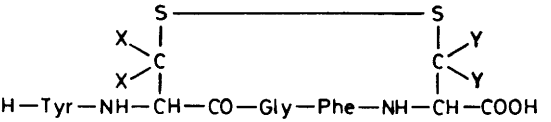
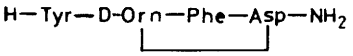


(5) X, Y = H or CH_3



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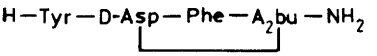

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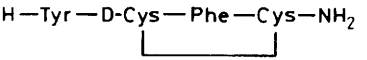

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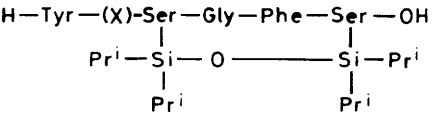
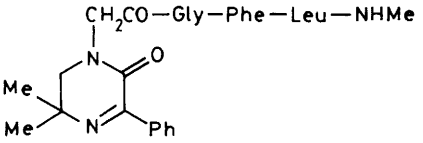
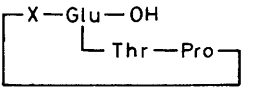


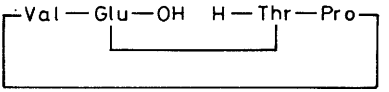
(9) (X) = D or L



(10)



(11) X = Val or Phe



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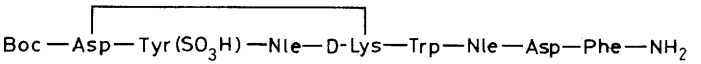

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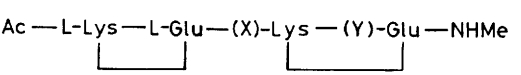


(14) (X), (Y) = D,D or L,L

ester proved the sweetest (280 times as sweet as sucrose) and its X-ray structure provides evidence for its conformation. The synthesis of cyclopropyl tyrosine (4) via oxazolone and thiazoline intermediates has also been reported[37]. Dipeptides with C-terminal α,α-disubstituted α-amino acids have been prepared[38] through reaction of N-protected amino acids with 2,2-disubstituted 3-amino-2H-azirines. Several Aib-containing analogues of bradykinin have been studied[39] using c.d. and ^{1}H n.m.r. Substitution of Aib for Pro^2 and/or Pro^3 in bradykinin stabilises a β-turn conformation in the N-terminal tetrapeptide moiety, but does not seem to promote biological potency. [Aib^2]-, [Aib^3]-, and [Aib^{2,3}]-bradykinin all have low biological activity so the 'cis proline' type conformation of Aib residues may not be preferred for recognition at the binding site. This is further supported by the fact that [Aib^7]-bradykinin, where the replacement is further away from the 'recognition site' has high activity. Further conformational details on Aib residues in model peptides have been obtained[40] from a study of the X-ray crystallographic data on Ac-Aib-OMe, Z-Aib-OBzl, Ac-Ala-Aib-OMe and Z-Aib-Ala-O-Trt.

3. Conformationally Restricted Cyclic and Bridged Analogues.

This continues to be an active field, with somatostatin and the enkephalins still demanding separate 'billing' as the most studied sequences as candidates for conformational restriction.

3.1 Somatostatin Analogues. - Any self-respecting solution-phase peptide synthetic chemist feeling vulnerable at the pace of automation in the field, should derive great satisfaction from the Merck group's large scale synthesis[41] of the highly potent somatostatin analogue cyclo-(MeAla-Tyr-D-Trp-Lys-Val-Phe). Mixed anhydride couplings and a cyclisation step using diphenylphosphoryl azide with $NaHCO_3$ as base readily lead to 40-50g batches of the cyclo-peptide with > 99% purity. The same cyclisation technique has also been used[42] to synthesise progressively smaller compounds possessing higher potency and activity than somatostatin, and which retain β-turn

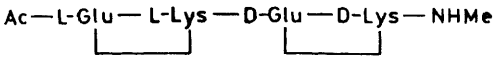
characteristics at the D-Trp-Lys residues as well as the usual requirement of an aromatic residue. Thus a series of amine derivatives based on cyclo-[D-Trp-Lys(Inoc)-δD,L-Aad(R)-Phe) where Aad is the aminoadipic acid residue, with R = $NH(CH_2)_nPh$ ranging from n = 1 to n = 3 were synthesised. All analogues were shown to have little ability to inhibit release of insulin, glucagon, and growth hormone, but the analogue with R = $NH(CH_2)_3Ph$ elicited an unexpected stimulation of glucagon secretion. Analogues containing further modifications to the Merck group's cyclic analogue have been investigated[43] by insertion of (aminomethyl)phenylacetic acid (AMPA) spacer groups as mimics for a Gly-Gly dipeptide unit. The analogue cyclo(Phe-D-Trp-Lys-Thr-ortho-AMPA), showed no growth hormone inhibition, and a 2D n.m.r. study revealed different conformations from the proposed bioactive one. This is believed to be due to the involvement of the ortho-AMPA NH bond. Replacement of this bridging unit by its meta and para-AMPA analogues revealed that the conformation of the meta-form had some analogy with the proposed bioactive form, but both analogues showed no inhibition potency in vitro. A cyclic analogue containing the retro-sequence of the 7-11 amino acids in somatostatin, cyclo-(Phe^{11}-Thr^{10}-Lys^9-Trp^8-Phe^7-D-Pro^6) using the D-Pro unit as bridge has now been synthesised[44] in a photoreactive form cyclo-[Phe(pNH(1-C^{14})Ac)-Thr-Lys($COC_6H_4pN_3$)-Trp-Phe-D-Pro] suitable for photoaffinity labelling. This shows high activity for cytoprotection of rat hepatocytes against cell poisons such as phallotoxins and galactosamine.

3.2 Enkephalins. - Conformational features of the previously reported cyclic pencillamine-containing enkephalin analogues of general formula (5) have been derived from n.m.r. studies[45] on [D-Pen^2, L-Cys^5]-, [D-Pen^2, D-Cys^5]-, [D-Cys^2, L-Pen^5]- [D-Cys^2, D-Pen^5]-, [D-Pen^2, L-Pen^5]- [D-Cys^2, D-Pen^5]-, [D-Pen^2, L-Pen^5]- and [D-Pen^2, D-Pen^5]-enkephalins. Main deductions were, (a) orientation of the Tyr^1 residue relative to the cyclic portion is fairly fixed, (b) D-Pen^2 -vs- Cys^2 substitutions have little effect on the conformation, (c) similar substitutions to (b) at position 5 do alter the conformation and (d) exchange of D-Pen^5 for L-Pen^5 alters the conformation as well. It is suggested that the gem-dimethyls of D-Pen^2 in [D-Pen^2, Pen^5]-enkephalin may cause

an unfavourable steric effect at both μ and δ-receptors. Ten analogues of the highly μ-receptor selective cyclic opioid peptide (6) have been prepared[46] by solid phase techniques. The mode of receptor binding of (6) is identical with that of dermorphin but differs from that of the structurally related β-casmorphins. The side-chain length of the aromatic residue at position 3(Phe) in (6) was found to be critical for receptor affinity and selectivity. Reducing the size of the ring [analogue (7)] with the introduction of 2,4-diaminobutyric acid (A_2bu) increased μ-receptor selectivity, whereas the more flexible cystine-containing analogue (8) was less selective in its receptor binding. Bridging via an 'external' reagent 1,3-dichloro-1,1,3,3-tetra-isopropyldisiloxane across the two seryl residues of [Ser^2, Ser^5]-enkephalin has given[47] the cyclic analogues (9). The analogues showed δ-receptor activity and n.m.r. studies support the presence of a 2→5 H-bonded β-turn conformation for the most biologically potent of the compounds. An even 'tighter' conformational restriction is present in the heterocyclic analogue (10) synthesised[48] as a potential Leu-enkephalin analogue.

Comparison of conformational properties of linear and cyclic δ-selective opioid ligands has been made[49] to determine the structural characteristics which could be responsible for their differential δ-selectivity. H-Tyr-D-Thr-Gly-Phe-Leu-ThrOH(DTLET) and H-Tyr-cyclo-(D-Pen-Gly-Phe-Pen) (DPLPE) were chosen for the n.m.r. spectroscopic comparison at 400 MHz in d_6DMSO. Similar backbone folding tendencies were discovered for both linear and cyclic analogues, so the hypothesis is introduced that the enhanced δ-selectivity of DPLPE is related to a very large conformational expense of energy needed to interact with the receptor, a feature not encountered in the case of DTLET.

3.3 Other Cyclic Analogues. - Five cyclic analogues of substance P, H-Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH_2 using the disulfide link as the conformational restrictor[50] have been prepared on a polymer matrix. Procedure is based on the attack of a 3-nitro-2-pyridine sulfenyl group on one cysteine residue by the thiol function of another cysteine residue. The yields of cyclic product were: [Hcy^5, Hcy^{11}]-SP (14%), [Cys^5,



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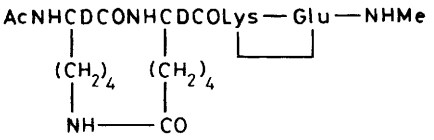

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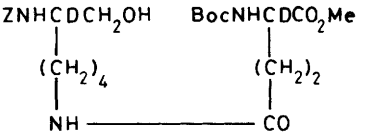

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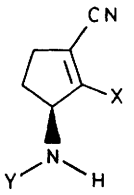

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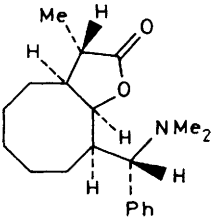
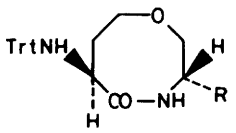
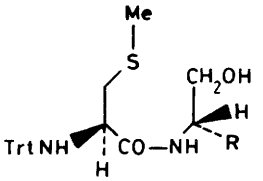


(20)

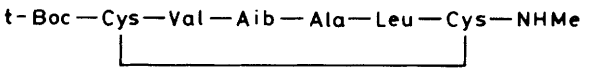
Cys^{9}]-SP(17%), [D-Cys^{5}, Cys^{8}]-SP(3%), [D-Cys^{4}, Cys^{7}]-SP(6%), [D-Cys^{3}, Cys^{6}]-SP(10%). As part of a search for new reagents useful for the treatment of sickle-cell anaemia, cyclic tetrapeptide analogues (11) and (12) have been synthesised[51] as mimics of the mutation site of haemoglobin S. Only analogue (11)(X = Val) showed any antigelling activity, albeit low. Cyclic analogues of the cholecystokinin sequence CCK (26-33) have been synthesised[52] and their biological properties investigated. Replacement of the crucial Gly^{29} residue in CCK(26-33) by a D-Lys residue in Boc-[$Nle^{28,31}$]-CCK(27-33) gave a derivative as active as CCK(26-33). When a similar linear derivative of CCK(27-33) was cyclised through amide bond formation between side-chains of Asp^{26} and D-Lys^{29} to give analogue (13) the potency to stimulate secretion of amylase from rat pancreas was 80 times lower than that of CCK(27-33). The analogue (13) acted as a weak antagonist of the CCK(27-33)-induced contractions of guinea pig ileum. The internuclear distances of the cyclic thymopoietin analogue cyclo-[D-Val-Tyr-Arg-Lys-Glu] have been determined[53] using 2D-nmr, n.O.e. measurements and a Molecular Dynamics simulation. Refinement of conformations by the Molecular Dynamics method can overcome the ambiguities which may arise from the inherent limitations of the n.m.r. techniques. The main difference between the calculated conformation and the previously postulated one obtained by model building based on n.m.r. data, is the bending of the ring between the α- and β-turn.

Lactam bridged peptides typified by compounds (14) and (15) have been synthesised[54] via active ester activation and high dilution conditions. Energy minimisation calculations, c.d. and n.m.r. studies showed that (14) and (15) exhibited characteristics associated with β-turns. An isotopically-labelled form (16) of these dilactam peptides has also been synthesised[55] starting from $H_2^{15}N(CH_2)_4CD(NHZ)CH_2OH$ and Boc[α^{2}H]-Glu(OSu)OMe to give (17) as a key intermediate. There is a continuing interest in devising non-peptidic mimics of the β-turn in order to produce more rigid equivalents. The β-enaminonitriles as amide carbonyl replacements have been studied[56] through the incorporation of 1,2-diamino-3-cyanocyclopentene (Mcc)(18) residue into cyclo-(Pro-Gly-Pro-DL-Mcc-Gly). The Mcc group seemed compatible with standard peptide synthesis conditions except for complete racemisation in





(21) R = Me, CH_2CHMe_2 or $CHMe_2$ (22) R = Me, CH_2CHMe_2 or $CHMe_2$



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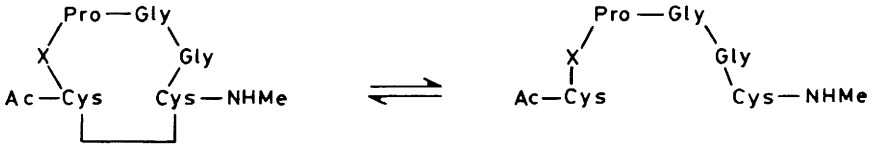
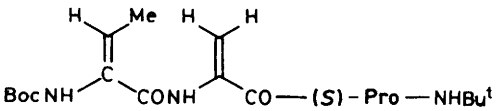


(29) X = Gly, Lys, Phe or Val



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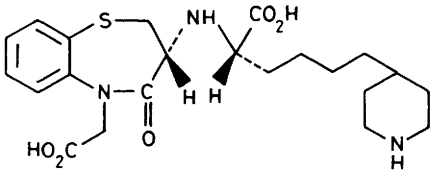


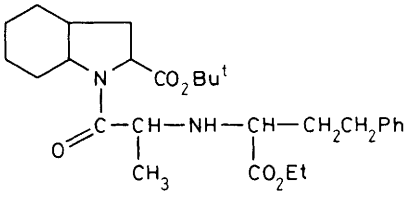
(31)

trifluoroacetic acid. This disadvantage was overcome by incorporating the unit (19) which did not racemise but suffers from a slight lability to acid in the enamine function. An interesting β-turn mimetic has also been developed[57] through the stereoscopic synthesis of model compound (20). Conformational restraint in a peptide sequence might be a possibility if the cyclic ether (21) was incorporated. This optically active unit has been prepared[58] by the displacement cyclisation of linear dipeptides (22).

N.m.r. studies at 270 MHz have been carried out[59] on (23) which is a model for disulfide loops of limited ring size. Data provide support for an intramolecular antiparallel β-sheet conformation with a chain reversal at the Aib/Ala segment with a type I′ β-turn. The chemical equilibrium (29) between oxidised (cyclic) and reduced (acyclic) forms of a series of hexapeptides have been examined[60] in aq. solution at pH 8.0. Conformational free energies have been determined, and reinforce a previous hypothesis by the authors that short range interactions dominate the tendency of a peptide chain to fold back on itself.

4. Dehydroamino Acid Analogues.

Rather disappointing biological activities[61] resulted from synthesising a series of substance P 6-11 fragment analogues, H-Gln-Phe-Phe-Sar-Leu-Met-NH_2 where the two phenylalanine residues were in turn replaced by Δ-Phe, and some substitutions made in position 9. In examples where Δ^Z-Phe has replaced D- and L-Phe in a number of β-turn sites, n.m.r. studies[62] have revealed the Δ^Z-Phe residues could adopt similar conformations in all cases to their saturated analogues. In linear peptides such as t-Boc-Pro-X-NHMe(X=D-Phe, Phe or Δ^Z-Phe), the Δ^Z-Phe did have a rigidifying influence so that a stronger preference for a turn conformation was exhibited. Δ^Z-Phe is a good conformationally homologous replacement for D-Phe in reverse turn regions in cyclic peptides. Similar deductions have been made from an X-ray crystallographic study[63] of t-Boc-L-Phe-ΔPhe-L-Val-OMe. In the asymmetric hydrogenation of the unsaturated tripeptide (30), Raney nickel proved to be the most effective catalyst giving, after acid hydrolysis (R)-alanine and (S)-butyrine in 94 and 54% asymmetric



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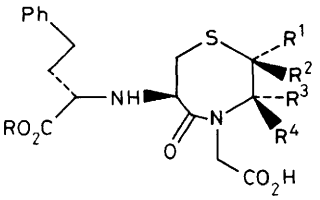
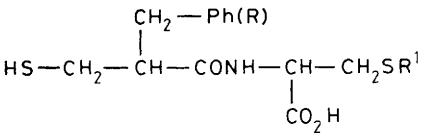
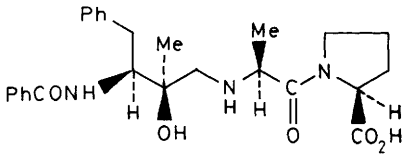


(33) R^1—R^4 = Ph, 2-thienyl or 3-thienyl



(34) R = H or OMe
R^1 = Me or Et



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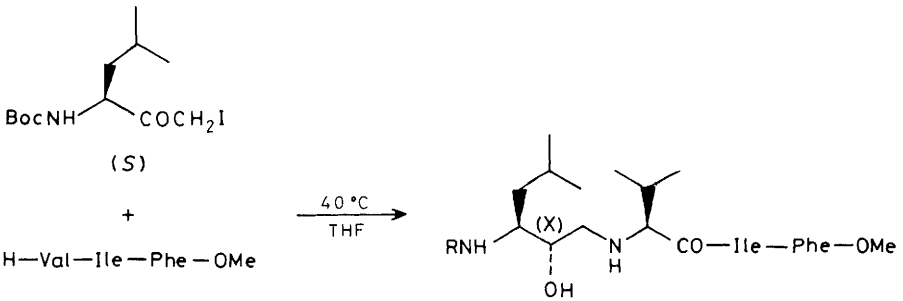


(36) R = Boc, X = *RS*
(37) R = H, X = *R*
(38) R = H, X = *S*

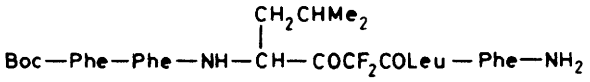
Scheme 4

yields respectively[64]. Numerous examples of the N-carboxy-α-dehydroamino acid anhydride method[65] for synthesis of dehydro-oligopeptides have been recorded, including an example of its use in the solid phase context[66].

5. Enzyme Inhibitors.

This field continues to be an area of intensive activity, especially as templates for designing possible medicinal products of the future. A wealth of data (over 400 references) on all enzymes which have been subjected to Quantitative Structure-Activity Relationship (Q.S.A.R.) studies has been gathered together in a comprehensive review[67].

5.1 Angiotension Converting Enzyme (A.C.E.) Inhibitors. - The A.C.E. inhibitor (31) (CV-5975) can now be synthesised using an improved method[68] which involves a kinetic resolution of a racemic intermediate [ethyl(RS)-6-(1-benzyloxycarbonyl-4-piperidyl)-2-hydroxyhexanoate]. Octahydroindole-2-carboxylic acid, octahydroisoindole-1-carboxylic acid and octahydro-3-oxoisoindole-1-carboxylic acid can replace proline in both sulfhydryl and non-sulfhydryl A.C.E. inhibitors giving compounds[69] equipotent to captopril and enalapril. Compound (32)(Cl-907)-indolapril in this bicyclic series has advanced to clinical evaluation. The effect of introducing hydrophobic substituents into the 2- and 3-positions of the thiazepinone ring has been monitored through the synthesis[70] of A.C.E. inhibitor analogues with many permutations of substituents in the ring as represented in (33). Pseudoequatorial substituents at positions 2 and 3 had potent *in vitro* inhibiting activity. Hypotensive effects almost equal to that of captopril in anaesthetised rats have been obtained[71] from sulfur-containing N-mercapto alkanoyl amino acids such as (34). A synthetic methodology has been worked out[72] for a series of tripeptide amino alcohols such as (35) as potential inhibitors of A.C.E. The rationale used was to replace the scissile amide bond in acyl tripeptide substrates with the $-CH(OH)(CH_3)CH_2NH-$ moiety. Angiotensin I analogues with a phosphonic acid group replacing C-terminal carboxyl groups have been shown[73] to be competitive inhibitors of A.C.E. When compared with captopril the peptide phosphonic acids showed similar K_i-pH profiles despite their



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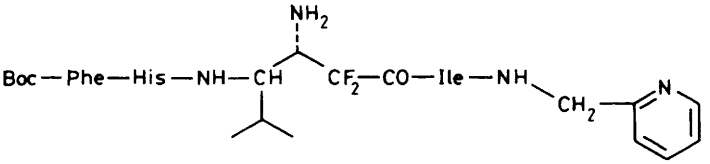

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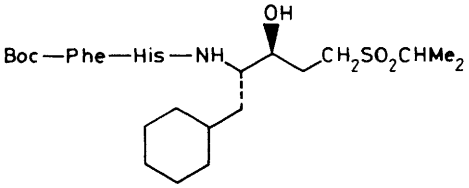

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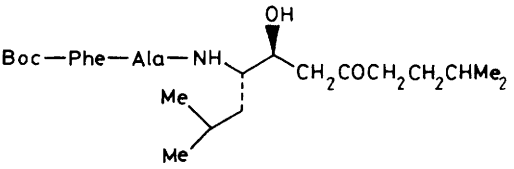

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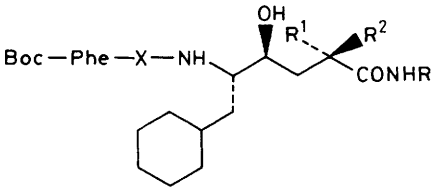
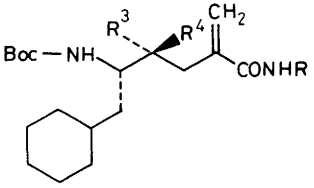
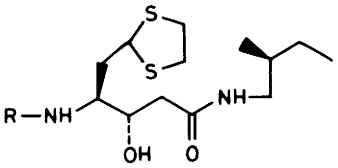


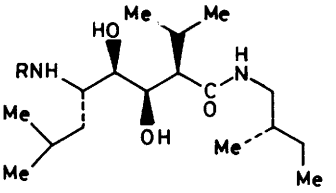
(43) $R^1R^2 = CH_2$



(44) R^3 = H, R^4 = OH, or R^3 = OH, R^4 = H



(45) R = Boc—Phe—His



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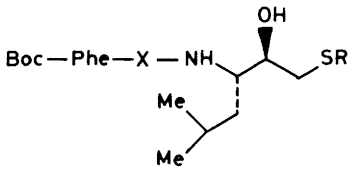
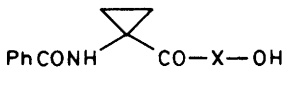


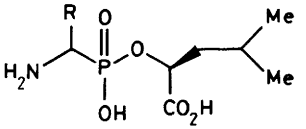
(47) X = His, Ala, Phe or Tyr

P5 P4 P3 P2 P1 P1′ P2′ P3′
—His—Pro—Phe—His—Leu—Leu—Val—Tyr—

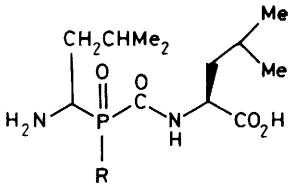
(48)



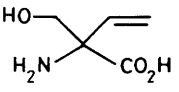
(51) X = Phe
(52) X = Pro



(53) R = CH_2CHMe_2 or CH_2Ph



(54) R = OH or NH_2



(55)

structural differences. Examples of the best inhibitors found in this class together with their K_i (μm) values were Z-His-Pro-Phe-His-Leu-PO(OH)$_2$ (0.74) and Z-Ile-His-Pro-Phe-His-Leu-PO(OH)$_2$ (0.54).

5.2 Renin Inhibitors. - A report last year (Godfrey et.al.) on an amino alcohol isostere has prompted[74] disclosure of another efficient route (scheme 4) to inhibitors such as (37) and (38). Separation of the diastereoisomers was carried out on a silica column. Improved potency over statine and statone containing analogues have been reported[75] for the difluorostatone-containing analogue (39). Difluorostatine derivatives for insertion into this sequence were prepared in 5 steps starting with $Me_2CHCH_2CHNH(Boc)CHO$ with $BrCF_2CO_2Et$ in the presence of activated Zn. (3S,4S)- and (3R,4S)- Diastereoisomeric forms of (40) have been synthesised[76] and their potency as inhibitors is weaker than difluorostatine-containing peptides. Non-peptidic replacements at the scissile bond have been used[77] in a series of analogues such as (41) and (42). Analogue (41) exhibited an IC_{50} of 7.6nM against human renin and showed high specificity for this enzyme. α,α,-Disubstituted analogues such as (43) and didehydroanalogues such as (44) have also been the subject of structure-activity studies[78]. Starting from Z-Asp it has been possible to synthesise[79] novel statine analogues which have been incorporated into (45) which is a potent renin inhibitor. The dihydroxyethylene isostere (46), synthesised[80] from a dioxolane derivative derived from L-leucine, in terms of its renin inhibition potency seems to fit the hypothesis that it could be a transition state mimic of the enzyme catalysed hydrolysis. A marked selectivity towards renin inhibition has been shown[81] by analogues of general structure (47) even though the molecules contain no functionality beyond what is formally the Val^{11} side-chain of angiotensinogen. It is believed the activity is due in part to the 'transition-state' characteristics of the alcohol group, although R groups in (47) that resemble a valyl side-chain improve potency as well. Substitution of cyclohexylalanine for Leu^{10}, together with a cyclohexylsulfonate C-terminal unit, provided a 10-fold boost in potency for the final inhibitor devised from the angiotensinogen sequence[82]. Angiotensinogen (6-13) fragment analogues such as

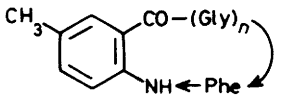
H-Pro-His-Pro-Phe-His-Phe-Phe-NH(CH_2)$_5$COOH, and H-Pro-His-Pro-D-Phe-His-D-Phe-D-Leu-Val-D-Phe-OH prepared by solid phase methodology[83] inhibited renin with IC_{50} values of 5.0×10^{-7}M and 3.6×10^{-7}M respectively. Further studies on the optimal binding aspects in a region of the renin enzyme that appears to be specific for spatial arrangements of aromatic groups reveal[84] that the metabolically labile Phe amide group can be exchanged with varied hydrophobic carboxy termini e.g. as in Boc-Phe-His-Sta-Leu-NHR where R can be CH_2Ph, (+)- and (-)-CH(Ph)CH_2Ph, and $CH_2C_6H_4Cl$(p), etc. On the basis of the minimal octapeptide sequence (48) of renin substrate a series of peptides based on statine (3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid, or (3S,4S)-4-amino-3-hydroxy-5-phenylpentanoic acid (AHPPA) at the P1P1′ positions have been synthesised[85]. In the biological potency studies the importance of the 3(S)-hydroxyl group has been demonstrated, while the AHPPA had several-fold less potency probably due to a steric conflict between the phenyl ring of AHPPA and the S1 subsite. Replacement of His at P2 by other basic residues also reduced potency, but in other work[86] when P2 His is replaced with other heterocycles in a series of angiotensinogen analogues containing statine analogues retro-inverted at the C-termini, maintenance or even enhancement of binding was found.

The conformational behaviour of pepstatin, Iva-Val-Val-Sta-Ala-Sta-OH, and of two derived renin inhibitors, Boc-Phe-Nle-Sta-Ala-Sta-OMe(49) and Boc-Phe-Nle-NHCH(iPr)CHOHCH$_2$CO-Ala-Sta-OMe(50) have been subjected to rigorous 400 MHz ^{1}H nmr analysis[87]. For pepstatin the solvated conformation resembles the structure found in the crystal of the pepstatin - Rhizopus chineus complex. There is a lack of large structural differences found in the conformations of (49) and (50), so it is suggested that the 100-fold lower inhibitory potency of (50) is mainly due to the unfavourable close contacts of the β-branched i-Pr in (50) with constituent amino acids of the enzyme when compared with the isobutyl group of statine in (49). Diastereoisomeric forms of Ac-Sta-NHMe have also been analysed[88] using X-ray techniques, i.r. absorption and ^{1}H n.m.r. Thirty four pepstatin analogues in which mainly position 2 was varied have been subjected[89] to Free-Wilson and correlation analysis. Crucial parameters were found to be n.m.r. chemical shifts of the α-carbon at position 2, the

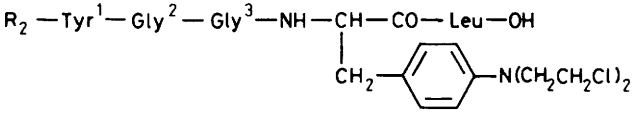
localised inductive effects and the van der Waals radius - related parameter. Out of the model comes a suggestion that the His-2 derivative should be a lead compound for further structure-activity studies. An efficient synthesis of statine via the diastereoselective epoxidation of cis-4-amino-allylic alcohol with m-chloroperbenzoic acid has been reported[90]. N-Protected statine and its analogues can also be synthesised[91] in high yield via chiral tetramic acid.

5.3 Other Inhibitors. - In an effort to study stronger interactions between substrate inhibitors and penicillinopepsin, statine derivatives containing basic side chains have been incorporated into the penicillinopepsin inhibitors. Statines with ornithinyl side chain (OrnSta) and lysinyl side chain (LySta) have been synthesised and incorporated to produce the inhibitors, Iva-Val-Val-[OrnSta]-OEt and Iva-Val-Val-[LySta]-OEt.[92] Both compounds were potent inhibitors of penicillinopepsin with K_i values 10-100 times smaller (2.1 and 1.1 nm) than the K_i of Iva-Val-Val-Sta-OEt(47nM). In contrast both are exceptionally weak inhibitors of porcine pepsin. Results have been correlated with the ability of the basic groups to bind to Asp-77 in penicillinopepsin. Cyclopropane-containing peptides (51) and (52) have been shown[93] to be irreversible inhibitors of carboxypeptidase A by a mechanism probably involving glutamate-270 of the enzyme as a nucleophile. Inactivation occurred approximately 2.3 times faster than hydrolysis in the case of (51), but while (52) is a potent inhibitor it showed no reactivity. Phosphorus amino acids and dipeptide analogues such as (53) and (54) have been evaluated[94] as inhibitors of leucine aminopeptidase (LAP) and shown to be very unexceptional inhibitors. The fact that these tetrahedral P analogues are less potent inhibitors of LAP than they are of other zinc peptidases suggests quite different mechanistic pathways.

In an attempt to design inhibitors of serine hydroxymethyl transferase, an enzyme which catalyses conversion of L-Ser to Gly, α-vinyl serine (55) has been synthesised[95], but showed very weak competitive inhibition. The-Gln-Val-Val-Ala-Gly- sequence which occurs frequently in several natural thiol proteinase inhibitors has been the template for other analogues to be considered as



(56) $n = 4$
(57) $n = 6$



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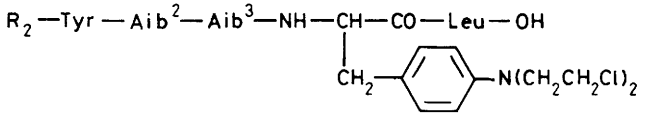

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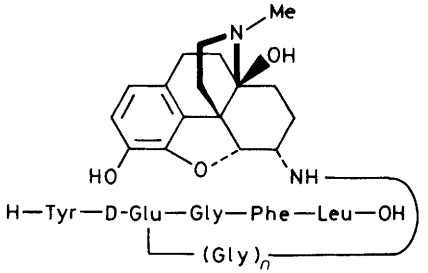
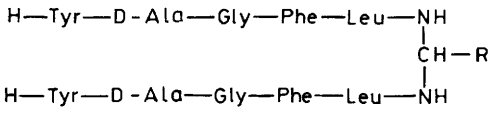


(60) $n = 0, 1,$ or 2



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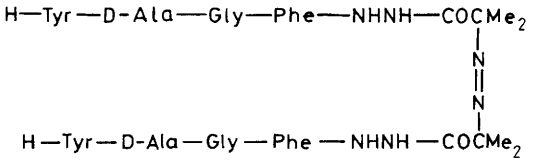


(62)

inhibitors. From the studies[96] it was concluded that Z-Gln-Val-Val-OMe was the smallest peptide to exhibit some effect on papain and was important for binding of the peptides to papain. Cyclic peptides (56) and (57) have been synthesised[97] in order to study their potential as 'suicide' substrates of α-chymotrypsin. There was enhancement in hydrolysis rate in going to the enlarged ring size of analogue (57), where the α-chymotrypsin specifically split the Phe-Methylanthranilic acid bond. It is suggested that if CH_3 in the anthranilic acid moiety was replaced by a functionalised $-CH_2X$ group it could be used in the design of 'suicide' substrates for serine proteases.

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

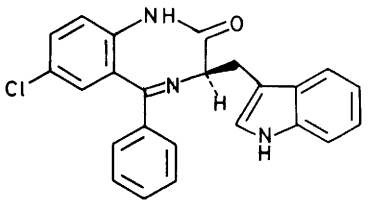
This particular section has generated fewer general themes this year whereby papers could be grouped together to represent a particular theme or development. The discussion therefore tends to be random but the perceptive reader should be able to identify some degree of order in areas such as opiate peptides, photoaffinity labels, peptide hormones and some cyclic peptides.

In an attempt to design irreversible δ-receptor antagonists a series of N,N-dialkylated leucine enkephalins have been synthesised[98]. In the sequence R_2Tyr-Gly-Gly-Phe-Leu-OH, R in turn was, allyl, cyclopropylmethyl, benzyl, n-propyl, n-pentyl, n-octyl and phenethyl. When tested in guinea pig ileum (gpi) and mouse vas deferens (mvd) at 1μM level all compounds except [N,N-di-2-phenethyl,Leu^5]-enkephalin showed antagonist activity against the δ-receptor agonist [$D\text{-}Ala^2$, $D\text{-}Leu^5$]-enkephalin, with the most potent being [N,N-dibenzyl, Leu^5]-enkephalin. Keeping the N,N-dialkyl substituents at the N-terminal, and substituting melphalan at position 4 as in (58) gave antagonists with lower activity[99]. At higher concentrations (10μM) the two active analogues (58, R=CH_2Ph) and the $Aib^{2,3}$-analogue (59, R=allyl) showed weak irreversible antagonism at the δ-receptor, with (59, R=allyl) showing antagonistic activity at the μ-receptor as well. Molecular modelling studies suggested that incorporation of modifed tyrosine residues into [$D\text{-}Ala^2$, Met^5]-enkephalin would give rise to μ-selective antagonists. Four analogues with Tyr

replaced in turn by m-Tyr, βCH_3m-Tyr, α,β-diCH_3m-Tyr, and N-phenethyl, m-Tyr, have been synthesised[100] and receptor binding studies (rat brain homogenates), analgesic agonist and antagonist activity (mouse tail flick test), and energy optimisation conformational studies carried out on them. Shift of tyrosine OH group to meta enhances the relative antagonist-v-agonist activity and addition of βCH_3 as well enhances μ-selectivity. Conformational studies indicate that all the analogues with high μ-receptor affinity have a common β-turn at the 2-3 positions. [βCH_3-m-Tyr, D-Ala^2, Met^5]-Enkephalin with nearly equal agonist/antagonist activity is a promising candidate for low physical addiction. Further examples of hybrid bivalent opiate/enkephalin pharmacophores such as (60) have been prepared[101] in an effort to investigate the co-existence of μ- and δ-recognition sites in the same opioid receptor complex. Only (60, n=0) had substantially greater antinociceptive potency in mice than its monovalent analogue H-Tyr-D-Glu-Gly-Phe-Leu-OH, or its oxymorphamine pharmacophore assessed independently. Binding data seem consistent with the simultaneous occupation of μ- and δ-sites by a single bivalent ligand (60, n=1), but they are also in harmony with interaction with an opioid receptor and an accessory binding site. Dimeric enkephalins[102] (61) with R = H, CH_2CHMe_2, CH_2Ph, CH_2COOH, $(CH_2)_3NHC(=NH)NH_2$, $CH_2CO_2CH_2Ph$, have been prepared. A key synthetic step is the conversion of Boc-Phe-Leu-$NHCH(R)CONH_2$ to Boc-Phe-Leu-NH-$CH(R)NH_2$ by treatment with [bis(trifluoroacetoxy)iodo]benzene. Analogue (61, R = CH_2CO_2H) showed good δ-receptor binding activity. Synthesis, conformational analysis and biological studies have been carried out[103] on poly-N-vinyl imidazole derivatives of (62), the N,N-bis-2-D-Ala, 5-des-Met-enkephalin hydrazide of azo-isobutyric acid. Compound (62) exceeded the initial tetrapeptide hydrazide in activity while modification by polyvinylimidazole leads to a decrease in activity *in vitro* and *in vivo*. Incorporation of polar amino acids[104] such as D-Arg or D-Glu into enkephalin analogues at position -2 has led to a sequence, Am-Tyr-D-Arg-Gly-EtPhe($4NO_2$)NH_2 (Am = $-C(=NH)NH_2$) which has high opiate activity and which is effective after oral administration. The DNA-intercalating agents ellipticine and 9-HO-ellipticine have been coupled[105] by quaternarisation of the pyridine nitrogen to the enkephalin

sequence H_2^+-Tyr-D-Ala-Gly-Phe-D-Leu- through a short -$(CH_2)_3$-NH- linker unit. These bifunctional molecules preserved high affinity for both DNA and opioid receptors. Two enkephalin O-glucopeptides, H-Tyr(β-D-Glc)-Gly-Gly-PheOH and H-Tyr(β-D-Glc)-Gly-Gly-Phe-Leu-OH have been synthesised[106] to determine the influence of the carbohydrate molecule on biological activity and conformation. In g.p.i. tests both analogues were inactive while in the m.v.d. test the second analogue was a full agonist but still more than a 1000 times less potent than [Leu^5]-enkephalin. The importance of having an unmodified Tyr moiety is thus confirmed once again.

The peripheral opioid activity in g.p.i, mouse, rabbit and rat vas deferens, has been determined[107] for β-endorphin (β-EP) homologues from six species. Good correlation was seen between binding potency and the charge value on the amino acid residues. Homologues with higher content of basic residues seemed to have a higher receptor binding activity. A series of twelve dermorphin tetrapeptides of the general fcrmula R-Tyr-D-Met(O)-Phe-X-R^1 have been studied[108]. H-Tyr-D-Met(O)-Phe-Gly-OMe was one of the most selective μ-receptor ligands reported to date, while compared to morphine H-Tyr-D-Met(O)-Phe-Sar-NH_2 and $NH_2C(=NH)$-Tyr-D-Met(O)-Phe-Sar-NH_2 showed lower affinity for μ, δ and κ-sites but stronger analgesia. Last year's discovery that synthetic dipeptide H-Lys-Trp(Nps)-OH exhibited a naloxone reversible antinociceptive effect comparable to that of an enkephalin analogue has led[109] to other analogues being synthesised for correlation of structure to activity. Peptides of general formula X-Trp(Nps)OR have been synthesised where X=Lys, Gly, Ala, Leu, Ser, Gln, His, Arg, Orn and Dpr (2,3-diaminopropionic acid). Analgesia was of the same order of magnitude when Lys is replaced by residues of similar or higher basicity. In the same series a series of sulfur substituents on the Trp residue have been investigated[110]. Only in H-Lys-Trp(S-C_6H_4-CO_2Me(o)) was a response similar to the original nitrophenyl analogue elicited. A new synthesis[111] of ($pGlu^5$, $MePhe^8$, Sar^9) substance P(5-11), using a polyacrylamide resin gave g.p.i. values of ED_{50} in agreement with previous values. Fmoc-Amino-acid active esters proved successful in synthesising substance P analogues with the



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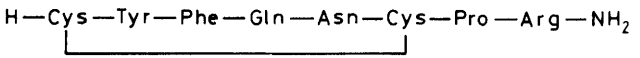

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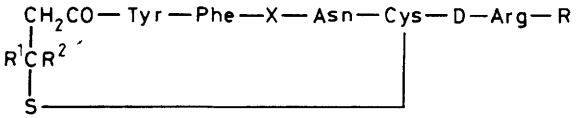
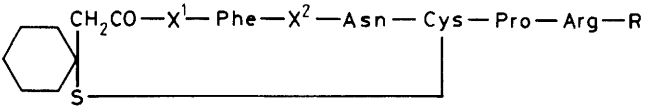
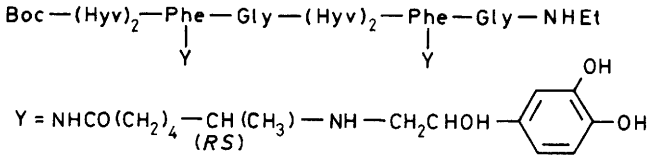


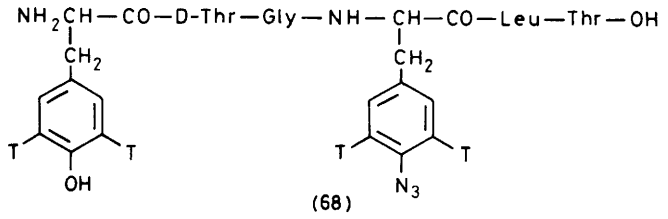
(65) R = NH_2, $R^1 = R^2 = H$, X = Gln, Val
$R^1 = R^2 = Me$, X = Val
$R^1R^2 = (CH_2)_5$, X = Val



(66) R = NH_2, X^1 = Tyr, Tyr (Me), X^2 = Gln
X^1 = D-Phe, D-Ile, X^2 = Ile
X^1 = D-Phe, D-Tyr (Et), Tyr (Et), X^2 = Val



(67) Y = $NHCO(CH_2)_4$—CH (CH_3) (*RS*) —NH—CH_2CHOH—



(68)

D-NH-CH(C_6H_4-4OH)-CO residues replacing Phe[7] and Phe[8] residues[112]. Analogues of [Orn[6]]-SP(6-11) have been synthesised[113] in which the SCH_3 group of the Met[11] side-chain is replaced by other functional groups. Substitution of Lys, Gln or Glu at position 11 all caused marked reductions in biological activity while a glutamate ester only had slightly reduced activity. Charged groups at position 11 are therefore not recommended for activity.

Earlier work has shown that the cholecystokinin sequence CCK(30-33) is the minimum fragment of CCK with nanomolar affinity for CNS receptors. Substitutions[114] in the middle dipeptide sequence of Boc-Trp-X-Phe-NH_2 (in Boc-CCK(30-33) X = Met-Asp) by X = Gly-Asp, Met-Gly, Gly-Gly and $(CH_2)_n$ (n = 0-4) tend to reduce affinity in the binding assay. In a comprehensive survey[115] of C-terminal fragments, peptides of cholecystokinin containing O-sulfated tyrosine and including CCK(27-32), CCK(27-31), CCK(27-30), CCK(27-29), CCK(26-27), CCK(25-27) together with their terminal amide analogues have been synthesised. Only in CCK(27-32) and CCK(25-27) was there significant biological response in the writhing test at a dose of 8-10 mg/Kg. Following up on a previous report of the design and synthesis of a new class of potent non-peptidal antagonists of CCK a detailed account[116] has appeared on the design, synthesis and structure-activity of benzodiazepine (63). Structure-activity relationships have been investigated[117] for both the N-terminal and C-terminal regions of the molluscan neuropeptide FMRF amide H-Phe-Met-Arg-Phe-NH_2. Hydrophobic or bulky groups substituted for Phe or Met contribute to the contractile effect. Side-chain lengths in the 2-position can influence the relaxing effect, while replacing the C-terminal Phe-NH_2 by D-Ala-cyclohexylamide gave rise to analogue with relaxing activity only. H-Met-Arg-Asp-dicyclohexylamide inhibited FMRF amide contraction selectively. Five neurokinin A (NKA) analogues substituted with Gly in positions 3,4,5,6 or 7 in H-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2 have been synthesised[118] using the Merrifield solid phase technique. Results with isolated g.p.i. and rat vas deferens suggest that Asp[4], Phe[6] and Val[7] may be essential for the intrinsic biological activity. Reduced opioid activity on g.p.i. has been recorded[119] for an analogue of dalargine, H-Tyr-D-Ala-Gly-Phe-Leu-Arg-OH where t-leucine residue replaces the leucine.

β-Alanine residues have been substituted[120] into positions 1, 5 and 8 to give three analogues of angiotensin II, H-Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7-Ile^8-OH. Only the [β-Ala^1]-angiotensin II exhibited antagonist activity. DL-Hexafluorovaline benzyl ester, DL-$(CF_3)_2CHCH(NH_2)CO_2Bzl$ has been used[121] for the substitution of hexafluorovaline into position 8 in [Sar^1,Leu^8]-angiotensin II. The analogue having the L-hexafluorovaline present was 20-100 times more active as angiotensin agonist or antagonist, than its D-analogue at the ng/mL dose range. At the microgram level both the D- and L- analogues were more effective than [Sar^1, Leu^8]-angiotensin II producing prolonged blockade of the pressor response toward angiotensin II. Introduction[122] of cyclopentylglycine (Cpg) and cyclohexylglycine(Chg) into the 5-position of the angiotensin II antagonist H-Sar^1-Arg^2-Val^3-Tyr^4-X^5-His-Pro-Lac-OH, has given rise to [Sar^1, Chg^5, Lac^8]-angiotensin II as the most potent antagonist in the series. [Aib^1, Leu^8]-Angiotensin II has a higher affinity than [$\underline{N}^4\underline{N}^4$-dipropyl-Asn^1,Leu^8]-angiotensin II, although both act as pure inhibitors. C.D. data[123] suggest conformational differences between the two inhibitors. Three analogues of arginine vasopressin (AVP) in which Pro at position 7 has been replaced[124] by stereochemically defined forms of 4-hydroxyproline(4HOPro) have been synthesised. [Trans-4HOPro^7]-AVP, the 1-desamino[trans-4HOPro^7]-AVP and the 1-desamino[cis-4HOPro^7]-AVP analogues showed a high antidiuretic and strikingly high uterine activity, and a better antidiuretic and uterine to pressor selectivity than AVP itself. Solid phase techniques continue to be fundamental to the search for potent vasopressin antagonists. Twenty four analogues of desGly-vasopressin with structures differing within the range (64)-(66) as well as their non-terminal amide analogues have been synthesised and tested[125] for agonist and antagonist potency. It appears from the results that a previously determined conclusion about the need for a terminal amide for V_2 agonism is no longer valid. Modifications in the C-terminal tripeptide tail of vasopressin antagonists such as (66) X^1 = D-Tyr(Et), X^2 = Val, the substitutions being carried out on the Pro-Arg tail in (66), have indicated[126] a difference in the way agonists and antagonists bind to vasopressin receptors and have highlighted the difference in structure-activity relationsips of agonists and antagonists. In

order to provide evidence for glucagon's role in diabetes, a search for a suitable glucagon-receptor antagonist has been made via the synthesis[127] of two new glucagon analogues [Asp^3, D-Phe^4, Ser^5, Lys^{17,18}, Glu^{21}]-glucagon and [D-Phe^4, Tyr^5, 3,5-I_2-Tyr^{10}, Arg^{12}, Lys^{17,18}, Glu]-glucagon. The former analogue when tested in normal rats lowered plasma glucose levels but did not do so in diabetic animals. [Tyr^{22}]-Glucagon and [desHis^1, Tyr^{22}]-glucagon have been synthesised using an improved solid phase procedure[128] on a Pam-resin. C.D. data showed that the Tyr^{22}-analogue contained increased β-sheet structure when compared to glucagon and it possessed 20-30% agonist activity in the rabbit blood glucose assay and 10% in the rat liver membrane adenylate cyclase assay. The analogue completely inhibited formation of c-AMP by natural glucagon with 50% inhibition at a ratio of 83.1 and pA_2=6.7. Data for the desHis^1-analogue agree with previous data that His^1 has a direct or indirect role in receptor binding. Reductive alkylation of the D-Lys side chain in position 6 and/or the L-Lys side-chains in position 8 of the LH-RH antagonist [N-Ac, D-Nal^1, D-Phe^{2,3} D-Arg^6, Phe^7, D-Ala^{10}]-LH-RH has been carried out[129] on resin bound peptides using aldehydes and ketones in the presence of $NaBH_3CN$. It is concluded from the biological results that the hydrophobocity of the Lys-derivative is of less importance than the flexibility of the alkyl group and thus its ability to shield the charged basic site in the moiety. Non-coded amino acids, neopentylglycine(Neo) and cycloleucine(Cle) have been inserted[130] into the position 8 of oxytocin H-Cys^1-Tyr^2-Ile^3-Gln^4-Asn^5-Cys^6-Pro^7-X^8-Gly^9-NH_2. Both analogues only had 15-30% of the uterotonic potency of oxytocin in in vitro tests, but the Cle^8-analogue had the same potency as oxytocin in vivo. Oxytocin analogues are among the series of peptides used to assess[131] a multivariate partial least squares method for studying QSAR for predicting the activity of new peptide analogues. Empirical energy calculations have also been applied to a series of TRH-analogues in order to incorporate the parameter into QSAR studies[132]. Results obtained suggest that modifications to the TRH structure can lead to greater potency in addition to improved stability.

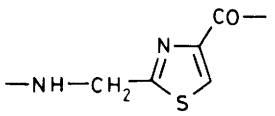
The contribution of each amino acid in the sequence to the biological activity of α-MSH, Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2 has been monitored through testing peptide fragments in the frogskin bioassay[133]. The central tetrapeptide Ac-His-Phe-Arg-Trp-NH_2 represents the minimum chain length for observable biological activity. Met^4, Gly^{10} and Pro^{12} are important potentiating amino acid residues, while Ser^1 and 3, Tyr^2, Glu^5 Lys^{11} and Val^{13} only make minimal contributions. Melanin-concentrating hormone (MCH) H-Asp-Thr-Met-Arg-Cys-Met-Val-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Glu-Val-OH, and analogues have undergone[134] *in vitro* bioassay studies using fish scales and a radioimmunoassay using a MCH specific antiserum. MCH(1-14) and Nps-Trp^{17}-MCH were equipotent to MCH. Modification of Tyr and Arg residues caused loss of activity while the configuration of the S-S loop is essential for activity. The pigment-dispersing activity of the pigment dispersing hormone (β-PDH) in fiddler crab *Uca pugilator* has been compared[135] to a number of its tyrosyl analogues. [Nle^{15}, Tyr^{16}]-βPDH and [Nle^{15}]-β-PDH, 31- and 16-fold more potent respectively than β-PDH were the best, the former being a candidate for iodination experiments on the Tyr residue. Phospho-hexapeptides such as Ac-Leu-Arg-Arg-Ala-Ser[$P(O)(OH)_2$]-Leu-Gly-R where R was OMe, or NHMe have been obtained[136] by phosphorylation of the serine residue with $(PhO)_2P(O)Cl$, and have been shown to be substrates for *cyclic*-AMP-dependent protein kinase. ATP-γ-Peptide esters, e.g. Ac-Ala-Ser(ATP)-Leu-OMe and Ac-Arg-Ala-Ser(ATP)-OMe have been made from the phosphopeptides by condensation with ADP-imidazolate. Substrates of *cyclic*-AMP-dependent protein kinase have also been successfully synthesised[137] from a series of seryl peptides by phosphorylation. The adamantyloxycarbonyl group proved to be a successful and highly lipophilic protecting group for the Arg-Arg residues in the substrates and purification of the final phosphopeptides was achieved by preparative reversed phase ion-pair chromatography. Despite the close similarity between norleucine (Nle) and methionine (Met) side-chains, i.e. a CH_2 group replacing the thioether, Nle-containing peptides have a markedly different behaviour with phospholipids, e.g. HCO-Nle-Leu-Phe-OMe behaves as a hydrophobic peptide when mixed with dimyristoylophosphatidyl choline and lowers the enthalpy of the lipid phase transition[138].

Its analogue HCO-Met-Leu-Phe-OMe is much more rapidly extracted into the aqueous phase. The influence of tyrosyl residues on the ionophoric properties of gramicidin A which can form ion channels in lipid bilayers and membranes, has been investigated as a result of the synthesis of [Tyr(Bzl)^{9,11,13,15}] and [Tyr^{9,11,13,15}]-gramicidin A by solid phase methods[139]. Their c.d. spectra showed a strong dependency on solvent and concentration while the single channel conductance of the tyrosyl-gramicidin A analogue was slightly lower than the parent molecule. Catecholamine conjugates of peptides have been synthesised[140] as part of an investigation to determine the effect of peptide carrier size on β-adrenergic activity of the norepinephrine moieties. The conjugate link Y in (67) was via an amine attached to the benzene ring of Phe. Increasing the size of the peptide did not seem to enhance potency but it could still be feasible to retain potency with quite large peptide carriers. As part of a study to gain evidence for the neurotransmitting role of γ-aminobutyric acid (H-γ-Abu-OH) in the nervous system of vertebrates a number of dipeptides containing this amino acid at the C-terminal position have been synthesised[141] and all were shown to be cross-reactive; when the antiserum had been pre-incubated with a cross-reactive compound the staining intensity of γ-AbuOH-like immunoreactivity was decreased or even completely abolished. Antitumour activity of a series of proline peptides derivatised at the N-terminal Pro by $ClCH_2CH_2N(NO)CO_2NSu$ has been assessed[142] and the synthesis of four MIF analogues containing N-terminal (S)-5-thioxoprolyl residue has been achieved[143] via mixed anhydride coupling of the proline analogue to a C-terminal dipeptide. N^3(4-Methoxyfumaryl)-L-2,3-diaminopropanoic residues have been introduced[144] at the N-terminal and C-terminal positions in a series of dipeptides. These peptides inhibit the growth of a number of microorganisms especially the pathogenic fungus Candida albicans, as a result of their efficient transport via peptide permeases as drug delivery systems. Conversion of N-terminal amino acid residues to N-methylated derivatives has been carried out via a retro aza Diels-Alder reaction[145] and tetrazole peptide analogues have been made[146] from Z- or Pht-protected dipeptide esters using imidoyl chloride and imidoyl azide intermediates. Practitioners interested in using substituted phenylalanines and tryptophan in

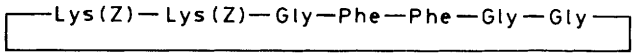
analogue studies will find useful data on preparation and derivatisation techniques for solid phase synthesis in a recent report[147].

Photo-labile, fluorescent and isotopically-labelled peptides continue to be useful probes for understanding the interactions and destination of peptides within their biological environment. In amphibia the antidiuretic pressor principle is arginine vasotocin. A photoreactive analogue 1-deamino [Lys^8(N^{ϵ}-4-azidobenzoyl)]-vasotocin has been prepared[148] by introducing the aryl azido group at the ϵ-amino group of Lys^8. After irradiation the osmotic water flow across the bladder wall was increased. [Arg^8]-Vasotocin (AVT) has been modified[149] by introducing Lys into position 7 which has then been acylated with suitable photoaffinity ligands e.g. 7-Lys(d-biotinyl)dAVT. 4-Azidophenyl-alanines as either D- or L- forms have been introduced to different positions[150] of luteinising hormone releasing hormone LH-RH and show promise for receptor labelling studies. A full report has now appeared on the novel fluorescent amino-acid, L-1-pyrenyl alanine (L-Pya) substituted[151] into positions 1, 4 or 5 of [$D\text{-}Ala^2$, Leu^5]-enkephalin. Mono Pya-enkephalins showed strong fluorescent intensities and potent binding affinities specific for opiate receptors. However, di-Pya analogues showed decreased receptor binding, but in $Pya^{1,4}$- and $Pya^{1,5}$-enkephalins the pyrenyl groups seem to be conformationally close to each other since they showed intramolecular excimer spectra. Thymopentin, H-Arg-Lys-Asp-Val-Tyr-OH, a pentapeptide representing the 32-36 residue sequence in thymopoietin has been derivatised[152] by fluorescein isothiocyanate and stilbene isothiocyanate at the N-terminal positions to give fluorescent probes. An irreversible photoaffinity probe for opioid δ-receptors, tritiated Tyr-D-Thr-Gly-Phe(pN_3) (68) has been synthesised[153] with a specific activity of 50 Ci/mmol. Catalytic transfer hydrogenation techniques have been applied[154] to the introduction of deuterium labels using $ND_4^+DCOO^-$ as the transfer reagent. [$D\text{-}Ala^2$, $pClPhe^4$]-Leu enkephalin in 80% $CDCOOD/D_2O$ yielded [$D\text{-}Ala^2$, $Phe(4\text{-}D)^4$]-Leu enkephalin with the level of D-incorporation monitored by FAB-MS techniques.

Mainstream discussion of cyclic peptides is the preserve of another Chapter in this book, but it is worth noting that the



(69)



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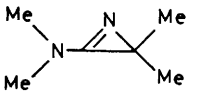

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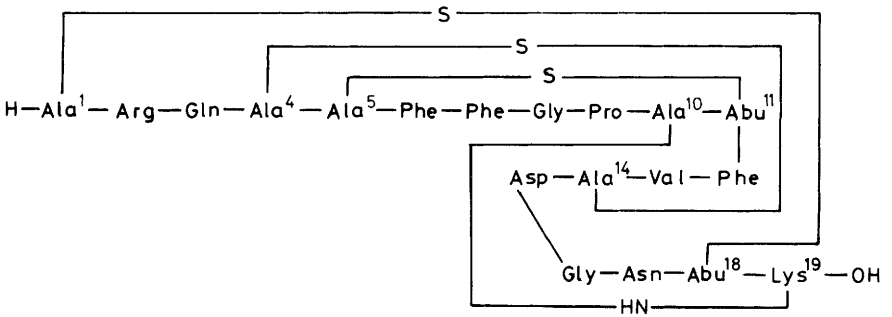
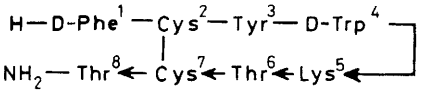


(72) Lanthionine Ala^4/Ala^{14}
β-Me-lanthionine Ala^1/Abu^{18}
Lysinoalanine Lys^{19}/Ala^{10}



(73)

cyclic peptides are also popular candidates for sequence substitutions. Thus in HC-toxin, cyclo-(L-Ala-D-Ala-L-Aoe-D-Pro) the sequence has been substituted[155] at the Aoe (2S,9S-2-amino-8-oxo 9,10-epoxydecanoic acid) position by Ada (2S-2-amino-9-decenoic acid). The cyclic tetrapeptide is only obtained with D-Pro in the C-terminal position. The epoxyketone side-chain of HC-Toxin has been replaced by chloromethyl ketone and diazomethyl-ketone functions[156], the former giving the most potent synthetic antimitogenic cyclic tetrapeptide analogue yet designed. L-Iodophenylalanine and L-tyrosine replacements of $L\text{-}Ala^3$ in HC-Toxin have also been reported[157]. A combination of synthesis of isomers and analogues, and n.m.r. studies have led to a new structure being proposed[158] for dolastatin 3. The new structure is cyclo-[Pro-Leu-Val-gly(Thz)-gly(The)] where glyThz is represented in (69). Modifications[159] have been made either in position 1 or 2 of the immunosuppressive fungal metabolite cyclosporin A, cyclo-[(Me)Bmt^1-Abu^2-Sar-MeLeu-Val-MeLeu-Ala-D-Ala-MeLeu-MeLeu-MeVal]. Substitutions included, [$MeThr^1$]-, Hyp^1 [Hyp^1, Nle^2]-, [$MeSer^1$]-, [$MeSer^1$,Nle^2]-, [$MeSer^1$]- [$MeSer^1$,Nle^2]-[$MeSer^1$,Nva^2]-, [$MeThr^1$,Nle^2]-, [$MeThr^1$,Nva^2]-, [MeSer,Thr^2]- and [Dab^1]-. The most reliable cyclisation yield was obtained using $[BtOP(NMe_2)_3^+PF_6^-]$. Six analogue thioether tripeptides containing L- and D-Ala and L- and D-cysteine have been synthesised[160] to study the helicity of the tryptathionine moiety of the phallotoxins. Synthesis of analogues containing the 6-hydroxytryptophan residue in amaninamide from Amanita virosa have proved difficult so analogues with the simple tryptophan residue have been synthesised[161]. The need for the OH group of hydroxyproline, and isoleucine residues to be present for binding to the enzyme RNA polymerase has been deduced from these analogue studies. An ionophoric model (70) which can mediate the transport of PheOMe.HCl through organic liquid membrane has been synthesised[162] using a soluble carbodiimide as the key ring closing reagent. Reactions of carboxylic acids with the azirine (71) followed by selective amide hydrolysis gives peptides[163] with repeating Aib residues, while cyclisation of $HOCH(Ph)CO(NHCMe_2CO)_nNMe_2$ (n = 2, 3, 4) with HCl at 100° gave cyclic depsipeptides. Analogues of AM-Toxin II e.g. [$L\text{-}Phe^3$-$L\text{-}Ser(Bzl)^4$]-AM Toxin II have been used as models[164] for

synthesising the cyclotetradepsipeptide by cyclisation through an ester bond formation.

7. Conformational Information derived from Physical Methods.

Technology continues to advance in the field of n.m.r. techniques and the amount of conformational and sequential details deduced improves significantly from year to year. This year again it has to receive pole listing in terms of the relative number of publications under this section.

7.1 Nuclear Magnetic Resonance Techniques. - The physicochemical environment inside opioid receptors has been simulated using a crown ether complex of H-Tyr-D-Ala-Phe-Gly-NH_2 the N-tetrapeptide fragment of dermorphin, and of other analogues. In this 500MHz study[165] the deduced conformations possess most of the features previously proposed for μ-agonists and are consistent with μ-receptor models deduced from rigid opiates. The considerable contributions of Bystrov and co-workers to the n.m.r. characteristics of gramicidin A has been the subject of a 32-reference review[166]. The unambiguous assignment of the individual protons in Z-His-Trp-Ser-TyrOH a component of LH-RH has been made[167] using 2D-n.m.r. techniques. A random coil backbone conformation is inferred from the data. Complete sequence determination and location of cyclic bridges in the nonadecapeptide $R_0$09-0198 (72) has been established[168] totally by homonuclear 2D-n.m.r. spectroscopy with the special application of RELAYED-NOESY techniques. The amino acid sequence shows a surprising analogy to the A.C.E. inhibitor ancovencin. Cyclic oligopeptides have proved to be useful models to test the application of heteronuclear coupling constants (e.g. $H^{\beta}C'$ couplings and $^{3}J(H^{\beta}{}^{15}N)$ constants) for determining side chain conformations[169]. Even higher sensitivity than the conventional COLOC treatment has been possible[170] using inverse heteronuclear shift correlation via long-range coupling to the carbonyl as witnessed in a study of cyclo(Phe-Thr-Ala-Trp-Phe-D-Pro). Substance P fragment Arg-Pro-Lys-Pro has been studied[171] by natural abundance ^{13}C-n.m.r. and 2D $^{13}C/^{1}H$ correlation techniques. Both isomeric forms (cis and trans about the Lys^3-Pro^4 bond) can

be assigned and the cis-form seems to be stabilised by a charge interaction between the Lys^3 side-chain amino group and the deprotonated C-terminal carboxyl group. High field n.m.r. studies[172] have confirmed that the cyclic analogue of somatostatin, (analogue 73), exists in water as a conformational equilibrium between two γ-turns involving residues 2, 3, and 4 and residues 3, 4 and 5 respectively. Many of the previous deductions made on the solution conformation of reduced glutathione (γ-Glu-Cys-Gly-OH) have been called into question by a new 400 MHz ^1H and ^{13}C-study[173] at various pH values. The tripeptide in water was shown to interconvert rapidly between an array of conformers and the carbon backbone was more rigid than anticipated, due to interaction between the α-amino and α-carboxyl Glu groups and the γ-Glu-Cys peptide bond CO and NH groups. The theoretical predictions (Calvin et al.) of the conformation are borne out by the present work. The introduction of a third frequency dimension as an extension of 2D-n.m.r. has provided evidence[174] of important advantages in the unravelling of the more severe overlap among cross peaks as exemplified by spectra derived from pyroGlu-His-Trp-Ser-Tyr-D-Ser-Leu-Arg-Pro-NHEt. The 17-membered ring in Boc-Cys-Ala-Aib-Gly-Cys-NHMe has been the subject of an n.m.r. study[175] which supports an intramolecular antiparallel β-sheet conformation at these residues nucleated by a γ-turn centred at the Aib residue. Interactions between a side-chain aromatic ring and a succeeding peptide bond in a sequence have been revealed[176] in a study of Boc-Phe-MeAla-OMe and Z-Ala(γ-thia)-Pip-OMe. Trans-Rotamers on the central -CON(R)- bond are stabilised in the D-L sequence while cis-forms prevail in the L-L-form. Cis/trans-Conformational equilibria has been shown[177] to exist around the benzoyl amide in Bz-Leu-His-OMe, and all the evidence (n.m.r. and c.d. data) indicates[178] that the conformation of Ac-Pro-Gln-Pro-Pro-Gln-NH_2 (a type I collagen α-1 chain C-telopeptide) is non-random and rigid and might form a stable nucleus around which the rest of the telopeptide could fold. Evidence from the solid state (X-ray crystallography), n.m.r. and conformational energy calculations has been brought to bear[179] on the conformational properties of 2,4-methanoproline in peptides, with n.m.r. evidence identifying 2,4-methanopyrrolidine asymmetry in Ac-L-Tyr-2,4MePro-NHMe. Methodology for analysing solid state n.m.r. measurements

of peptides has been presented[180], which involves using the computer programme LINK to bring together orientations from two peptide planes and determine the backbone torsion angles. Differences observed in the chemical shift tensors have been shown[181] to be the result of particular lattice-dependent interactions present in the solid form of a series of peptides, N-Ac[1-^{13}C]-Gly-X-NH_2 where X was [^{15}N-Gly], DL-[^{15}N]-Tyr, L-[^{15}N]-Phe and DL-[^{15}N]-Ala.

7.2 X-Ray and Related Techniques. -Crystal and molecular structures of several small peptides, such as glutathione[182] (γ-L-Glu-L-Cys-Gly-OH), aspartame hydrochloride[183], α-L-Asp-L-Ala-OH and β-L-Asp-L-Ala-OH[184]. The cyclic peptide[185] cyclo(L-Asp-L-Ala) is also part of this series of X-ray crystallographic studies. Similar studies on cyclo-[Gly-Pro-D-Phe-Gly-L-Val) have revealed[186] not only the familiar type II β-turn but a chain reversal the authors define as a helical H_R-turn which is right-handed in the cyclic peptide studied. The crystal structure[187] of [Phe^4,Val^6]-antamanide pentahydrate was found to be similar to the other polymorphs of the cyclopeptide confirming that it is only complexation with Li^+ and Na^+ which gives rise to significant conformational changes. X-ray data on H-L-Tyr-L-Phe-OH[188] and H-Gly-Gly-Phe-OH HCl[189] confirm an all trans amide bond arrangement with one bond in the latter peptide showing significant deviations from planarity.

7.3 Circular Dichroism and Infra-red Spectroscopic Studies. - Repeat peptides of elastin have been studied[190] by c.d. techniques and solid state i.r. spectroscopy. The C-terminal positions of the peptides were linked to polyethyleneglycol monomethyl ether via a photosensitive 3-nitro-4-bromomethylbenzoyl handle, and the spectra revealed significant β-turn structure up to the dodecapeptide sequences. Gradual attachment of side-chains to the ring, of a series of oxytocin analogues[191] has given rise to c.d. spectra which show evidence of substantial reduction in conformational mobility of the backbone, but the conformation of the disulfide group is independent of the peptide moiety. Differences in the c.d. spectra of a series of oxytocin analogues substituted at the 7-position have been interpreted[192] as being

due to the variations in the magnitude of angle Ø of the amino acid substituted at 7. Results from c.d. and Raman spectroscopy on the 14-membered disulfide models, Boc-Cys-Pro-X-Cys-NHMe with X = Gly, Ala, Aib, Leu or D-Ala, correlated well[193] with X-ray studies previously reported, and are consistent with β-turn conformations and a trans-gauche-gauche-arrangement about the C^{α}-C^{β}-S-S-C^{β}-C^{α} bonds. In order to clarify previous conflicting results on the conformation of dynorphin A, a laser-Raman spectroscopic study reveals[194] that dynorphin A(1-13) appears to have a mixture of extended β-pleated sheet and random structure. C.d. and i.r. measurements carried out in solvents of different polarity show[195] that the chemoattractants OHC-Met-Leu-Phe-OMe and analogues preferably adopt a folded 'active' conformation stabilised by a 3→1 H-bond forming a γ-turn.

7.4 Computational/Theoretical Methods. - Conformational energy computations on Ac-2,4-MePro-NHMe and the dipeptides Ac-2,4-MePro-X-NHMe and Ac-X-2,4-MePro-NHMe where X = L-Ala or L-Tyr have shown[196] that, when compared to their prolyl analogues, there is a stronger preference for a partially folded conformation. Potential energy calculations[197] have aided the design of an LH-RH analogue containing an extension of Gly-Cys at the C-terminus and capable of adopting a conformation virtually identical to the type II' turn around Gly^{6}-Leu^{7} in the native LH-RH. Empirical force-field calculations have been used[198] to analyse the effect of crystal packing forces and of hydration on the conformation of enniatin B. Molecular mechanics approaches have been applied[199] to cyclo(D-Ala-Gly-Pro-D-Phe)$_2$ cyclo(Gly-Pro-D-Phe)$_2$ to reveal that the latter has more conformational freedom than the former while an analogue of the cyclooctapeptide containing L-Phe instead of the D-Phe was found to be less rigid. Cyclic tripeptides[200] and cyclohexapeptides[201] have been subjected to energy minimisation calculations. In the former four different conformations were found to be possible, (i) an all cis-form with very little non-planarity, (ii) an all cis-form with larger non-planarity, and (iii) two possible conformations of 2 cis- and 1-trans. All the conformations can accommodate both Sar and Pro residues. Conformation (i) and one of the forms in (iii) can accommodate only homo-isomeric sequences of Pro residues while the

other can accommodate hetero-isomeric Pro residues. All four conformations are known to occur in the solid state, but in the work on the cyclohexapeptides not all the conformational possibilities have been observed. The cyclic hexapeptides can be made up of all possible combinations of 4→1 H-bond types I, I′, II and II′ β-turns, and exclusive features of 3→1 H-bonded γ-turns were found to be possible in 3-fold and S_6-symmetric cyclic hexapeptides.

References

1. "Peptides 1986", Proc. of the 19th European Peptide Symposium, Porto Carras, Greece, ed. D. Theodoropoulos, Walter de Gruyter publ. Berlin, 1987.
2. A. J. Douglas, B. Walker, D. T. Elmore and R. F. Murphy, Biochem. Soc. Trans., 1987, 15, 927.
3. M. Kruszynski, Pol. J. Chem., 1986, 60, 95.
4. D. W.Brown, M. M. Campbell, M. S. Chambers and C. V. Walker, Tetrahedron Lett., 1987, 28, 2171.
5. T. F. M. La Cour, Int. J. Pept. Protein Res., 1987, 30, 564.
6. M. Goodman, J. Coddington, D. F. Mierke and W. D. Fuller, J. Am. Chem. Soc., 1987, 109, 4712.
7. M. Rodriguez, P. Dubreuil, J-P. Bali and J. Martinez, J. Med. Chem., 1987, 30, 758.
8. T. Tancredi, A. Motta, P. A. Termussi, M. Goodman and P. Lucietto, Bull. Magn. Res., 1986, 8, 214.
9. H. Ogawa, G. T. Burke, J. D. Chanley and P. G. Katsoyannis, Int. J. Pept. Protein Res., 1987, 30, 460.
10. Y. Sasaki, W. A. Murphy, M. L. Heiman, V. A. Lance and D. H. Coy, J. Med. Chem., 1987, 30, 1162.
11. A. Aumelas, M. Rodriguez, A. Heitz, B. Castro and J. Martinez, Int. J. Pept. Protein Res., 1987, 30, 596.
12. M. Rodriguez, M-F. Lignon, M-C. Galas, P. Fulcrand, C. Mendre, A. Aumelas, J. Laur and J. Martinez, J. Med. Chem., 1987, 30, 1366.
13. H. Oyamada, and M. Ueki, Bull. Chem. Soc. Jap., 1987, 60, 267.
14. M. Lebl, E. E. Sugg, G. Van Binst, P. V. Elst, D. Tourwe, J. Slaninova and V. J. Hruby, Int. J. Pept. Protein Res., 1987, 30, 318.
15. A. Spaltenstein, P. A. Carpino, F. Miyake and P. B. Hopkins, J. Org. Chem., 1987, 52, 3759.
16. Y-K. Shue, G. M. Carrera Jr. and A. M. Nadzan, Tetrahedron Lett., 1987, 28, 3225.
17. R. E. TenBrink, J. Org. Chem., 1987, 52, 418.
18. M. Maeda, K. Kawasaki, J. Watanabe and H. Kaneto, Pept. Chem. (1986), 1987, 24, 201.
19. G. Grundke, W. Keese and M. Rimpler, Liebigs Ann. Chem., 1987, 729.
20. L. Nakonieczna and A. Chimiak, Synthesis, 1987, 418.
21. J. Viret, J. Gabard and A. Collet. Tetrahedron, 1987, 43, 891.
22. A. F. Spatola and J. V. Edwards, Biopolymers, 1986, 25 (Suppl), S.229; A. F. Spatola, C. Mapelli, J. Humphrey, J. V. Edwards, T. F. Burks and J. E. Shook, in Ref. 1, 1987, p.381.
23. M. Kondo, H. Uchida, H. Kodama, H. Kitajima and Y. Shimohigashi, Chem. Lett., 1987, 997.
24. G. Osapay, I. Szilágyi and J. Seres, Tetrahedron, 1987, 43, 2977.
25. B. Koehler and U. Schoellkopf. Liebigs Ann. Chem., 1987, 267.
26. K. Rolka, K. Kolasa, Z. Kleinrok, and G. Kupryszewski, Pol. J. Chem., 1986, 59, 1269.

27. S. Funakoshi, M. Kubota, N. Kosuga, J-P. Xu, H. Yajima, W. Li, S.Tamura, S. Kaneko, M. Satoh and H. Takagi, Chem. Pharm. Bull (Jpn.)., 1987, 35, 2561.
28. D. H. Coy, W. A. Murphy, V. A. Lance and M. L. Heiman, J. Med. Chem., 1987, 30, 219.
29. T. Hashimoto, Y. Uchida, K. Okimura and K. Kurosawa, Chem. Pharm. Bull. Jpn., 1987, 35, 3442.
30. Y. Uchida, K. Okimura, K. Kurosawa, N. Sakura, K. Hirose, T. Hashimoto, Bull. Chem. Soc. Jpn., 1987, 60, 1561.
31. T. Hasimoto, Y. Uchida, M. Nishijima, N. Sakura and K. Hirose, Bull. Chem. Soc. Jpn., 1987, 60, 1207; T. Hashimoto, N. Sakura, K. Hirose, Y. Uchida, M. Nishijima and T. Moro, ibid., 1987, 60, 827.
32. M. Narita, S. Honda and H. Umeyama, Bull. Chem. Soc. Jpn., 1987, 60, 4127.
33. C. F. Barbas III and C-H. Wong, J. Chem. Soc. Chem. Commun., 1987, 533.
34. A. L. Margolin, D-F. Tai and A. M. Klibanov, J. Am. Chem. Soc., 1987, 109, 7885.
35. M. Kondo, M. Kimura, K. Sato and H. Horimoto, Bull. Chem. Soc. Jpn., 1987, 60, 1391.
36. C. Mapelli, M. G. Newton, C. E. Ringold and C. H. Stammer, Int. J. Pept. Protein Res., 1987, 30, 498.
37. M. Suzuki, G. F. Orr and C. H. Stammer, Bioorg. Chem., 1987, 15, 43.
38. P. Wipf and H. Heimgartner, Helv. Chim. Acta., 1987, 70, 354.
39. J. R. Cann, R. E. London, C. J.Unkefer, R. J. Vavrek and J. M. Stewart, Int. J. Pept. Protein Res., 1987, 29, 486.
40. G. Valle, M. Crisma, F. Formaggio, C. Toniolo and G. Jung. Liebigs Ann. Chem., 1987, 1055.
41. S. F. Brady, R. M. Freidinger, W. J. Paleveda, C. D. Colton, C. F. Homnick, W. L. Whitter, P. Curley, R. F. Nutt and D. F. Veber, J. Org. Chem., 1987, 52, 764.
42. T. A. Lyle, R. M. Freidinger, R. F. Nutt, C. F. Homnick, R. Saperstein and D. F. Veber, Int. J. Pept. Protein Res., 1987, 29, 244.
43. P. Vander Elst, E. Van den Berg, H. Pepermans, L. Vander Auwera, R. Zeeuws, D. Tourwe and G. Van Binst. Int. J. Pept. Protein Res., 1987, 29, 318; P. Vander Elst, D. Gondol, C. Wynants, D. Tourwe and G. Van Binst, ibid, 1987, 29, 331.
44. H. Kessler, A. Haupt, M. Frimmer and K. Ziegler, Int. J. Pept. Protein Res., 1987, 29, 621.
45. H. I. Mosberg, Int. J. Pept. Protein Res., 1987, 29, 282.
46. P. W. Schiller, Th.M-D. Nguyen, L. A. Maziak, B. C. Wilkes and C. Lemieux, J. Med. Chem., 1987, 30, 2094.
47. J. S. Davies, E. J. Tremeer, R. C. Treadgold, J. Chem. Soc., Perkin Trans I. 1987, 1107.
48. A. A. Carr, M. W. Dudley, E. W. Huber, J. M. Kane and F. P. Miller, J. Het. Chem., 1987, 24, 239.
49. J. Belleney, B. P. Roques and M-C. Fournie-Zaluski, Int. J. Pept. Protein Res., 1987, 30, 356.
50. O. Ploux, G. Chassaing and A. Marquet, Int. J. Pept. Protein Res., 1987, 29, 162.
51. L. Sheh, M. Mokotoff and D. J. Abraham, Int. J. Pept. Protein Res., 1987, 29, 509.
52. B. Charpentier, Ch. Durieux, I. Menant and B. P. Roques, J. Med. Chem., 1987, 30, 962.
53. J. Lautz, H. Kessler, R. Boelens, R. Kaptein and W. F. Van Gunsteren, Int. J. Pept. Protein Res., 1987, 30, 404.
54. N. J. Manesis and M. Goodman, J. Org. Chem., 1987, 52, 5331; N. Manesis, M. Hassan, R. Glaser and M. Goodman, Biopolymers, 1986, 25(suppl.) S.97.
55. N. J. Manesis and M. Goodman, J. Org. Chem., 1987, 52, 5342.
56. D. S. Kemp and J. S. Carter, Tetrahedron Lett., 1987, 28, 4641; 4645.
57. M. Kahn and B. Chen, Tetrahedron Lett., 1987, 28, 1623.

58. K. Barlos, D. Papaioannou, S. Patrianakou, C. Sanida and Th. Tsegenidis, J. Chem. Soc., 1987, 474.
59. R. Kishore, S. Raghothama and P. Balaram, Biopolymers 1987, 26, 873.
60. P. J. Milburn, Y. Konishi, Y. C. Meinwald and H. A. Scheragga, J. Am. Chem. Soc., 1987, 109 4486.
61. T. Wasiak, A. Janecka and W. Koziolkiewicz, Pol. J. Chem., 1986, 60, 477.
62. A. C. Bach II and L. M. Gierasch, Biopolymers, 1986, 25(suppl.) S.175.
63. T. P. Singh, M. Haridas, V. S. Chauhan, A. Kumar and D. Viterbo, Biopolymers, 1987, 26, 819.
64. M. Takasaki and K. Harada, J. Chem. Soc. Chem. Commun., 1987, 571.
65. C. G. Shin, Y. Yonezawa and T. Obara, Heterocycles, 1986, 24, 1561; C. G. Shin, Y. Yonezawa and M. Ikeda, Bull. Chem. Soc. Jpn., 1986, 59, 3573; ibid., 1986, 59, 3696.
66. C. G. Shin, N. Takamatsu and Y. Yonezawa, Agric. Biol. Chem., 1986, 50, 797.
67. S. P. Gupta. Chem. Rev., 1987, 87, 1183.
68. M. Kori, K. Itoh and H. Sugihara, Chem. Pharm. Bull, Jpn., 1987, 35, 2319.
69. C. J. Blankley, J. S, Kaltenbronn, D. E. DeJohn, A. Werner, L. R. Bennett, G. Bobowski, U. Krolls, D. R. Johnson, W. M. Pearlman, M. L. Hoefle, A. D. Essenburg, D. M. Cohen and H. R. Kaplan, J. Med. Chem., 1987, 30, 992.
70. H. Yanagisawa, S. Ishihara, A. Ando, T. Kanozaki, S. Miyamoto, H. Koike, Y. Iijima, K. Oizumi, Y. Matsushita and T. Hata, J. Med. Chem., 1987, 30, 1984.
71. T. Komori, K. Asano, Y. Sasaki, H. Hanai, S. Morimoto and M. Hori, Chem. Pharm. Bull. Jpn., 1987, 35, 2388; T. Komori, K. Asano, Y. Sasaki, H. Hanai, R. Seo, S. Morimoto, M. Hori and M. Takaoka, ibid., 1987, 35, 2382.
72. J. D. Godfrey, E. M. Gordon, D. J. Von Langen, Tetrahedron Lett., 1987, 28, 1603.
73. A. Páldi, M. Móra, S. Bajusz and L. Gráf, Int. J. Pept. Protein Res., 1987, 29, 746.
74. R. J. Arrowsmith, D. E. Davies, Y. C. Fogden, C. J. Harris and C. Thompson, Tetrahedron Lett., 1987, 28, 5569.
75. K. Fearon, A. Spaltenstein, P. B. Hopkins and M. H. Gelb. J. Med. Chem., 1987, 30, 1617.
76. S. Thaisrivongs, H. J. Schostarez, D. T. Pals and S. R. Turner, J. Med. Chem., 1987, 30, 1837.
77. G. Bolis, A. K. L. Fung, J. Greer, H. D. Kleinert, P. A. Marcotte, T. J. Perun, J. J. Plattner and H. H. Stein, J. Med. Chem., 1987, 30, 1729.
78. D. J. Kempf, E. de Lara, H. H. Stein, J. Cohen and J. J. Plattner, J. Med. Chem., 1987, 30, 1978.
79. H. L. Sham, C. A. Rempel, H. Stein and J. Cohen, J. Chem. Soc. Chem. Commun., 1987, 683.
80. S. Thaisrivongs, D. T. Pals, L. T. Kroll, S. R. Turner and F-S. Han, J. Med. Chem., 1987, 30, 976.
81. J. R. Luly, N. Yi, J. Soderquist, H. Stein, J. Cohen, T. J. Perun and J. J. Plattner, J. Med. Chem., 1987, 30, 1609.
82. J. F. Dellaria, R. G. Maki, B. A. Bopp, J. Cohen, H. D. Kleinert, J. R. Luly, I. Merits, J. J. Plattner and H. H. Stein, J. Med. Chem., 1987, 30, 2137.
83. R. Paruszewski, P. Jaworski, M. Strzalkowska, Pol. J. Chem., 1986, 60, 121.
84. M. G. Bock, R. M. DiPardo, B. E. Evans, K. E. Rittle, J. Boger, M. Poe, B. I. LaMont, R. J. Lynch, E. H. Ulm, G. P. Vlasuk, W. J. Greenlee and D. F. Veber, J. Med. Chem., 1987, 30, 1853.
85. K. Y. Hui, W. D. Carlson, M. S. Bernatowicz and E. Haber, J. Med. Chem., 1987, 30, 1287.
86. S. H. Rosenberg, J. J. Plattner, K. W. Woods, H. H. Stein, P. A. Marcotte, J. Cohen and T. J. Perun, J. Med. Chem., 1987, 30, 1224.

87. P. Roy, M. Delepierre, J. Wagnon, D. Nisato and B. P. Roques, Int. J. Pept. Protein Res., 1987, 30, 44.
88. C. Toniolo, G. Valle, G. M. Bonara, F. Lelj, V. Barone, F. Fraternali, G. Callet, J. Wagnon and D. Nisato, Int. J. Pept. Protein Res., 1987, 30, 583.
89. D. Nisato, J. Wagnon, G. Callet, D. Mettefeu, J-L. Assens, C. Plouzane, B. Tonnerre, V. Pliska and J-L. Fauchère, J. Med. Chem., 1987, 30, 2287.
90. H. Kogen and T. Nishi, J. Chem. Soc. Chem. Commun., 1987, 311.
91. P. Jouin, B. Castro and D. Nisato, J. Chem. Soc., Perkin Trans I. 1987, 1177.
92. F. G. Salituro, N. Agarawal, Th. Hofmann and D. H. Rich, J. Med. Chem., 1987, 30, 286.
93. S. K. Ner, C. J. Suckling, A. R. Bell and R. Wrigglesworth, J. Chem. Soc. Chem. Commun., 1987, 480.
94. P. P. Giannousis and P. A. Bartlett, J. Med. Chem., 1987, 30, 1603.
95. S. J. B. Tendler, M. D. Threadgill and M. J. Tisdale, J. Chem. Soc., Perkin Trans I. 1987, 2617.
96. N. Teno, S. Tsuboi, Y. Okada, W. Itoh and H. Okamoto, Int. J. Pept. Protein Res., 1987, 30, 93.
97. J-P. Mazaleyrat, M. Reboud-Ravaux and M. Wakselman, Int. J. Pept. Protein Res., 1987, 30, 622.
98. J. A. Lovett and P. S. Portoghese, J. Med. Chem., 1987, 30, 1144.
99. J. A. Lovett and P. S. Portoghese, J. Med. Chem., 1987, 30, 1668.
100. A. K. Judd, J. A. Lawson, C. M. Olsen, L. R. Toll, W. E. Polgar, E. T. Uyeno, C. J. Keys and G. H. Loew, Int. J. Pept. Protein Res., 1987, 30, 299.
101. P. S. Portoghese, D. L. Larson, G. Ronsisvalle, P. W. Schiller, T. M-D. Nguyen, C. Lemieux and A. E. Takemori, J. Med. Chem., 1987, 30, 1991.
102. Y. Shimohigashi, M. Maruyama, T. Ogasawara, T. Koshizaka, M. Waki, T. Kato, N. Izumyia, T. Costa and A. Hertz. Pept. Chem (1986), 1987, p.41.
103. G. P. Vlasov, E. N. Krasnikova, N. Ya. Kozhevnikova, N. G. Illarionova and I. Denisov, Biopolymers, 1987, 26, 1489.
104. G. W. Hardy, L. A. Lowe and T. W. Smith, Ref. 1, 1987, p.435.
105. P. Rigaudy, Ch. Garbay-Jaureguiberry, A. Jacquemin-Sablon, J-B. Le Pecq and B. P. Roques, Int. J. Pept. Protein Res., 1987, 30, 347.
106. L. Varga, S. Horvat, C. Lemieux and P. W. Schiller, Int. J. Pept. Protein Res., 1987, 30, 371.
107. C. L. Ho, J. L. Ko and C. H. Li, Int. J. Pept. Protein Res., 1987, 29, 521.
108. M. Marastoni, S. Salvadori, G. Balboni, P. A. Borea, G. Marzola and R. Tomatis, J. Med. Chem., 1987, 30, 1538.
109. M. T. Garcia-Lopez, R. Gonzalez-Muriz, R. Herranz, M. T. Molinero and J. Del Rio, Int. J. Pept. Protein Res., 1987, 29, 613.
110. M. T. Garcia-Lopez, R. Bonzalez-Muriz, M. T. Molinero, J. Naranjo and J. Del Rio, J. Med. Chem., 1987, 30, 1658.
111. B. Calas, R. Michelot, J. P. Lecear, A. Cave, J. Parello and P. Potier, Int. J. Pept. Protein Res., 1987, 29, 170.
112. K. M. Sivanandaiah and N. S. Ragaraju, Indian J. Chem., 1986, 25B, 1045.
113. C. Poulos, G. Stavropoulos, J. R. Brown and C. C. Jordan, J. Med. Chem., 1987, 30, 1512.
114. D. C. Horwell, A. Beeby, C. R. Clark and J. Hughes, J. Med. Chem., 1987, 30, 729.
115. K. Iuchi, M. Nitta, T. Tanaka, K. Ito, Y. Morimoto and G. Tsukamoto, Chem. Pharm. Bull, Jpn., 1987, 35, 4307.
116. B. E. Evans, K. E. Rittle, M. G. Bock, R. M. DiPardo, R. M. Freidinger, W. L. Whitter, N. P. Gould, G. F. Lundell, C. F. Homnick, D. F. Veber, P. S. Anderson, R. S. L. Chang, V. J. Lotti, D. J. Cerino, T. B. Chen, P. J. King, K. A. Kunkel, J. P. Springer and J. Hirshfield, J. Med. Chem., 1987, 30, 1229.

117. K. Kouge, H. Soma, Y. Katakai, H. Okai, M. Takemoto and Y. Muneoka, Bull. Chem. Soc. Jpn., 1987, 60, 4343; K. Kouge, Y. Katakai, H. Soma, M. Kondo, H. Okai, M. Takemoto and Y. Muneoka, ibid., 1987, 60, 4351.
118. T. Hashimoto, K. Kurosawa and Y. Uchida, Bull. Chem. Soc. Jpn., 1987, 60, 3071.
119. J. Pospisck, Z. D. Bespalova, D. Zhanna, E. Kovarikova, M. I. Titov, T. Barth and K. Medzihradszky, Coll. Czech. Chem. Commun., 1987, 52, 1867.
120. R. Paruszewski, R. Matusiak, P. Jaworski, S. Gumulka and I. Wisniewska, Pol. J. Chem., 1986, 60, 301.
121. K-h. Hsieh, P. Needleman and G. R. Marshall, J. Med. Chem., 1987, 30, 1097.
122. O. Nyeki, K. Sz. Szalay, L. Kisfaludy, E. Karpati, L. Szporny, G. B. Makara and B. Varga, J. Med. Chem., 1987, 30, 1719.
123. P. Cordopatis, A. Michel, J. Matsackas, Gazz. Chim. Ital., 1987, 117, 287.
124. A. Buku, I. L. Schwartz, N. Yamin, H. R. Wyssbrod and D. Gazis, J. Med. Chem., 1987, 30, 1509.
125. M. Manning, A. Misicka, A. Olma, W. A. Klis, K. Bankowski, E. Nawrocka, M. Kruszynski, A. Kolodziejezyk, L. L. Cheng, J. Seto, N. C. Wo and W. H. Sawyer, J. Med. Chem., 1987, 30, 2245.
126. F. El-Fehail Ali, H-L. Chang, W. F. Huffman, G. Heckman, L. B. Kinter, E. F. Weidley, R. Edwards, D. Schmidt, D.Ashton-Sue and F. L. Stassen, J. Med. Chem., 1987, 30, 2291.
127. B. Gysin, D. G. Johnson, D. Trivedi and V. J. Hruby, J. Med. Chem., 1987, 30, 1409.
128. G-S. Lu, S. Mojsov and R. B. Merrifield, Int. J. Pept. Protein Res., 1987, 29, 545.
129. S. J. Hocart, M. V. Nekola and D. H. Coy, J. Med. Chem., 1987, 30, 1910.
130. J. Hlavacek, J. Pospisek, J. Slaninova, W. Y. Chan and V. J. Hruby, Coll. Czech Chem. Commun., 1987, 52, 2317.
131. S. Hellberg, M. Sjostrom, B. Skagerberg and S. Wold, J. Med. Chem., 1987, 30, 1126.
132. D. J. Ward, P. W. Finn, E. C. Griffiths and B. Robson, Int. J. Pept. Protein Res. 1987, 30, 263.
133. V. J. Hruby, B. C. Wilkes, M. E. Hadley, F. A. Al-Obeidi, T. K. Sawyer, D. J. Staples, A. E. DeVaux, O. Dym, A. M. de L. Castrucci, J. Med. Chem., 1987, 30, 2126.
134. I. Kawazoe, H. Kawauchi, T. Hirano and N. Naito, Int. J. Pept. Protein Res., 1987, 29, 714.
135. J. P. Riehm and K. R. Rao, Int. J. Pept. Protein Res., 1987, 29, 415.
136. T. B. Johnson and J. K. Coward, J. Org. Chem., 1987, 52, 1771.
137. L. Grehn, B. Fransson and U. Ragnarsson, J. Chem. Soc., Perkin Trans I. 1987, 529.
138. R. M. Epand and K. E. Raymer, Int. J. Pept. Protein Res., 1987, 30, 515.
139. Y. Trudelle and F. Heitz, Int. J. Pept. Protein Res., 1987, 30, 163.
140. M. Fukuda, C. M. Beechan, M. Verlander, M. Goodman, M. M. Khan, and K. L. Melmon, Int. J. Pept. Protein Res., 1987, 29, 300.
141. K. Ienaga, K. Higashiura and H. Kimura, Chem. Pharm. Bull. Jpn., 1987, 35, 1249.
142. H. Suli-Vargha, A. Jeney, K. Lapis, and K. Medzihradsky, J. Med. Chem., 1987, 30, 583.
143. T. P. Andersen and A. Senning, Liebigs Ann. Chem., 1987, 59.
144. R. Andruszkiewicz, H. Chmara, S. Milewski and E. Borowski, J. Med. Chem., 1987, 30, 1715.
145. P. A. Grieco and A. Bahsas, J. Org. Chem., 1987, 52, 5746.
146. K-L. Yu and R.L. Johnson, J. Org. Chem., 1987, 52, 2051.
147. J. Porter, J. Dykert and J. Rivier, Int. J. Pept. Protein Res., 1987, 30, 13.
148. F. Fahrenholtz, P. Eggena, E. Kojro, D. Gazis and I. L. Schwartz, Int. J. Pept. Protein Res., 1987, 30, 577.

149. A. Buku, P. Eggena, H. R. Wysbrod, I. L. Schwartz, D. Gazis, L. I. Somoza and J. D. Glass, J. Med. Chem., 1987, 30, 1526.
150. Z. Mackiewicz, S. Bélisle, D. Bellabarba, N. Gallo-Paget, J. G. Lehoux, G. Lagace and E. Escher, Helv. Chim. Acta., 1987, 70, 423.
151. H. Mihara, S. Lee, Y. Shimohigashi, H. Aoyagi, T. Kato, N. Izumiya and T. Costa, Int. J. Pept. Protein Res., 1987, 30, 605.
152. A. A. Amoscato, G. F. Babcock, R. M. Sramkoski, B. A. Hynd and J. W. Alexander, Int. J. Pept. Protein Res., 1987, 29, 177.
153. G. Gacel, E. Fellion, B. P. Roques, R. Genet, J. L. Morgat and P. Fromageot, J. Labelled Compd. Radiopharm., 1987, 24, 867.
154. M. K. Anwer, R. A. Porter and A. F. Spatola, Int. J. Pept. Protein Res., 1987, 30, 489.
155. R. Jacquier, R. Lazaro, H. Ranirisehenо and Ph. Viallefont, Int. J. Pept. Protein Res., 1987, 30, 22.
156. R. E. Shute, B. Dunlap and D. H. Rich, J. Med. Chem., 1987, 30, 71.
157. R. E. Shute and D. H. Rich, Tetrahedron Lett., 1987, 28, 3419.
158. U. Schmidt, R. Utz, A. Lieberknecht, H. Griesser, B. Potzolli, J. Bahr, K. Wagner and P. Fischer, Synthesis, 1987, 233; 236; J. J. Stezowski, H. W. Pohlmann, E. Haslinger, H. Kalchhauser, U. Schmidt and B. Pozolli, Tetrahedron, 1987, 43, 3923.
159. I. J. Galpin, A. K. A. Mohammed and A. Patel, Tetrahedron Lett., 1987, 28, 6517.
160. G. Zanotti, B. Beijer and Th. Wieland, Int. J. Pept. Protein Res., 1987, 30, 323.
161. G. Zanotti, C. Mohringer and Th. Wieland, Int. J. Pept. Protein Res., 1987, 30, 450.
162. T. Katagi and H. Kataoka, Heterocycles, 1987, 26, 2109.
163. D. Obrecht and H. Heimgartner, Helv. Chim. Acta, 1987, 70, 329.
164. K. Ueda, M. Waki and N. Izumiya, Int. J. Pept. Protein Res., 1987, 30, 33.
165. M. A. Castiglione-Morelli, F. Lelj, A. Pastore, S. Salvadori, T. Tancredi, R. Tomatis, E. Trivellone and P. A. Termussi, J. Med. Chem., 1987, 30, 2067.
166. V. F. Bystrov, A. S. Arsen'ev, I. L. Barsukov and A. L. Lomize, Bull. Magn. Res., 1986, 8, 84.
167. W. Zhang, M. J. O. Ateunis and F. A. M. Borrams, Bull Soc. Chim (Belg.)., 1987, 96, 373.
168. H. Kessler, St. Steurengel, D. Gillesen and T. Kamiyama, Helv. Chim. Acta., 1987, 70, 726.
169. H. Kessler, Ch. Griesinger and K. Wagner, J. Am. Chem. Soc., 1987, 109, 6927.
170. W. Bermel, Ch. Griesinger, H. Kessler and K. Wagner, Magn. Res. Chem., 1987, 25, 325.
171. A. Otter and G. Kotovych, J. Magn. Res., 1987, 74, 293.
172. C. Wynants, E. Sugg, V. J. Hruby and G. Van Binst, Int. J. Pept. Protein Res., 1987, 30, 541.
173. M. J. York, G. R. Beilharz and P. W. Kuchel, Int. J. Pept. Protein Res., 1987,29, 638.
174. Ch. Griesinger, O. W. Sorensen and R. R. Ernst, J. Am. Chem. Soc., 1987, 109, 7227.
175. R. Kishore, S. Raghotama and P. Balaram, Int. J. Pept. Protein Res., 1987, 29, 381.
176. W. Zhang, M. J. O. Anteunis and F. A. M. Borremans, Int. J. Pept. Protein Res., 1986, 28, 593.
177. P. G. Harrison and N. W. Sharpe, Int. J. Pept. Protein Res., 1987, 29, 293.
178. A. Otter, P. G. Scott and G. Kotovych, J. Am. Chem. Soc., 1987, 109, 6995.
179. S. Talluri, G. T. Montelione, G. Van Duyne, L. Piela, J. Clardy and H. A. Scheraga, J. Am. Chem. Soc., 1987, 109, 4473.

180. P. L. Stewart, K. G. Valentine and S. J. Opella, J. Magn. Resonance, 1987, 71, 45.
181. T. G. Oas, C. J. Hartzell, T. J. McMahon, G. P. Drobny and F. W. Dahlquist, J. Am. Chem. Soc., 1987, 109, 5956.
182. C. H. Gorbitz, Acta. Chem. Scand., 1987, B.41, 362.
183. C. H. Gorbitz, Acta. Chem. Scand., 1987, B.41, 87.
184. C. H. Gorbitz, Acta. Chem. Scand., 1987, B.41, 679.
185. C. H. Gorbitz, Acta. Chem. Scand., 1987, B.41, 83.
186. A. N. Stroup, A. L. Pheingold, A. L. Rockwell and L. M. Gierasch, J. Am. Chem. Soc., 1987, 109, 7146.
187. I. L. Karle and Th. Wieland, Int. J. Pept. Protein Res., 1987, 29, 596.
188. R. Murali and E. Subramanian, Int. J. Pept. Protein Res., 1987, 29, 187.
189. R. Murali and E. Subramanian, Int. J. Pept. Protein Res., 1987, 29, 374.
190. S. Abdel Rahman, M. Elsafty, and A. Hattaba, Collect. Czech. Chem. Commun., 1987, 52, 1356.
191. J. Hlavacek, I. Fric, P. Malon, K. Jost and K. Blaha, Collect. Czech. Chem. Commun., 1987, 52, 1841.
192. E. Gizdala, B. Lammek and Z. Grzonka, Pol. J. Chem., 1986, 59, 1153.
193. R. Kishore, H. Ishizaki, A. T. Tu, A. Ravi and P. Balaram, Int. J. Pept. Protein Res., 1987, 30, 474.
194. R. S. Rapaka, V. Renugopalakrishman, T. W. Collette, J. C. Dobbs, L. A. Carreira and R. S. Bhatnagar, Int. J. Pept. Protein Res., 1987, 30, 284.
195. G. Vertuani, S. Spisani, M. Boggian, S. Traniello and A. Scatturin, Int. J. Pept. Protein Res., 1987, 29, 525.
196. L. Piela, G. Némethy and H. A. Scheraga, J. Am. Chem. Soc., 1987, 107, 4477.
197. C. A. Morrison, R. V. Fishleigh, D. J. Ward and B. Robson, F.E.B.S. Letters, 1987, 214, 65.
198. S. Lifson, C. E. Felder and M. Dobler, Acta Crystall., 1987, B.43, 179.
199. K. Romanowska and K. D. Kopple, Int. J. Pept. Protein Res., 1987, 30, 289.
200. C. Ramakrishnan, K. Ramnarayan and G. Manjula, Int. J. Pept. Protein Res., 1987, 29, 657.
201. P. K. C. Paul and C. Ramakrishnan, Int. J. Pept. Protein Res., 1987, 29, 433.

4
Cyclic, Modified, and Conjugated Peptides

BY P. M. HARDY

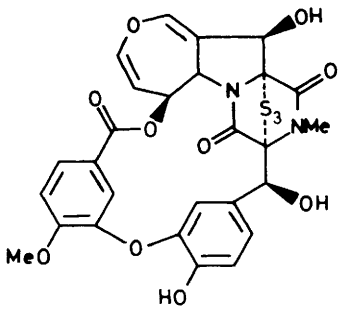
1 Introduction

The borderline between peptides and non-peptidic organic compounds becomes increasingly blurred with each successive year as far as this chapter is concerned. In synthesis, particularly with the growth of interest in amide-bond analogues and the introduction of other non-classical features, unequivocal classification of compounds within the headings adopted also becomes more difficult. The reader is therefore advised to browse around in the chapter, as, for example, a compound containing a dehydro-amino acid may not be listed under dehydropeptides but in another category because of other structural features in the molecule. Pressure on space (cf. 287 references this year compared to 243 in 1986) does not usually allow mention of a compound under more than one heading. The borderline in linear peptides between 'modified protein constituents' and 'other unusual amino acids' is also a rather grey area, although for continuity no changes in classification have been made this year.

2 Cyclic Peptides

2.1 Naturally Occurring Dioxopiperazines (Cyclic Dipeptides)- A new epitrithiodioxopiperazine from Emericella striata, emestrin B (1), differs from emestrin only in its possession of a third atom of sulphur.[1] The trisulphide analogue of gliotoxin, gliotoxin E, (cf. Vol. 19 p. 190) has been isolated from Penicillium terlikowskii and two other species. It is equipotent with gliotoxin in a macrophage cell adherence assay.[2] The antibiotic aspirochlorine was isolated in 1975 from Aspergillus flavus, and suggested to be (2). The structure has now been revised to (3), making it both the first epidithiopiperazine-2,5-dione derived from glycine and the first natural product with an N-methoxyl amide moiety. The very high amide carbonyl frequency (1724 cm^{-1}) which lead to the previous incorrect structural formulation remains to be explained, however.[3]

The total synthesis of saframycin B (4) and its congeners



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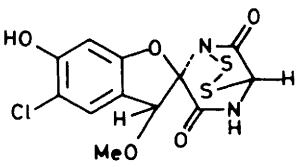

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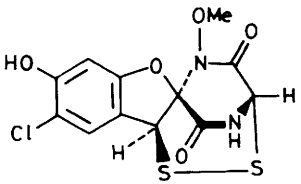

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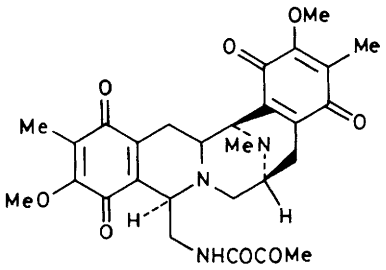

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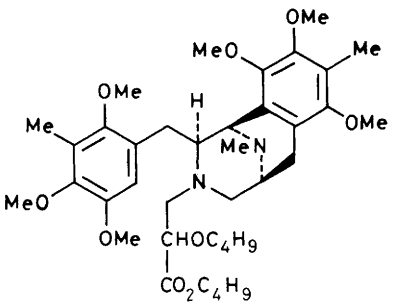

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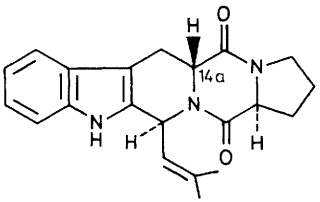

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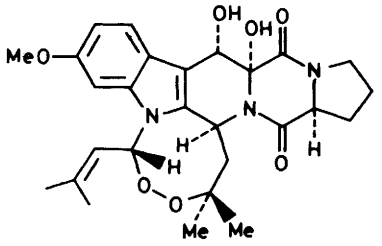

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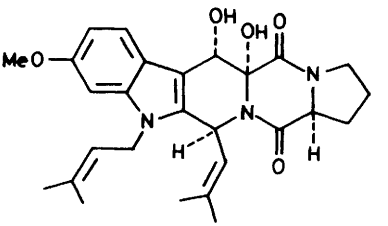

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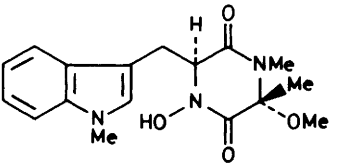

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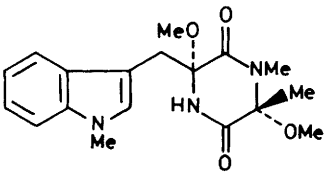

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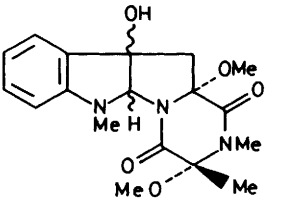
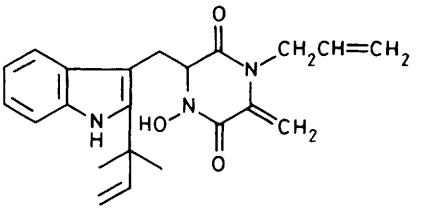


(11)

Reagents: i, methylene blue, -78 °C, O_2, $h\nu$; ii, -78 °C, Me_2S

Scheme 1



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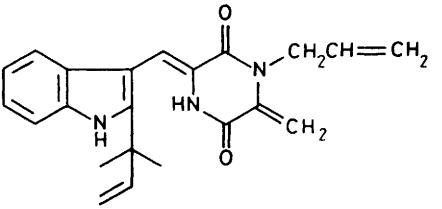

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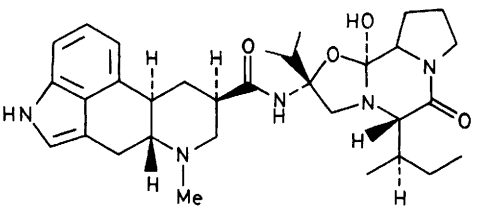

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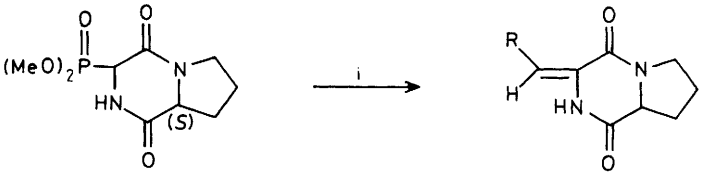
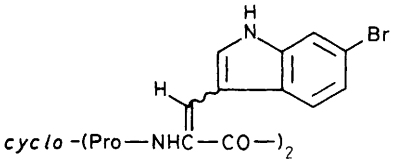


(15)

Reagents: i, RCHO, $KOBu^t$, CH_2Cl_2

Scheme 2



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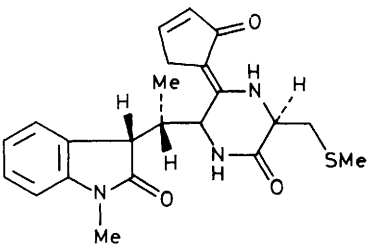

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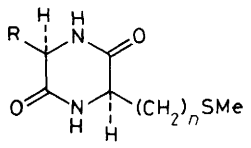
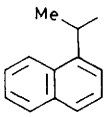
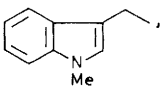
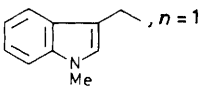


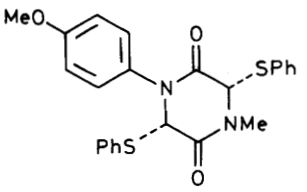
a, R =  , $n = 2$; b, R =  , $n = 2$; c, R =  , $n = 1$

(18)

has been achieved. The key step in the sequence of reactions was the stereoselective intramolecular cyclisation of the amino-acetal (5) brought about by trifluoroacetic acid.[4] This work also gives access to a simple and efficient synthesis of a variety of (Z)-3-arylidene-6-arylmethyl-2,5-piperazinediones having highly oxygenated rings.[5] The synthesis of a fumitremorgin (fungal metabolites capable of eliciting tremors in vertebrates) has been reported by two groups. The ^{1}H-n.m.r. spectrum of synthetic (6) differs from that of natural fumitremorgin C (whose structure was reported in 1977), and since the stereochemistry of the natural material is known except for the 14a position, the synthesis suggests that the latter is epimeric at this position.[6] The other synthesis also concerns this epimer, but in this case no comparison has been made with fumitremorgin C.[7] In both syntheses the cyclic dipeptide was the last ring system to be constructed.[6,7] The stereochemical course of the formation of the eight-membered peroxide ring in verruculogen (7), a tremorgenic metabolite of Penicillium verruculosum, has been established by ^{13}C-, ^{2}H-, and ^{18}O- labelling. The co-metabolite fumitremorgin B (8) is the substrate for conversion by a dioxygenase.[8]

O-Alkylation of the N-hydroxypiperazine (9) followed by base treatment in the presence of methanol gives (10), which will undergo oxidative ring closure using singlet oxygen. The tetra-cyclic compound (11) is generated as a mixture of diastereoisomers with a cis-ring junction between the five membered rings (Scheme 1). These compounds are potential precursors of the sporidesmins; the stereochemistry of (10) was confirmed by X-ray.[9] To investigate the proposed role of N-hydroxyamino acid derivatives in the biosynthesis of neoechinulin , (12) has been prepared. On O-tosylation followed by base treatment N(1)-allyl neoechinulin B (13) was formed. However, attempts to remove the N-allyl group using rhodium catalysts led to the decomposition of (13).[10] A new semisynthetic ergot peptide alkaloid (14) containing L-allo threonine has been constructed. Its CNS effects suggest it might benefit patients who have cognitive and affective deficits which threaten their independence and necessitate drug therapy. Its renal and cardiovascular effects are also novel.[11]

Neither of the stereoisomeric dioxopiperazines obtained by the reaction of N-Boc-6-bromoindol-3-carbaldehyde with (15) (Scheme 2) has proved identical to barettin, a metabolite of the cold water sponge Geodia baretti. Barettin has now been



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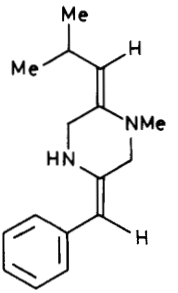

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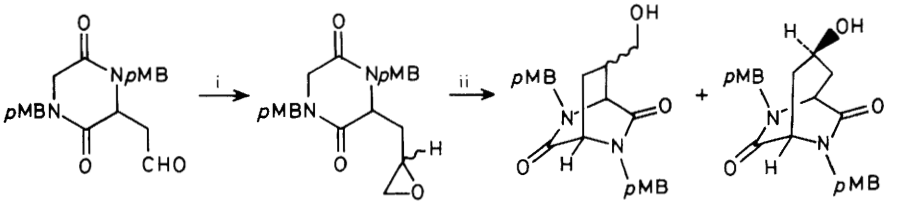
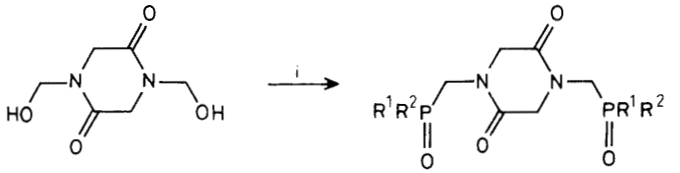


*p*MB = *p*-methoxybenzyl ratio 2·8 : 1·0

Reagents: i, $H_2C{=}S(O)Me_2$; ii, $Me_3SiN(Li)SiMe_3$, THF, −78 °C

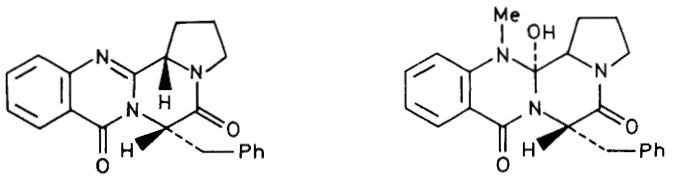
Scheme 3



(21) (22)

Reagent: i, $R^1R^2P(OEt)$, xylene, 120–140 °C
a, $R^1 = R^2 = OEt$
b, $R^1 = OEt$, $R^2 = Me$

Scheme 4



(23) (24)

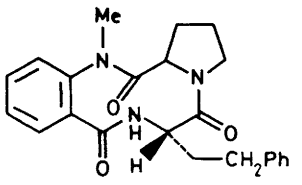
suggested to be the cyclic tetrapeptide (16).[12] The compound (17) was shown in 1986 to have potent and specific platelet activating factor (PAF) inhibitory activity (IC_{50} 0.37 μM). Of a series of structurally simpler analogues (18), the most potent were the D-D stereoisomers (18a, b, and c) showing IC_{50}'s respectively of 0.18, 2.3, and 7.9 μM.[13] Of another series of PAF-inhibitor analogues containing phenyl rather than bicyclic substituents, the most active material was (19), IC_{50} 0.69 M. This is PAF-specific, having no effect on platelet aggregation induced by other means.[14] The first naturally occurring dioxopiperazine containing an E-dehydroleucine residue, (3Z, 6E)-1-N-methylalbonursin (20) has now been synthesised.[15]

Thiolate addition to the C-5 exo-methylene group of some bicyclomycin analogues has been examined. There is a lack of correlation between simple thiolate susceptibility and anti-microbial activity, indicating that this reaction alone cannot be used as the biomechanistic template.[16] A convenient and practical synthesis of bicyclomycin-related cyclic dipeptides using an intramolecular enolate epoxide cyclisation has been developed (Scheme 3). The products correspond to the products of 4-exo-tet and 5-endo-tet ring closures.[17]

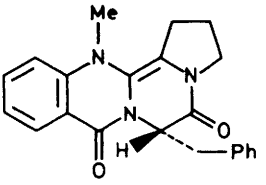
2.2 Other Dioxopiperazines- Analysis of 65 X-ray structures of cyclic dipeptides has shown that they fulfil a recently-proposed non-equilateral puckering equation.[18] On this basis the statistically most-favoured conformers for cyclo(Pro-Xxx), cyclo(Phe-Xxx), cyclo(MeAla-Xxx), and cyclo(Cys-Cys) have been put forward.[19] The crystal structures of cyclo(Asp-Ala),[20] cyclo(Met-Pro),[21] and cyclo(NMe-Phe-Phe)[22] have been published, but show no great surprises.

A new synthesis of (Z)-3-alkylidene-(S)-6-alkyl-2,5-piperazinediones involves the coupling of a Boc-α-amino acid to an N-carboxy-α-dehydroamino acid anhydride with carbodiimide to give the N-acyl derivative. On N-deprotection cyclic dipeptide formation occurs.[23] The N-hydroxymethyl cyclic dipeptide (21) can be phosphorylated (Scheme 4). Treatment with phosphodiesterase will then give the free disphosphonic acid from (22a) or the phosphonic/phosphinic acid from (22 b). With alkaline phosphatase, (22a) gives the monophosphonester.[14]

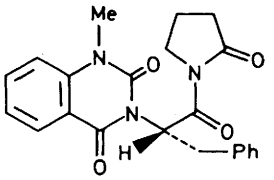
The racemisation of cyclo(Ala-Gly) at 120 °C in aqueous phosphate buffer at pH 8.0, a pH value near the maximum for racemisation, has been examined. At equilibrium the mole fractions



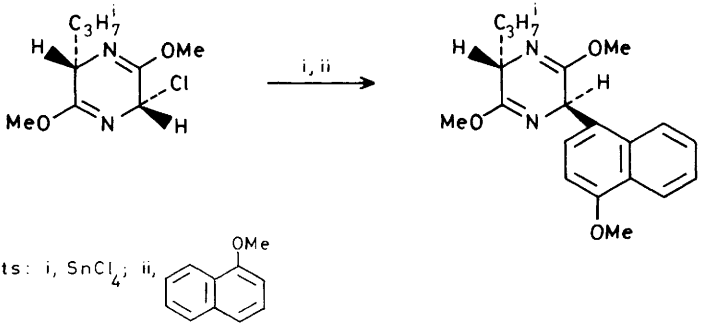
(25)



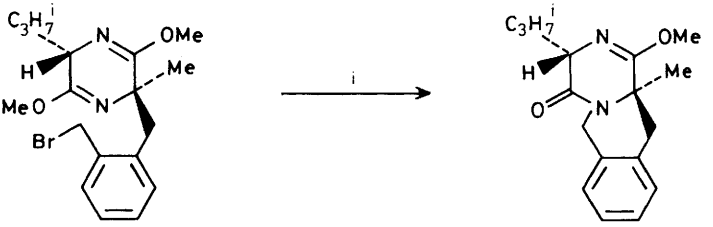
(26)



(27)



Scheme 5

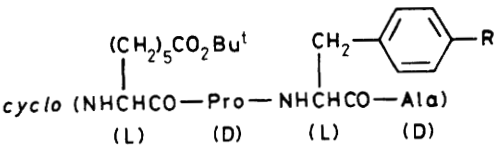


Scheme 6A

of Ala-Gly, cyclic dipeptide, and Gly-Ala are 0.57, 0.22, and 0.21 respectively. The rate constant for racemisation of cyclo-(Ala-Gly) is twice that of Gly-Ala and seven times that of Ala-Gly.[25] The complexation of cyclo(Ala-Ala) enriched to 25% in ^{17}O with the cobaltous ion in aqueous solution has been investigated using ^{17}O- and ^{14}N-n.m.r. spectroscopy. The larger ^{17}O chemical shifts and greater ^{17}O-nuclei sensitivity extend the range of kinetic and dynamic studies relative to ^{1}H by one to two orders of magnitude; the oxygen atoms are also involved directly in complexation.[26] Cyclo(L and D-Npg-Gly), where Npg is 2-amino-4,4-dimethylpentanoic acid (neopentylglycine), have been prepared as models for studies on the physical properties and conformations of peptides.[27]

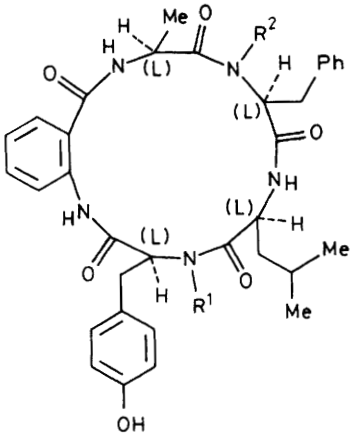
Under alkaline conditions, whereas anthraniloyl-Phe-Pro-ONp only gives the acylamidine (23), N-methylanthraniloyl-Phe-Pro-ONp gives a mixture of azacyclol (24) and cyclotripeptide (25). Both are stable in the solid state, but in solution in polar solvents (25) slowly forms (24). If Val replaces Phe, a similar mixture of products is formed, but if Gly replaces Phe, only the azacyclol is produced. If D-Pro replaces Pro, the only isolable compound is the ketene aminal derivative (26). Although stable in the solid state, in ethyl acetate in the presence of silica (26) gives (27), presumably via a dioxetane derivative.[28]

The utility of the alkylation of bislactim ethers of dioxopiperazines continues to be explored. Asymmetric Friedel-Crafts alkylation (Scheme 5) gives products containing bound α-arylglycines, but even using the mildest possible hydrolysis conditions (0.1 M HCl, 2 eq, 20 °C) to give the free α-arylglycine, 5-10% epimerisation takes place.[29] If α,ω-dibromo alkylating agents are used to alkylate the bislactim ether of cyclo(Val-Ala), 2-methyl-1-azacycloalkane-2-carboxylic acids can be prepared asymmetrically. After the initial C-alkylation, the intermediates are thermally cyclised (e.g. Scheme 6A).[30] Optically active γ-nitro-α-amino acids have also been prepared by alkylation of bislactim ethers.[31] As model compounds for the synthesis of constrained peptides, a series of di- and tetrapeptides containing adjacent glutamic acid and lysine residues linked also though their side chains have been prepared, e.g. Ac-Lys-Glu-NHMe and Ac-Lys-Glu-D-Lys-D-Glu-NHMe.[32] To aid in assigning the amide-proton resonances in the n.m.r. spectrum of the latter, it was isotopically labelled with deuterium in the C_α position of the



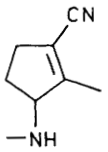
R = I, $PhCH_2O$—, HO—

(28)



a; $R^1 = R^2 = Me$
b; $R^1 = H$, $R^2 = Me$
c; $R^1 = Me$, $R^2 = H$

(29)



(30)

first two amino acids and with ^{15}N in the N-terminal lysine chain.[33]

2.3 Cyclic Tripeptides- A theoretical study of cyclic tripeptides suggest four favourable conformations, two containing all cis peptide units and two with two cis and one trans peptide units. The results compare reasonably well with the available crystal structure information.[34] A theoretical conformational analysis of the μ-selective cyclic opioid peptide analogue H-Tyr-D-Orn-Phe-Asp-NH_2 indicates that although the Tyr and Phe side-chains enjoy considerable rotational freedom, only a limited number of low-energy side-chain configurations are favoured.[35] Ten analogues of this tetrapeptide have been made; of these H-Tyr-D-Cys-Phe-Cys-NH_2 was less selective, but H-Tyr-D-Asp-Phe-Orn-NH_2 was more μ-selective.[36]

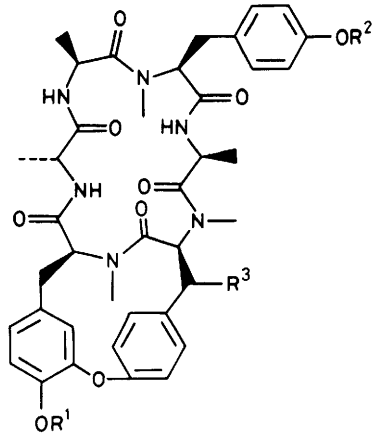
2.4 Cyclic Tetrapeptides- Peptides with sequences related to HC-toxin have been found to adopt a conformation locked by three γ-turns. When divalent cations are bound there is a 'transconformation' of the peptide backbone. These results show that the nature of the unusual residue in position 3 does not influence the conformation.[37] All four possible linear precursors of the HC-toxin related cyclic peptide cyclo(Ala-D--Ala-Ada-D-Pro) (where Ada is (2S)-2-amino-9-decenoic acid) have been prepared and their cyclisation examined. The best yield was obtained when D-Pro was C-terminal, using the succinimidyl ester for ring closure. The product was converted to HC-toxin itself by a two step sequence, a shorter route than hitherto described.[38] Four novel analogues of HC-toxin and chlamydocin in which the epoxy-ketone side chain is replaced by a chloromethyl or diazomethyl ketone have been prepared using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) cyclisations. The antimitogenic activities of both HC-toxin and chlamydocin chloromethyl ketones were 3-4 fold lower than the parents, while HC-toxin diazomethyl ketone was inactive.[39] Three new HC-toxin analogues containing p-substituents in the phenylalanine ring (28) have been prepared by a novel methodology involving a hydroxide-mediated Fmoc/methyl ester double deprotection and an improved BOP-Cl cyclisation.[40]

A set of five empirical rules to predict the conformations of cyclic tetrapeptides and cyclic tetradepsipeptides on the basis of primary structure have been put forward. N.m.r. experiments show that cyclo(Pro-Leu-D-Tyr(Me)-Ile) and

cyclo(Pro-D-Leu-D-Tyr(Me)-Ile) adopt cis-trans-trans-trans and cis-trans-cis-trans backbones respectively in accord with the rules. These are the first diastereoisomeric cyclic peptides with trans(LD) and cis(DD) secondary amide bonds.[41] Combined FAB/tandem MS has been applied to tentoxin, which 'serves as a textbook example for the problems often encountered with the sequencing of cyclopeptides when classical approaches are used.' The analysis was completed in about five hours, and high resolution MS was important in resolving ambiguities due to the presence of C_2H_4/CO units in some amino acids.[42] Cyclo-(Val-D-Val)$_2$ has been prepared by a mixed anhydride cyclisation but only in 3% yield. It was synthesised as part of a series of cyclic peptides made to examine if they can stack to form tubular structures which may act as conducting pores across suitable films. Using the same cyclisation technique, cyclo-(Val-D-Val)$_{3 \text{ and } 4}$ were obtained in 19% and 4% yields respectively.[43]

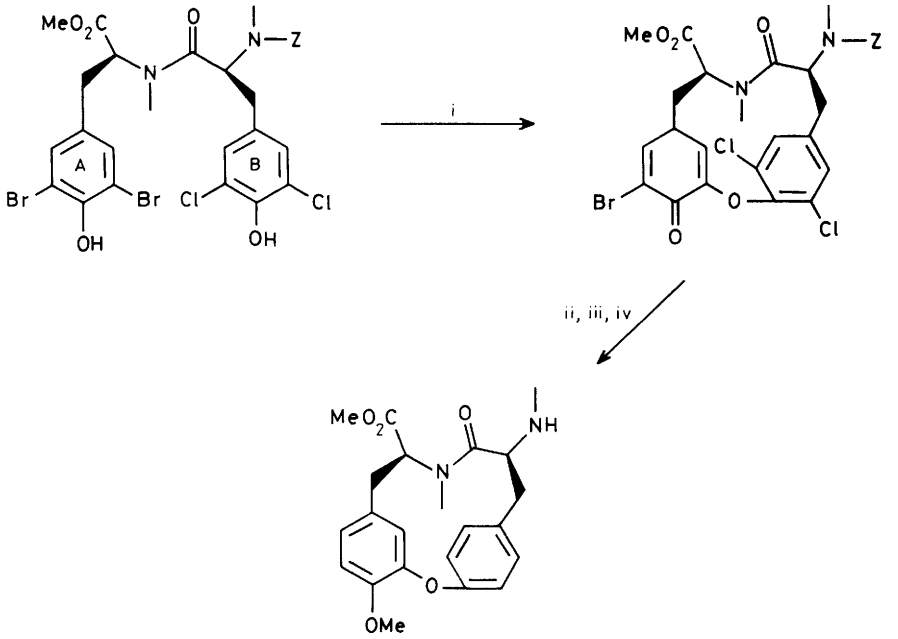
Cyclo(D-Trp-Lys-δ-D and L-Aad-Phe) have been prepared with the Aad (α-aminoadipic acid) side-chain carboxyl linked to $NH_2(CH_2)_nPh$, where n=1, 2 and 3. One compound, with n=3 and D-Aad, caused an unexpected stimulation of glucagon secretion while having little effect on either insulin or growth hormone secretion.[44] A computer simulation and a ^{1}H-n.m.r. study of H-Tyr-cyclo(D-A_2bu-Gly-Phe-D-Leu), whose opioid activity shows little μ or δ selectivity, has led to the postulation that it is the relative orientation of the Phe side-chain to the backbone and to the Tyr side-chain that is important for μ-receptor selectivity.[45] As agents that might be useful for the treatment of sickle-cell anaemia, cyclo(Val-Glu[Thr-Pro]-OH), cyclo(Phe-Glu--[Thr-Pro]-OH), and the lactone homologue H-Thr-Pro-Val-Glu-OH have been prepared to try and mimic the tetrapeptide region around the mutation site of haemoglobin S. The first of these showed very low antigelling activity, while the other two caused increased gelling.[46]

Five cyclic analogues of substance P have been prepared by solid-phase, [Hcy^5,Hcy^{11}]-SP (Hcy = homocysteine), [Cys^5,Cys^9]--SP, [D-Cys^5,Cys^8]-SP, [D-Cys^4,Cys^7]-SP, and [D-Cys^3,Cys^6]-SP. The polymer-bound linear sequences were converted to cyclic disulphides before cleavage from the resin. Cyclisation was effected by attack of a free thiol group on a 3-nitro-2-pyridine-sulphenyl group; no biological activities are given.[47] Preferential



a; $R^1 = R^2 = Me$, $R^3 = H$
b; $R^1 = R^3 = H$, $R^2 = Me$

(31)



Reagents; i, thallium tetranitrate, ii, Zn–90% HAc; iii, CH_2N_2; iv, Pd/C, MeOH, H_2, KOAc

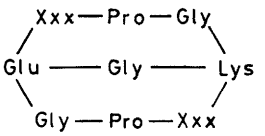
Scheme 6B

conformations of the δ-selective opioid peptide Tyr-cyclo-[D-Pen--Gly-Phe-Pen] and cyclo(Tyr-D-Thr-Gly-Phe-Leu-Thr) have been examined by 400 MHz n.m.r. Similar conformers are suggested for these compounds.[48] A conformational study of a series of enkephalin analogues bridged through D- and L-penicillamine or D- and L-cysteine residues in positions 2 and 5 (the compounds were first reported in 1983) has found that some analogues with similar opioid potency and receptor selectivity display disparate conformational features.[49]

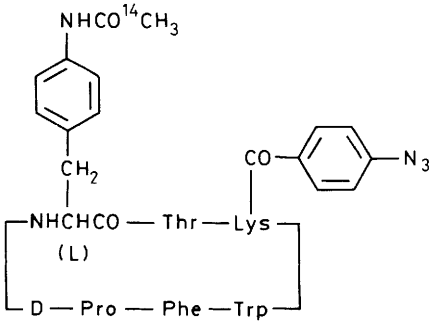
As part of an investigation into peripheral cholecystokinin receptor heterogeneity, three cyclic analogues of CCK_{26-33} have been prepared. Boc-Asp-Tyr(SO_3H)-Nle-D-Lys-Trp-Nle-Asp-Phe-NH_2 stimulated amylase secretion from rat pancreas one eightieth as effectively as CCK_8, and is a weak antagonist of CCK_8-induced contraction of GPI. Boc-Asp-Tyr(SO_3H)-Nle-D-Lys-Trp-Nle-Asp-NH_2 and Boc-Tyr(SO_3H)-Asp-Gly-Trp-Lys-Asp-Phe-NH_2 were inactive in all bioassays.[50]

2.5 Cyclic Pentapeptides- Three macrocycles containing an anthranilic acid residue as part of a peptide ring, the cyclo-aspeptides (29), have been isolated from Aspergillus sp. NE-45, a fungus from Nepalese forest soil. They have no antifungal or antibacterial activity at concentrations lower than 100 μg/disc.[51] A cyclic pentapeptide cyclo(Pro-Gly-Pro-DL-Mcc-Gly) has been synthesised in which the proline analogue Mcc (30) contains a cyanovinyl group in place of an amide carbonyl. Although the Mcc residue proved compatible with most routine operations of peptide synthesis, TFA at 25 °C for 1 h and acids of comparable strength under similar conditions caused complete racemisation of the Mcc.[52] Starting from 4-hydroxyproline, the peptide analogue (31) has been prepared. In this case, Bredt's rule inhibits epimerisation of the bicyclic Mcc derivative, but treatment with TFA results in partial hydrolysis of the enamine function.[53]

A conformation for the cyclic thymopoetin derivative cyclo-(D-Val-Tyr-Arg-Lys-Glu) has been deduced using the restrained molecular dynamics approach which satisfies all n.O.e. constraints. The main difference to a previously postulated structure is the bending of the ring between the β- and γ-turn. In addition, due to this bending an H-bond is formed between the Lys-NH and the Tyr-CO.[54] The crystal structure of cyclo(Gly-Pro-D-Phe-Gly-Val) contains a type II β-turn but instead of a γ-turn the conformational angles of the Val are approximately those of an RH γ-helix


Xxx—Pro—Gly
Glu — Gly — Lys
Gly—Pro—Xxx

(32)

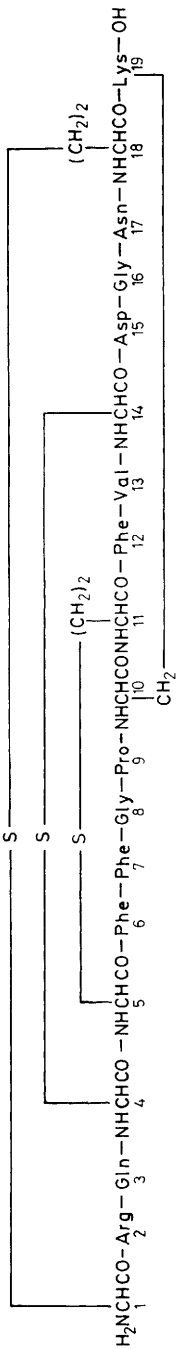

NHCO14CH3
CH2
CO
N3
NHCHCO — Thr — Lys
(L)
D — Pro — Phe — Trp

(33)

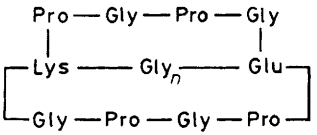
and there is no H-bond present in this turn. It is proposed that this type of chain reversal be termed a helical turn (H_R turn).[55] Cyclo(Val-D-Val-Val-D-Val-D-Val) has been prepared by a mixed anhydride cyclisation,[43] (cf section on cyclic tetrapeptides), and ^{1}H-n.m.r. studies of Boc-Cys-Ala-Aib-Gly-Cys-NHMe in d_6-DMSO support an intramolecular antiparallel β-sheet conformation at the Cys-Ala Gly-Cys residues nucleated by a γ-turn at the Aib.[56]

2.6 Cyclic Hexapeptides- The first total synthesis of deoxybouvardin (31a) and RA-VII (31b), compounds with antitumour activity, has been reported. In the key step, intramolecular oxidative coupling of protected (2,6-dibromotyrosyl)-2,6-dichlorotyrosine with thallium trinitrate gave the highly strained 14-membered ring system (Scheme 6B). If bromine atoms replace chlorine in ring B, none of the desired product is formed. The halogen atoms are removed by zinc and acetic acid before extending the peptide chain.[57] Two bicyclic nonapeptides containing two cyclohexapeptide rings (32; Xxx is either Ala or Leu) have been prepared and examined as cation complexing agents. The Leu compound contains one cis and one trans Pro-Leu bond and two β-turns. It binds Ca(II) tightly compared with other ions, forming both 1:2 and 1:1 complexes depending on the metal salt concentration. Results on the Ala analogue are very similar as far as cation binding is concerned, but the unbound cyclopeptide possesses a different conformation.[58] A photoreactive and radioactive derivative (33) has been prepared of a cyclic somatostatin analogue which has a high cytoprotective activity for rat hepatocytes against such cell poisons as phallotoxins and galactosamine. It labels the same proteins in rat liver cell membrane that are modified by photolysable derivatives of bile acids, phalloidin, and antamanide.[59]

Details have been reported of a synthesis of the somatostatin analogue cyclo(N-MeAla-Tyr-D-Trp-Lys-Val-Phe) designed to supply multihundred gram quantities of the cyclopeptide. These are required for assessment of its safety and efficiency; it is currently undergoing clinical evaluation as an agent for improved control of glucose levels in insulin-dependent diabetic patients. The linear hexapeptide was built up using mixed anhydride couplings and cyclised with diphenylphosphoryl azide.[60] Three other papers concern disulphide-bridged somatostatin analogues. A series of ψ[CH_2NH] modifications of H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2


H2NCHCO-Arg-Gln-NHCHCO-NHCHCO-Phe-Phe-Gly-Pro-NHCHCONHCHCO-Phe-Val-NHCHCO-Asp-Gly-Asn-NHCHCO-Lys-OH
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
S
S
S
(CH2)2
(CH2)2
CH2

(34)



n = 1 or 2

(35)

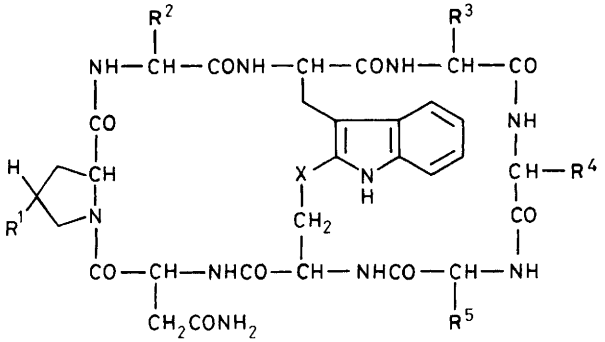


Table 1 Amaninamide analogues

R^1	R^2	R^3	R^4	R^5	X	K_i*
H	CHMeEt	H	CHMeEt	H	S	880
H	CHMeEt	H	CHMeEt	H	SO(*R*)	3880
H	CHMeEt	H	CHMeEt	H	SO(*S*)	8000
OH	CH_2CHMe_2	H	CHMeEt	H	S	1400
OH	$CHMe_2$	H	CHMeEt	H	S	128
OH	Me	H	CHMeEt	H	S	2700
OH	CHMeEt	Me	CHMeEt	H	S	72000
OH	CHMeEt	H	Me	H	S	22400
OH	CHMeEt	H	CHMeEt	Me	S	110

* relative to α-amanitin = 1

have been prepared using a new solid phase method for direct introduction of the peptide bond isostere. Compounds modified at the amide carbonyls of Cys^2 and Lys^2 showed the highest activities, being respectively 20 and 8 times more potent than somatostatin-14. The results appear to offer support for a proposed type II β-turn conformation centred on the D-Trp-Lys portion of the molecule.[61] In d_6-DMSO L- and D-Phys-Cys-Phe-D-Trp--Lys-Thr-Cys-D-Thr(ol) show a predominant conformation with a type II' β-turn involving Phe^3 to Thr^6. A clearcut correlation exists for these and other closely related analogues between the predominant conformation at the cystine bridge and the biological activity.[62] A similar study has been made of D-Phe-Cys-Tyr-D-Trp--Lys-Thr-Cys.[63]

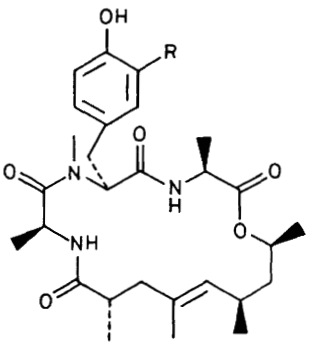
Boc-Cys-Val-Aib-Ala-Leu-Cys-NHMe has been prepared as a conformation model for disulphide loops of limited ring size. It adopts a type I' β-bend with three intramolecular H-bonds.[64] A method for obtaining side-chain conformations of cyclic peptides unambiguously using heteronuclear coupling constants derived from 2D-n.m.r. has been developed. The procedure is demonstrated with cyclo(Phe-Thr-Lys(Z)-D and L-Trp-Phe-D and L-Pro) stereoisomers.[65] Cyclo(Leu-D-Leu)$_3$ and cyclo(Phe-D-Phe)$_3$ have been prepared by mixed anhydride cyclisations,[43] and a theoretical study of cyclic hexapeptide conformations which accommodate H-bonded β- and γ-turns in the backbone has been made.[66] A molecular mechanics investigation of the flexibility of some cyclic hexa- and octapeptides has concluded that the former have greater freedom.[67]

2.7 Higher Cyclic Peptides- A new nonadecapeptide (34) from Streptoverticilium griseoverticillatum has interesting immunopotentiating activity. The constitution was established by homonuclear 2D-n.m.r., success being made possible by the use of a new variant of the RELAYED-NOESY technique. The amino acid composition is identical with that of cinnamycin and differs from duramycin by only one residue. The sequence also exhibits a surprising homology to the ACE inhibitor ancovenin; all the S-bridges are identical, the smallest being a cyclic heptapeptide, and only six out of the nineteen amino acids differ. Ancovenin, however, contains no lysinoalanine bridge. The configurations of the residues involved in the sulphide bridges are as yet unknown.[68] Homologous bicyclic cyclo[11]- and cyclo[12]-peptides (35) have been prepared (no details are given) and found to preferentially bind divalent ions, forming stable 1:1 and possibly 1:2 Ca(II) : .

Table 2 Cyclosporin analogues

K	DesoxyBmt	Val	Sar	MeLeu	Val	MeLeu	Ala	D-Ala	MeLeu	MeLeu	MeVal
L	Bmt	Abu									
M	MeBmt	Nva			Nva						
N	MeBmt	Nva								Leu	
O	MeLeu	Nva									
P	Bmt	Thr									
Q	MeBmt	Abu		Val							
R	MeBmt	Abu				Leu?				Leu?	
S	MeBmt	Thr		Val							
T	MeBmt	Abu								Leu	
U	MeBmt	Abu				Leu					
V	MeBmt	Abu					Abu				
W	MeBmt	Thr									Val
X	MeBmt	Nva							Leu		
Y	MeBmt	Nva				Leu					
Z	Mao	Abu									

Bmt = (4*R*)-4-[(*E*)-2-butenyl]-4-methyl-L-Thr
Mao = methaminooctanoic acid



a; R = I, b; R = Br

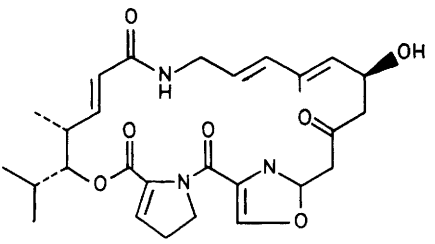
(36)

BCP-type peptide complexes. In the free state both peptide conformations are an average of many rapidly interconverting forms.[69]

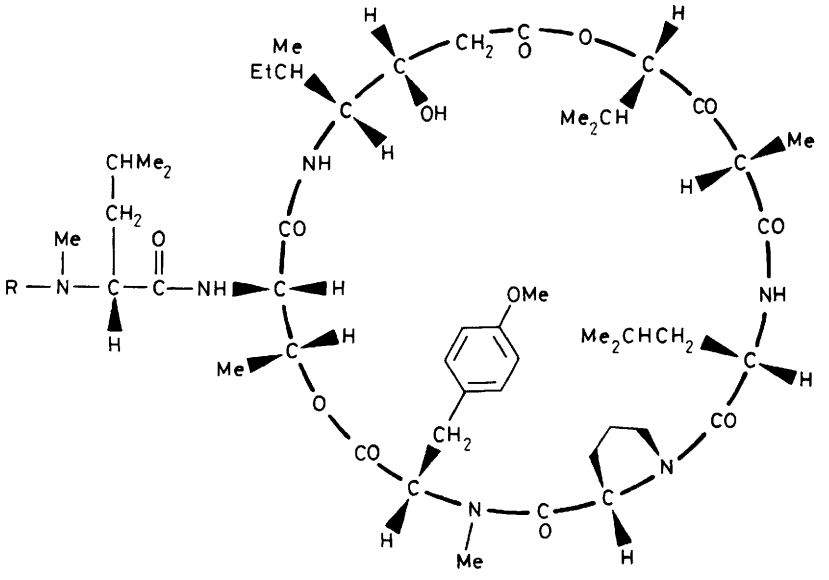
Two cyclo-octapeptides have been made as ionophore models. The ability of cyclo(Lys(Z)$_2$-Gly-Phe$_2$-Gly$_3$) to transfer H-Phe--OMe·HCl/LiPF$_6$ from 0.08 M HCl through $CHCl_3$ to 0.1 M HCl as receiving phase is about one half that of 18- crown-6,[70] while the first demonstration of peptide-mediated Ca(II) uptake into mammalian cells has been reported using cyclo(Glu(OBzl)-Sar-Gly--(N-n-decyl)-Gly)$_2$. The fluorescent dye quin-2 trapped in the cytoplasm of human and dog lymphocytes was used as an indicator to monitor the influx of calcium from the extracellular matrix.[71] Nine analogues (Table 1) of amaninamide, a poisonous component of white Amanita mushrooms, have been synthesised. They show widely differing inhibitory activities on RNA polymerase B from calf thymus.[72]

After 20 years of attempts, the X-ray crystal structure of cyclolinopeptide A, cyclo(Pro$_2$-Phe$_2$-Leu-Ile$_2$Leu-Val)m has been achieved by combined application of a rotational function coupled with packing criteria. The Pro-Pro bond is cis, all other amide bonds trans, and there are five transannular hydrogen bonds. The conformation of much of the peptide skeleton is very close to that shown in the solid state by antamanide.[73] Like its parent, the biologically active [Phe^4, Val^6]-antamanide crystallises in different forms depending on the solvent. A new X-ray of a polymorph obtained from acetone/water shows a conformation similar to results in other solvents, but the molecular packing is entirely different.[74]

The synthesis of [Aib$^{1,1'}$]-gramicidin S (GS) and [Aib^1]-semi GS has been reported. Both are inactive against Gram-positive organisms, and the c.d. spectrum of the former is markedly different to GS itself.[75] [(Z)-DehydroPhe$^{4,4'}$]-GS has also been made; its backbone conformation resembles GS, but there are stronger Val NH and Leu CO H-bonds. It shows strong antibiotic activity with the same spectrum of action as GS.[76] A further series of cyclosporins, K to Z (Table 2), from Tolypocladium inflatum, have been sequenced. All these cyclo-undecapeptides are less immunosuppressive than cyclosporin A.[77] On the synthetic side, a series of 10 cyclosporin analogues, [(Me)Thr^1]-, [Hyp^1]-, [Hyp^1,Nle^2]-, [(Me)Ser^1]-, [(Me)Ser^{1}Nle^2]-, [(Me)Ser^1,Nva^2]-, [(Me)Thr^1,Nle^2]-, [(Me(Thr)^1,Nva^2]-, [Me(Thr)^1,-Thr^2]-, and [Dab^1]-cyclosporins, have been reported. A number

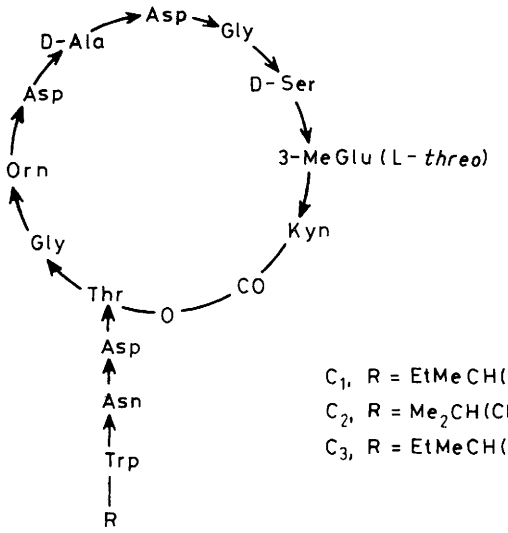


(37)

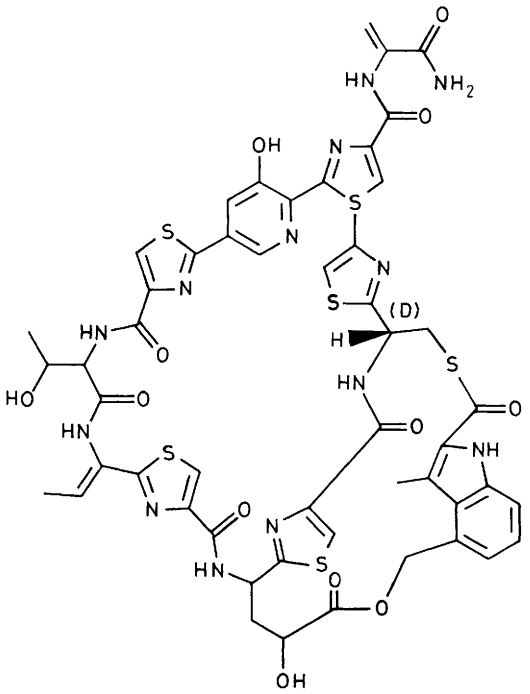


a; R = H b; R = MeCHOHCO—Pro— (L) c; R = MeCHOHCO— (L)

(38)


Asp
Gly
D-Ser
3-MeGlu (L-threo)
Kyn
CO
O
Thr
Gly
Orn
Asp
D-Ala
Asp
Asn
Trp
R
C1, R = EtMeCH(CH2)6CO—
C2, R = Me2CH(CH2)8CO—
C3, R = EtMeCH(CH2)8CO—

(39)


HN
NH2
O
OH
N
S
(D)
H
HO
NH
OH

(40)

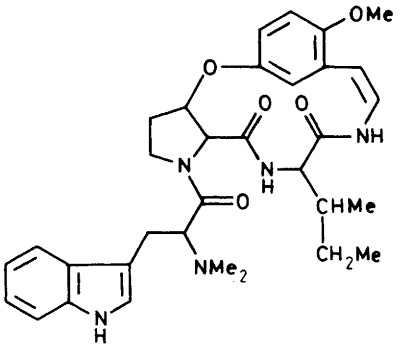
of cyclisation methods were investigated, and the Castro reagent $Bt\text{-}OP(NMe_2)_3^+PF_6^-$ found to give the most reliable yield of product when used at high dilution. No biological activities are given.[78]

2.8 Cyclic Depsipeptides- Two novel cyclodepsipeptides, geodiamolides A and B (36), have been isolated from a Geodia sp. of marine sponge collected off Trinidad. They show some activity against the fungus Candida albicans, and their structures suggest a mixed peptide-polypropionate biogenesis. It is not yet certain if these compounds are metabolites of the sponge or of micro-organisms inhabiting it.[79] Feeding studies with various stereospecifically labelled precursors have shown that incorporation of (S)-serine into the oxazole ring of virginiamycin M, (37) proceeds with loss of the 3-(pro-S)-hydrogen.[80]

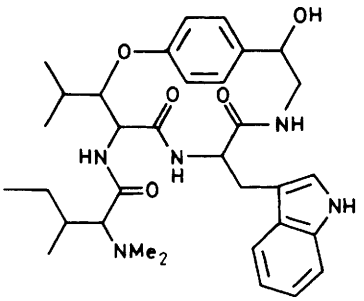
Two analogues of AM-toxin II, [Phe^3, $Ser(Bzl)^4$]- and [$Ser(Bzl)^4$]-AM-toxin II, have been prepared to explore the preparations of cyclodepsipeptides by cyclisation through ester bond formation. Using the water-soluble carbodiimide/DMAP route, yields on ring closure of 16% and 19% respectively were obtained.[81] Enantio AM-toxin I has also been synthesised, but showed no necrotic activity on apple leaves up to 100 μg/ml.[82] The actinomycin-related peptide lactone Z-Thr-D-Val-Pro-Sar-MeAla-] has been made in order to observe the behaviour of the unprotected cyclodepsipeptide after hydrogenolytic deprotection. An O,N-acyl shift occurred over a period of hours to give the cyclic pentapeptide. This contrasted with the more rapid rearrangement of O-hippuryl-Thr-OMe.[83]

The total synthesis of didemnin A (38) has been achieved, proving that it in fact contains isostatine and not statine as originally thought. The final cyclisation was effected between the threonine carboxyl and the amino group of isostatine using water-soluble carbodiimide and HOBt. The product was further elaborated into didemnins B and C.[84]

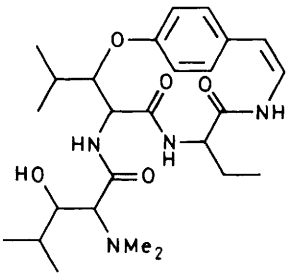
A complex of new acidic lipopeptolide antibiotics (39) has been isolated from Streptomyces roseosporus. They all have the same cyclopeptide ring, but differ at the end of the side chain. Their structures were determined using a combination of Edman degradation and GC-MS analysis of peptides obtained from partial hydrolysis.[85] A new theonellapeptide, Ic, has been isolated from the same Okinawan sponge as theonellapeptide Id (see p.207



(41)



(42)



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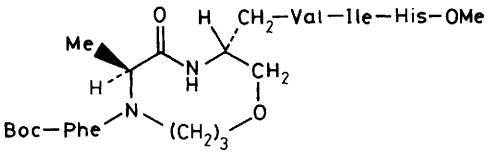

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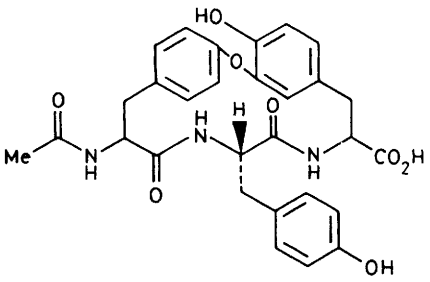

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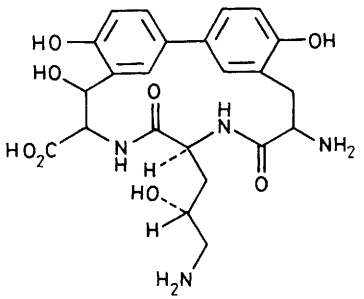


(46)

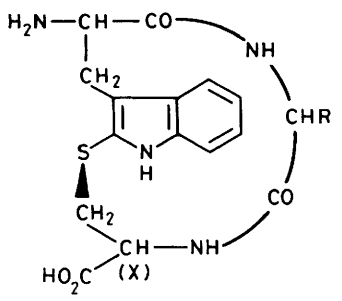
SPR 19). It differs only in having the β-Ala between the Thr and D-Leu residues replaced by Me-β-Ala.[86]

2.9 Cyclic Peptides Containing Thiazole and Oxazoline Rings- A reisolation of dolastatin 3 from the Indian ocean sea hare Dolabella auricularia has led to it now being formulated as cyclo(Val-Pro-Leu-Gln(Thz)-Gly(Thz)). This structure has been proved by synthesis; preparation of an isomer containing D-Gln(Thz) gave a product clearly different from the natural material.[87] A dolastatin 3 analogue cyclo(Pro-Leu-Val-Gly(Thz)-Gly(Thz)) has also been made. X-ray shows a cis Gly(Thz)-Pro peptide bond and two intramolecular H-bonds. The crystal packing demonstrates that it is a very hydrophobic molecule with a tendency to self associate, forming a network of interlocking tubes filled with toluene molecules.[88] Ulicyclamide has now been synthesised by a polystyrene solid-phase method (its conventional synthesis was reported in 1985). The coupling agent used was diethylphosphorocyanidate; linkage of the thiazole-containing residues required longer reaction times. The linear peptide was removed from the resin by methanolysis in DMF or by trimethylsilyl triflate, and cyclised with diphenyl phosphorazidate.[89]

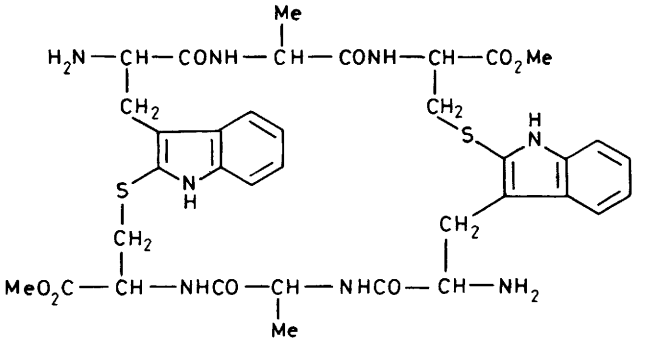
The crystal structure of ascidiacyclamide has been determined, the first one of an ascidian cytotoxic cyclic peptide. The backbone peptide chain takes a saddle-shaped conformation, with a rectangular overall shape having thiazole and oxazoline rings at alternate corners. There are no intramolecular H-bonds. All the N-H bonds are directed towards the interior of the ring, and the NH bonds and the nitrogen lone pairs are all directed to a point at the centre of the molecule.[90] On the basis of radiolabelling experiments, it is speculated that nosiheptide (40), whose structure was elucidated in 1977, arises from the dodecapeptide H-Ser-Cys-Thr_2-Cys-Glu-Cys-D-Cys-Cys-Ser-Cys-Ser-OH through connections of the C atoms 3 of Ser-1 and Ser-12 and attachment of a modified tryptophan.[91]

2.10 Cyclic Peptides Containing Other Nonprotein Ring Components- Three new peptide alkaloids have been characterised. Nummullarine R (41), from Z. nummularia, differs from sativanine E in having Ile in place of Leu as the amino acid bound to the styrylamine nitrogen,[92] and Discaria febrifuga, a South American shrub, has yielded discarine K (42), which also contains tryptophan.[93] Melifoline (43), from the Indian plant Melochia carcharifolia, contains 2-aminobutyric acid and N,N-diMe-β-OH-leucine in novel

Table 3 Cyclic tryptathionine peptides



	X	R
a	L	H
b	D	H
c	L	Me(L)
d	D	Me(L)
e	L	Me(D)
f	D	Me(D)



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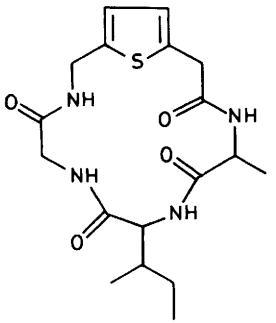
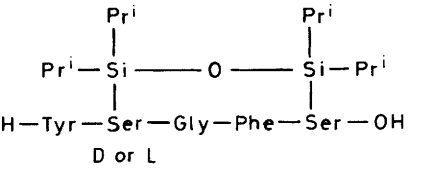


(48)



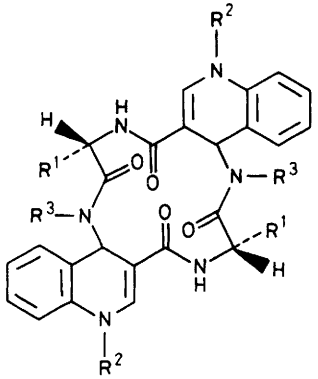
(49)

positions.[94]

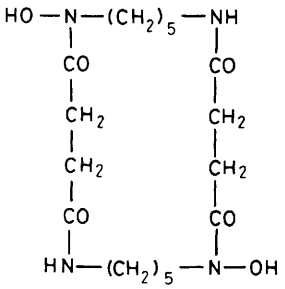
The cyclic peptide (44) was designed as a human renin inhibitor but found inactive. ^{1}H-N.m.r. indicates a cis Phe-Ala peptide bond and a preferred conformation for the macrocyclic ring.[95] A potent ACE inhibitor from Micromonospora halophytica K-13 (45) contains as ring components tyrosine and isodityrosine, the latter of unknown configuration. Isodityrosine has previously been found as a component of plant cell-wall glycoprotein.[96] The complete stereochemistry of the antibiotic WS-43708A, first reported in 1985, has now been determined (46). All the amino acid α-C's are of (S)-configuration, and the benzylic position of the C-terminal residue is of (R)-configuration. This appears to be the only antibiotic reported outside the vancomycin group to possess a biphenyl component. No evidence for binding of WS-43708A to the cell wall analogue N-Ac-D-Ala-D-Ala has been found.[97]

In order to investigate the influence of the configuration of the component amino acids on the conformation of cyclic tryptathionine tripeptides, six cyclic peptides containing D- or L-Ala and D- or L-Cys have been prepared (Table 3). The c.d. spectra of the peptides are similar, showing mirror images of the c.d. of phalloidin, but the D-Cys compounds differ from the L-Cys compounds by their weakly positive ellipticity values around around 270 nm. The cyclic hexapeptide (47) was also obtained from the D-Cys/L-Ala linear peptide when cyclisation took place in 2% solution. Its c.d. spectrum is very similar to that of phalloidin, and it forms rather a strong complex with Cu(II) ions.[98] A cyclic peptide (48) incorporating 5-(aminomethyl)-2-thiopheneacetic acid has been prepared by an azide cyclisation (Gly C-terminal) in 6.5% yield. This spacer induces a conformation not found in cyclopentapeptides, which have a comparable ring size. An intramolecular H-bridge could not be confirmed, but one of the NH protons is shielded from the solvent.[99]

Two diastereoisomeric disiloxane bridged cyclic enkephalin analogues (49) have been made. ^{1}H-N.m.r. studies support the presence of a 2 → 5 H-bonded β-bend conformation for the D-Ser compound, which was more potent than Met-enkephalin and 60 times more potent than H-Tyr-Ser-Gly-Phe-Ser-Oh. The feasibility of bridging two seryl side chains in close proximity by a disiloxane bridge was demonstrated in a preliminary experiment in which cyclo(Ser_2) was treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane to give the bicyclic compound in 25% yield.[100]



(50)



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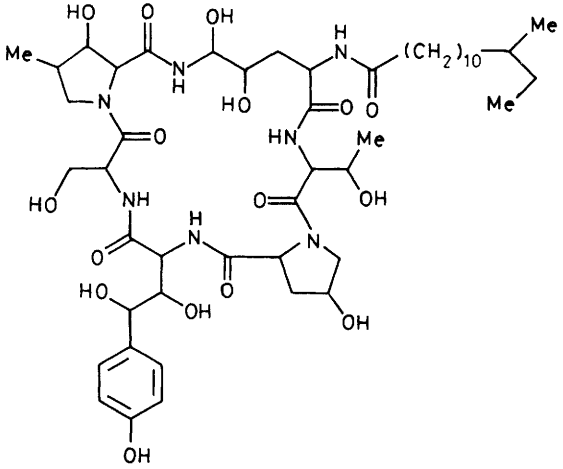

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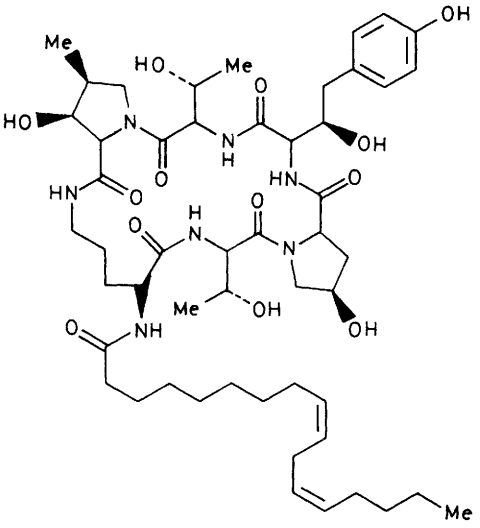


(53)

Quaternary salts of the amides of N-(3-(quinoline-carbonyl) amino acids cyclise with high diastereoselectivity under the influence of base to give 14-membered rings (50). These behave as model NAD compounds, oxidising 2-propanol in the presence of $ZnCl_2$ to give in high yield acetone and the 1,2-dihydroquinoline system.[101] From the marine bacterium Alteromanus haloplanktis isolated from deep-sea mud near Japan has been isolated bisucaberin (51), a new siderophore which sensitises tumour cells to macrophage-mediated cytolysis. It is the lower homologue of nocardamine; its activity is specifically inhibited by ferric ion.[102] The active sequence of somatostatin Phe^7-D-Trp^8-Lys^9-Thr^{10} has been cyclised through an o-(aminomethyl)phenylacetic acid unit designed to mimic Gly-Gly containing a cis-restrained peptide bond. A 2D n.m.r. study shows this biologically inactive material to have a different conformation to the bioactive one. It contains a type II' β-turn, but not the two H-bonds stabilising it.[103]

Five cyclic peptide β-turn models have been made. In cyclo($NH(CH_2)_nCOGly$-Pro-Gly-Gly), where n = 2 or 4, both compounds adopt a type II β-turn,[104] as does cyclo($NH(CH_2)_nCOGly$-Ser(OBu^t)$_2$-Gly) when n = 4. When, in the latter, n = 2, the c.d. spectrum shows a strong -ve bond near 200 nm, which is interpreted as a sign of the lack of β-turn structure in the compound.[105] Cyclo-($NH(CH_2)_4CO$-Gly-Pro-Ser(OBu^t)-Gly), however, forms a type I β-turn.[104] A new lipopeptide antifungal antibiotic from Aspergillus sydowi, mulundocandin, has been identified as (52), an echinocandin type of compound. No stereochemical assignations have yet been made. This material is active against yeasts and filamentous fungi.[106] Derivatives of the two unusual β-hydroxy amino acids in echinocandin D have been prepared by employing glycine enolate aldol methodology. These have been incorporated into a new synthesis of echinocandin D, (53) which was first made in 1986.[107]

A 2D-n.m.r. study of the gonadotropin-releasing hormone antagonist cyclo(Δ^3-Pro-D-pClPhe-D-Trp-Ser-Tyr-D-Trp-NeLeu-Arg-Pro--β-Ala) shows a mixture of the two slowly interconverting conformations. One has all-trans peptide bonds, but the other has a cis-β-Ala-Δ^3-Pro bond. Both forms consist of a type II' D-Trp-NMe--Leu and a type II Δ^3-Pro-D-pClPhe β-turn connected by extended antiparallel β-like strands.[108] Two cyclic peptides containing 5-methylanthranilic acid (MeAnt), cyclo(Phe-MeAnt-$Gly_{4 \text{ and } 6}$), have been prepared. The larger of the two acted as a reasonable substrate for chymotrypsin, suggesting that this peptide may be used as a core structure in the design of suicide substrates

Table 3a Novel renin inhibitors

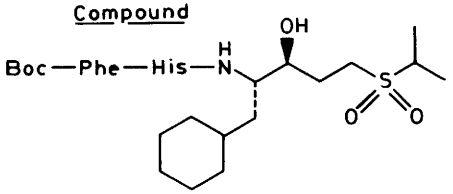
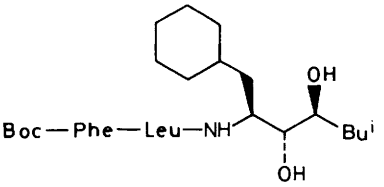
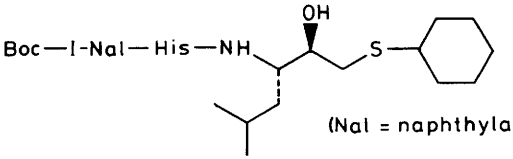
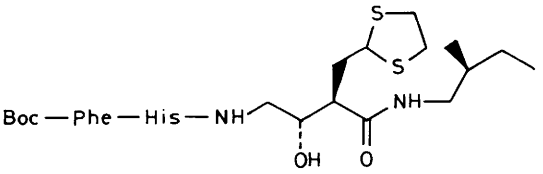
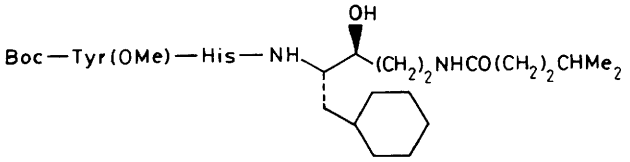
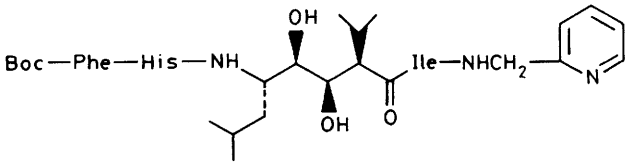
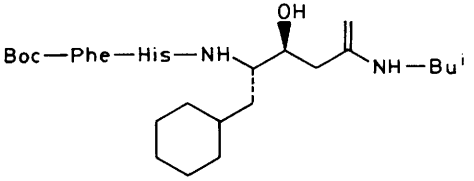
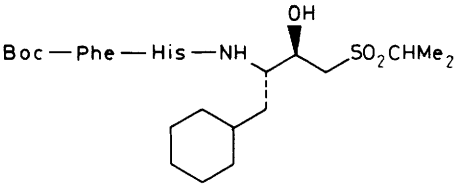
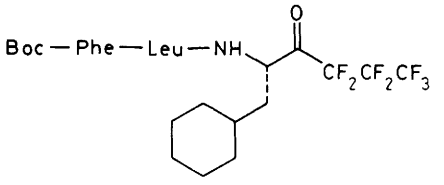
Compound	IC_{50}(nM)	Ref
	7·6	110
	0·06	111
 (Nal = naphthylalanine)	13	112
	1	113
	4	114
	0·35	115
	1·5	116

Table 3a - continued



	2·0	117



	3·0	118

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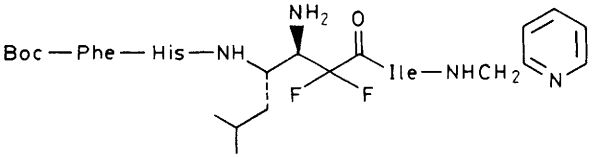

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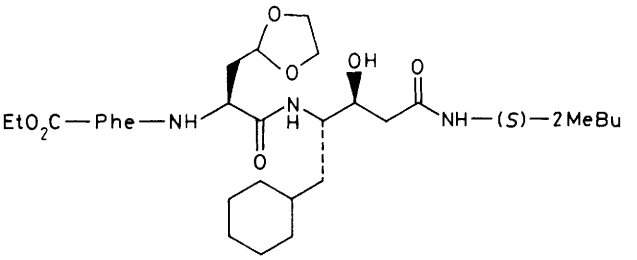
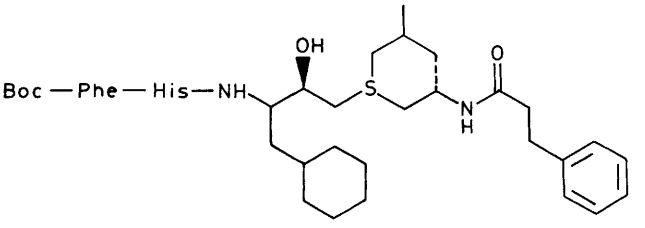
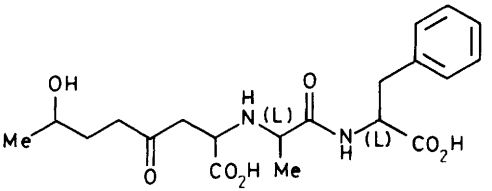


	0·8	120

Boc—α-MePro—Phe—His—Leu ψ[CHOHCH$_2$]—Val—Ile—Amp	1·8	121
Boc—Phe—His—Sta—Leu—(—)-α-phenethylamide	21	122
Boc—Phe—Phe—difluorostatone—Leu—Phe—NH$_2$	7	123



	7	123a



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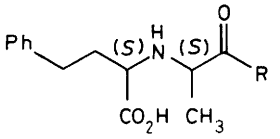
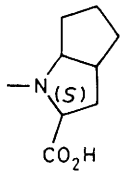
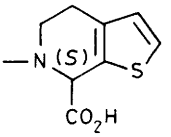


a; R = 

b; R = 

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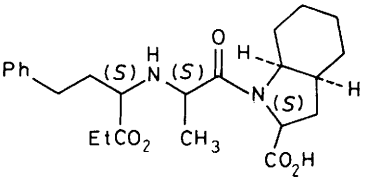
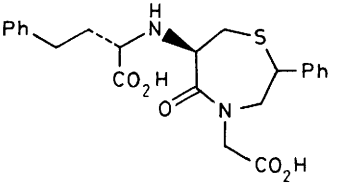


(57)



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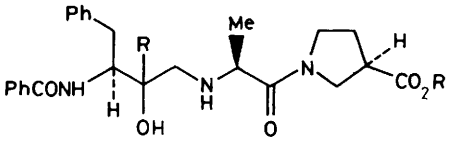
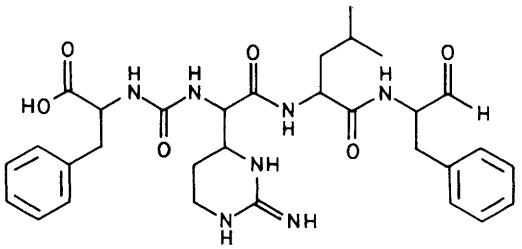


R = H or Me

(59)



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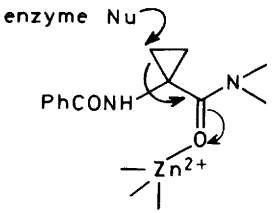

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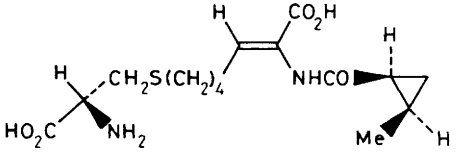


(62)

for serine proteases.[109]

3 Modified Linear Peptides

3.1 Enzyme Inhibitors- Novel renin inhibitors are listed in Table 3a. Only the most potent compound from each publication is reported. In compound (54) the electron withdrawing effect of the perfluoroalkyl group stabilises the ketone hydrate, which closely mimics the tetrahedral transition state of the hydrolysis of the scissile Leu^{10}-Val^{11} amide bond of the angiotensinogen sequence.[118] The interaction between a computer model of human renin and a number of peptides has been examined and compared to the apparent renin inhibitory effectiveness of these compounds in vitro.[124] A 2D-n.m.r. study of pepstatin and two derived renin inhibitors Iva-Val-Val-Sta-Ala-Sta and Boc-Phe-Nle-NHCH(iPr)$CHOHCH_2$CO-Ala-Sta--OMe shows for all three compounds similar extended conformations in d_6-DMSO and d_5-pyridine, with the hydrophobic lateral chains pointing away from the peptide backbone. In the case of pepstatin the conformation resembles that found in the crystal of the pepstatin-Rhizopus chinensis complex.[125] Free-Wilson and correlation analysis have been combined to study a series of 34 pepstatin N- & C-protected analogues of the type Xxx-Yyy-Sta-Ala-Sta. A statistically significant correlation was found between the inhibitory activity of the analogues in an enriched plasma preparation and structural parameters of the amino acid side-chain in position 2.[126]

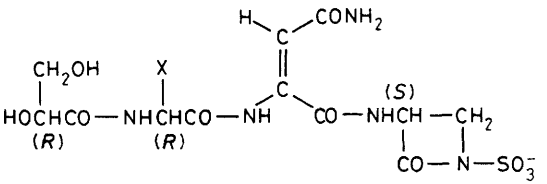
An ACE inhibitor (55) has been isolated from Daratomyces putredenis; its N-carboxymethyl dipeptide structure resembles enalapril, and it has an IC_{50} of 14 nM.[127] The X-ray structures of the ACE inhibitors ramiprilat (56a)[128] and SBG 107 (56b)[129] have been determined. To a large extent the crystal structure of the former is influenced by the non-stoichiometric content of the methanol molecules in channels. Preparation of a series of ACE inhibitors containing saturated bicylic amino acids in place of proline has led to the development of indolapril (57), IC_{50} 8 nM, which has advanced to clinical evaluation.[130] Incorporation of a perhydro-1,4-thiazepin-5-one ring has also proved effective, e.g. (58), IC_{50} 3.7 nM.[131] On the other hand, replacement of a secondary alcohol by a tertiary alcohol (59) has proved extremely detrimental to the ACE inhibitory activity of tripeptide-derived amino-alcohols.[132] Of a group of angiotensin I analogues with C-terminal phosphonic acid groups the most potent is Z-Ile-His-

-Pro-Phe-His-Leu-PO(OH)$_2$, IC_{50} 0.54 μM, which shows a similar IC_{50} -pH profile to captopril despite their structural differences.[133]

Three series of compounds have been examined as human leukocyte elastase inhibitors. Of a group of chloromethyl ketones Boc-Ala-Tyr-Leu-Val-CH_2Cl proved the most effective. The similarity of its inhibition effect on human spleen fibrinolytic protease suggests that the two enzymes have quite similar 3D-structures around the active centre.[134] Of some long-chain alkyl-amides, Boc-Val_3-$NH(CH_2)_{11}CH_3$ was the most effective (K_i 0.21 μM). This was non-toxic (LD_{50} >3.0 g/kg) after oral administration to rats and mice.[135] Finally, of a number of ω-carboxy-alkanoyl derivatives of Ala-Pro and Ala_2-Pro, the strongest inhibitor for leukocyte elastase was 4-carboxybutyryl-Ala_2-Pro-propylamide. The corresponding ethylamide was a better inhibitor of pancreatic elastase.[136]

The crystal structures of several enzyme-inhibitor complexes have been revealed this year. The active site serine of α-lytic protease from Lysobacter enzymogenes when bound to Boc-Ala-Pro-Val--boronic acid forms a covalent, nearly tetrahedral, adduct with the boronic acid moiety. The complex is stabilised by seven intermolecular H-bonds, and H-bonding between Asp-102 and His-57 remains intact. There is little change in the position of the protease residues on complex formation.[137] When rhizopus pepsin is bound to D-His-Pro-Phe-His-PheΨ[CH_2NH]-Phe-Val-Tyr the inhibitor lies within the major groove of the enzyme with the reduced peptide bond located in the active site with close contacts to the two catalytic aspartyl groups. The active site water molecule that is normally held between the two carboxyl groups is displaced by the inhibitor, as are a number of other water molecules seen in the binding groove of the native enzyme.[138] When Pro-Thr-Gly--PheΨ[CH_2NH]-Phe-Arg-Glu is bound to another aspartic proteinase, fungal endothiopepsin, it also adopts an extended conformation with the Ψ[CH_2NH] bond close to the essential aspartyl side chains of the enzyme.[139]

A ^{1}H-, ^{13}C-, and ^{19}F-n.m.r. study of a complex of α-chymo-trypsin with Ac-Leu -[1-^{13}C]Phe-CF_3 shows the CF_3CO present as an ionised hemiketal, pK_a ca. 4.9, about 4.2 units lower than the pK_a of model hemiketals. This provides direct and convincing evidence that serine proteases are able to stabilise the oxyanions of tetrahedral adducts. His-57 also participates in a tetrahedral adduct, its pK_a being elevated in the complex with Asp and Ser.[140] A kinetic investigation of the inhibition of cathepsin G by

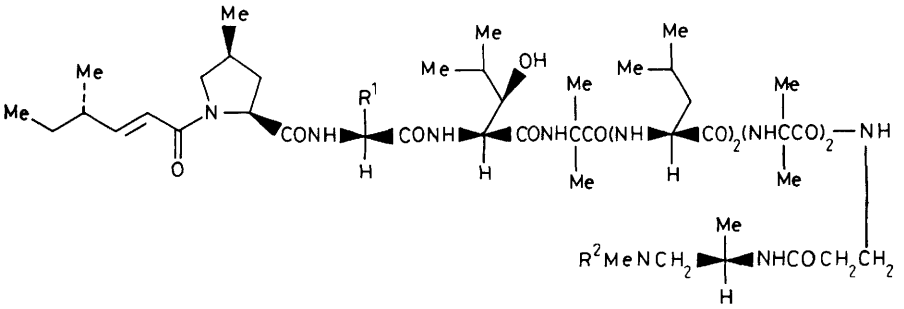


X = Me, CH_2OH, or H

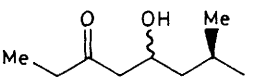
(63)

IIa	Ac-Aib-Ala_2-Aib-Aib-Gln-Aib_3-Ser-Leu-Aib-Pro-Leu-Aib-Ile-Gln_2-Trpol			
IIIa	Iva	Leu	Ile	Trpol
IIIb	Aib	Val	Leu	Trpol
IVb	Iva	Val	Ile	Trpol
Vb	Aib	Leu	Ile	Pheol
VIa	Iva	Leu	Ile	Pheol
VIb	Aib	Val	Ile	Pheol
VII	Iva	Val	Ile	Pheol

(64)



For a and b, R^1 =



For d, $R^1 = Me_2CHCH_2$—

For a and d, R^2 = Me

For b, R^2 = H

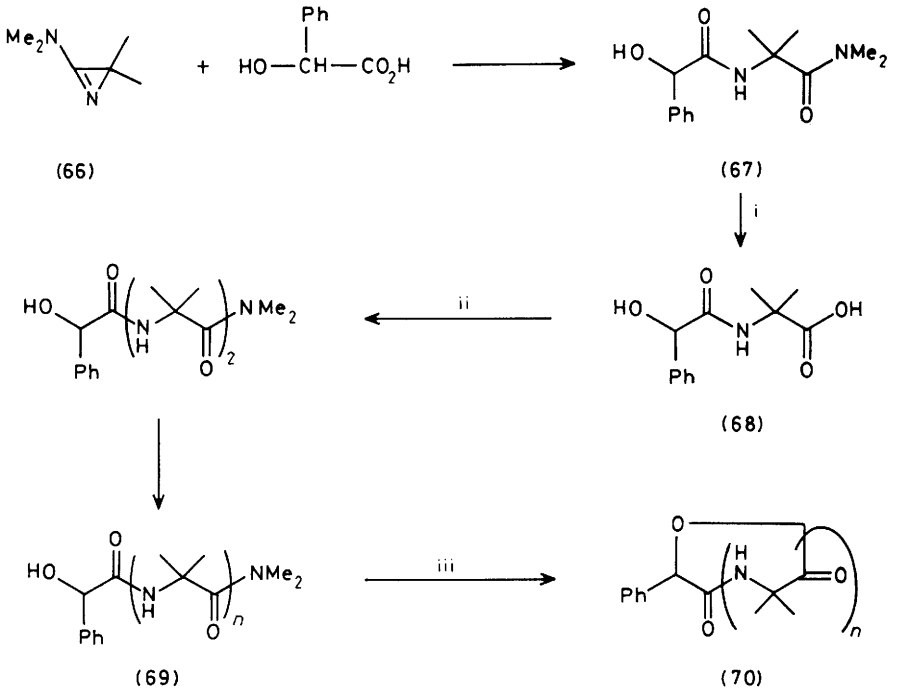
(65)

chymostatin (60) suggests a mechanism involving the general base-catalysed formation of an enzyme-bound hemiacetal, followed by a conformational change to produce the final, stable complex of enzyme and inhibitor.[141] Addition of a peptide chain to the chymotrypsin inhibitor Ac-Leu-ambo-Phe-CF_2R (R = H; K_i 25 μM) to try and utilise interactions with binding subsites on the leaving group side of the cleaved peptide has been very successful, e.g. when R = $-CH_2CH_2CONH$-Leu-Arg-OMe, K_i = 0.014 μM.[142]

Peptides containing lysine and ornithine side-chain analogues of statine have been prepared. Both Iva-Val-Val-[LySta] or [OrnSta]-OEt, where LySta and OrnSta are 4,8-diamino-3-hydroxy-octanoic acid and 4,7-diamino-3-hydroxyheptanoic acid respectively, are potent inhibitors of penicillopepsin but exceptionally weak inhibitors of porcine pepsin.[143] N-Benzoyl-1-aminocyclopropyl-carbonyl-Phe and Pro have been shown to be irreversible inhibitors of carboxypeptidase A, probably by a mechanism involving Glu-270 as a nucleophile (61).[144] β-Lactamase renal dipeptidase deactivates the potent antibiotic imipenem in the kidney, producing low antibiotic levels in the urinary tract. (Z)-2-(Acylamino)-3-substituted propenoic acids have been developed as competitive inhibitors of the enzyme. One of these compounds, cilastatin (62) has the desired pharmacological properties and has been chosen to improve the efficiency and safety margin of the antibiotic.[145]

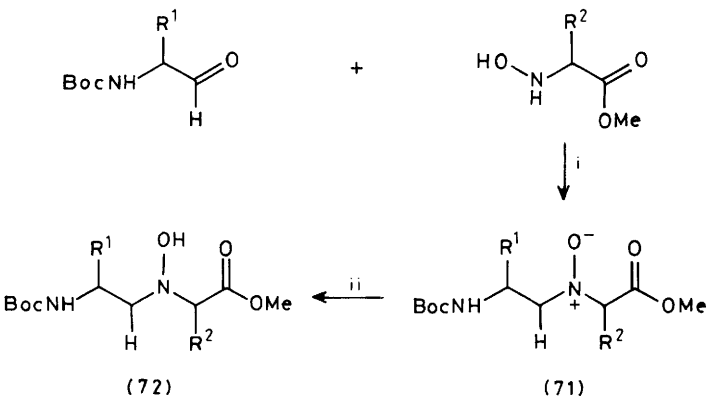
A series of N-carboxyalkyl derivatives of Leu-Ala have been synthesised and tested as inhibitors of thermolysin, but were not much more effective than compounds containing non-coordinating functionalities of similar size.[146] Caricostatin, a thiol proteinase inhibitor produced by a strain of Nigrosabulum novosp., has been identified as Ac-Leu-D,L-arginal. It differs from thiolstatin only in that Leu replaced Phe; it is only a very weak inhibitor of trypsin.[147] Five phosphorus-containing analogues Z-Gly-Leu-Xxx (Xxx = NH_2,Gly,Phe,Ala, or Leu) in which the Gly-Leu peptide bond is replaced by a phosphonate ester ($-PO_2^-O-$) have been made; their inhibition constants towards thermolysin show a direct relation with those of the corresponding phosphonamidate analogues examined previously, but they are bound about 840 times less strongly.[148] The mode of binding of Z-GlyΨ[PO_2^-O]Leu-Leu-OH to thermolysin has been determined by X-ray crystallography and shown to be virtually identical to Z-GlyΨ[PO_2^-NH]Leu-Leu-OH.[149]

3.2 Dehydropeptides- Three new monobactams containing an αβ-unsaturated residue have been isolated from Cytophaga johnsonae (63).



Reagents: i, HCl, CH_3CN, H_2O, 60–70 °C; ii, $HOCH(Ph)CO_2H$; iii, HCl, $PhCH_3$, 100 °C

Scheme 7



Reagents: i, $CaCl_2$, Et_2O; ii, $NaBH_4$, EtOH, 0° C

Scheme 8

They only have weak antibiotic activity against *E. coli*, a mutant supersensitive to β-lactam antibiotics.[150] [D-Ala^2,(2*R*,3*S*)-Acc^E Phe^4,Leu^5]-enkephalin (where Acc = 1-aminocyclopropanecarboxylic acid) and its 2*S*,3*R*-isomer have been prepared. The latter proved inactive in all bioassays, but the former showed a very high affinity for δ-receptors in rat brain and a very low one for μ-receptors. Its inactivity in both the MVD and GPI assays suggests that δ-receptors in the central and peripheral nervous systems are different.[151]

The remaining papers in this section concern the conformation of peptides containing dehydrophenylalanine. The crystal structure of Z-DL-Phe-Δ^ZPhe-OH shows the unsaturated residue to adopt an LH α-helical conformation,[152] while that of Boc-Phe-Δ^ZPhe--Val-OMe shows a sharp turn in the backbone at the αC of the dehydrophenylalanine.[153] N.O.e. studies of the latter and Boc-Phe-Δ^ZPhe-Leu or Ala-OMe in $CDCl_3$ support in all three cases a type II β-turn with one intramolecular H-bond.[154] Three cyclic pentapeptides, *cyclo*(Gly-Pro-Δ^ZPhe-D-Ala-Pro), *cyclo*(Δ^ZPhe-Pro--Gly-D-Ala-Pro), and *cyclo*(Gly_2-Δ^ZPhe-D-Ala-Pro), have been prepared and compared with their counterparts containing D-Phe in place of Δ^ZPhe. The results indicate that Δ^ZPhe is a conformationally homologous replacement for D-Phe in reverse turn regions of a peptide backbone, and it may be used as a means of encouraging the uptake of turn conformers in otherwise flexible peptides.[155]

3.3 Peptides Containing α,α-Dialkylamino Acids- The structures of a further eight trichorzianines (64), membrane active peptaibophols from *Trichoderma harzianum*, have been determined. All amino acids and amino alcohols are of the L-configuration except isovaline. The sequences were largely determined by positive ion FAB-MS.[156] Five novel Aib-containing peptide antibiotics, the leucinostatins, have been isolated from *Paecilomyces marquandii*. The structures of components A, B, and D are given in (65).[157] Leucinostatins H and K have the same sequences as components D and A respectively, but the 1-(dimethylamino)-2-aminopropane unit exists as the *N*-oxide. They have significantly lower biological activities.[158]

In MeCN at 20 °C, 3-(dimethylamino)-2,2-dimethyl-2*H*-azirine (66) will react with α-hydroxy acids to give diamides (67; Scheme 7). These can be selectively deprotected by mineral acid to give the carboxylic acids (68). Repetition of these two steps allows the build up of linear peptides (69), which can then be cyclised. In this way, cyclic peptides (70) containing 2, 3, and 4 Aib

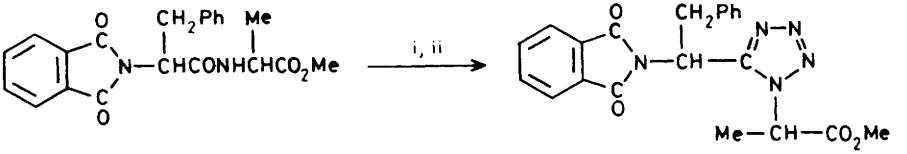
residues have been prepared.[159] Peptides containing repeated Phe-Phe-Aib sequences form very stable helices which are very soluble in a range of solvents. On coupling Boc$(Phe_2\text{-}Aib)_m$-OH (LL + DD) to H$(Phe_2Aib)_n$-OBzl (LL + DD) (m and n = 1 to 4) using DCC, diastereoselectivity for the coupling increased with the size of peptide substrates; for example for m=4, n=3, the ratio of all L- or all D-product to product containing both L- and D-residues is 8.2 to 1. This selectivity is ascribed to secondary structure-secondary structure interaction.[160]

A series of esters of L-aspartyl-1-aminocyclopropane-carboxylic acid has been prepared and their sweetness examined. The n-propyl ester is 300 times as sweet as sucrose; overall, the structure-activity relationship of these esters parallels that of some L-Asp-D-Ala esters.[161] A ^{13}C-n.m.r. study of Z-Aib_{10}-OBu^t in CH_2Cl_2 shows that at room temperature the RH and LH 3_{10} helices interconvert 1200 times per second.[162] [Aib^1,Leu^8]-Angiotensin II has been prepared by solid phase and found to be a potent inhibitor, coming close in activity to the Sar^1-analogue.[163] Some conformational studies on Aib-containing peptides are listed in Table 4.

Table 4 Application of physical methods to Aib Peptides

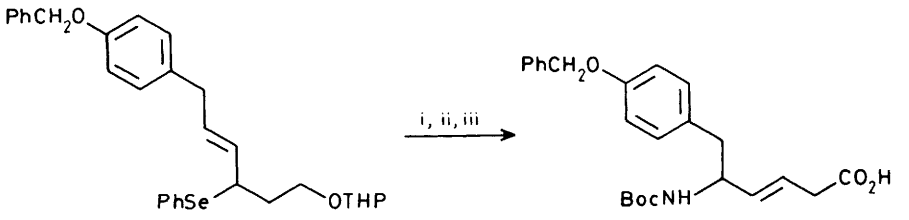
Peptide	Technique used	Ref.
Alamethicin	^1H-n.m.r.	164
Alamethicin	Raman and c.d.	165
[Aib^2]-,[Aib^3]-,[Aib^7]-, [$Aib^{2,3}$]-, and [$Aib^{2,3,7}$]-bradykinin	^1H-n.m.r. and c.d.	166
Boc-Aib-Pro-OBzl	X-ray	167
Tfa- and H-Aib_2-OBu^t	X-ray	168
Ac-Ala-Aib-OMe	X-ray	169
Z-Aib-Ala-OBu^t	X-ray	169
H-Aib_3-OH	X-ray	170
Boc-Trp-Ile-Ala-Aib-Ile-Val-Aib-Leu--Aib-Pro-Ala-Aib-Pro-Aib-Pro-Phe-OMe	X-ray	171

3.4. Amide-Bond Analogues- The preparation of N-blocked 'nitrono' derivatives (71) from Boc-α-amino aldehydes has been described (Scheme 8). So far only derivatives of amino acids with alkyl



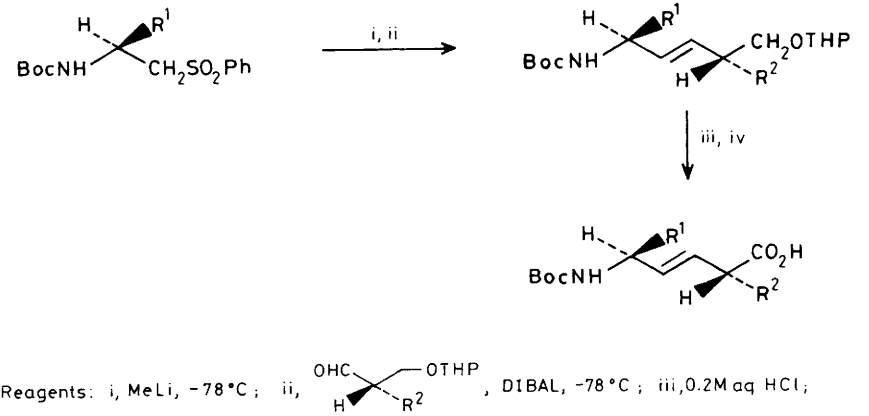
Reagents: i, PCl_5; ii, HN_3

Scheme 9



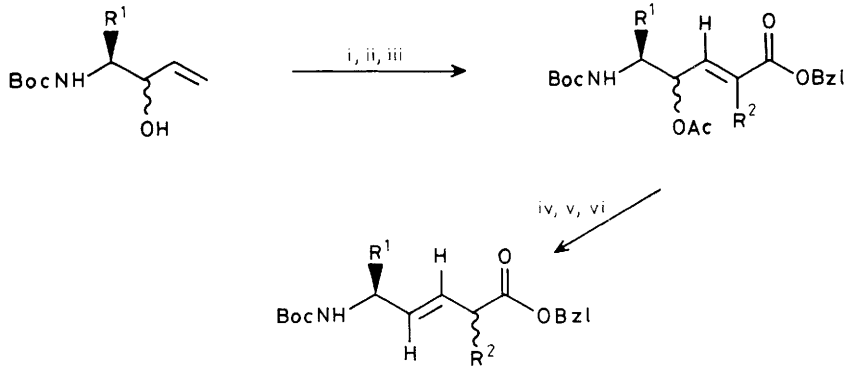
Reagents: i, Bu^tOCONH_2, NEt_3, NCS^-, MeOH, 0 °C; ii, 0·2M aq HCl; iii, Jones oxidation

Scheme 10



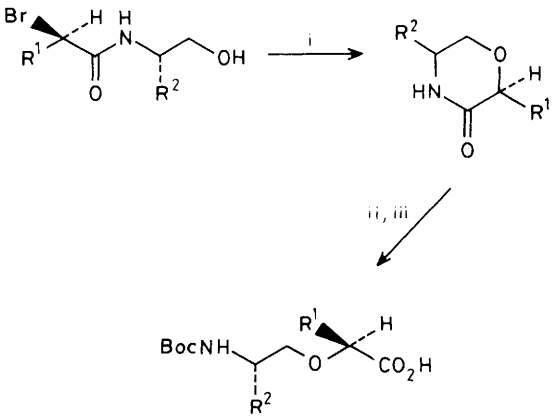
Reagents: i, MeLi, −78 °C; ii, , DIBAL, −78 °C; iii, 0.2M aq HCl; iv, Jones oxidation

Scheme 11



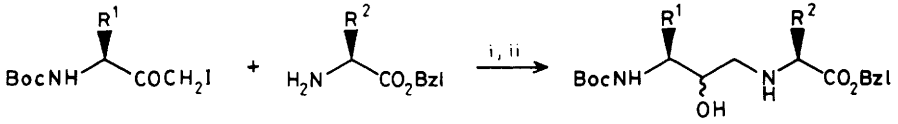
Reagents: i, Ac_2O, pyr; ii, NMO, $NaIO_4$, OsO_4; iii, $Ph_3P{=}CR^2CO_2Bzl$; iv, Na_2CO_3, MeOH, 20 °C, 45 min; v, Ph_3P, CBr_4, THF; vi, Zn, HOAc

Scheme 12



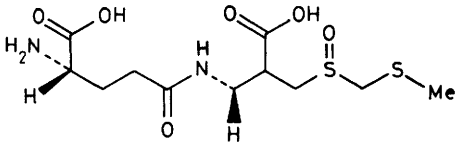
Reagents: i, NaH — THF, 0–10 °C; ii, 6MHCl, 100 °C; iii, Boc_2O, NaOH, H_2O, Bu^tOH

Scheme 13

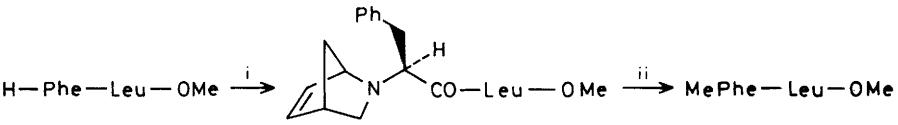


Reagents i, THF, 40 °C; ii, $NaBH_4$—MeOH

Scheme 14

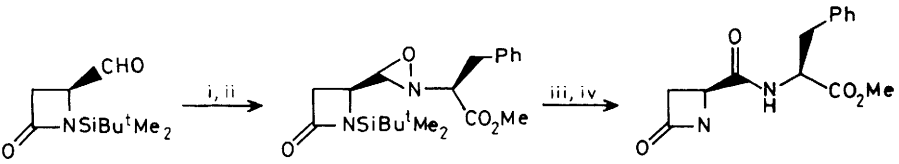


(73)



Reagents : i, cyclopentadiene—CH_2O ; ii, CF_3CO_2H, $CHCl_3$, $SiEt_3$, 20 °C, 2h

Scheme 15



(74)

Reagents: i, PhOMe, Et_2O, -20 °C, 3Å molecular sieve ; ii, MCPBA, CH_2Cl_2, 0 °C ; iii, HF, pyr ; iv, u.v., CH_2CN, 7h, 15 °C

Scheme 16

or benzyl side chains have been examined. Borohydride reduction of (71) gives methylene(hydroxyamino) dipeptides (72).[172] Another novel amide bond analogue involves replacement by a tetrazole ring in a two-step process (Scheme 9). However, racemisation of the N-terminal amino acid occurs during the conversion, and base hydrolysis of the Pht-PheΨ[CN_4]Ala-OMe causes racemisation of the C-terminal amino acid.[173] Phosphorus-containing peptide bond analogues have earlier been mentioned (Ref. 147 and 148); H-Phe or LeuΨ[PO_2^-O]Leu-OH and H-LeuΨ[PO_2NH]Leu-OH have also been examined as inhibitors of leucine aminopeptidase, but only have very modest activity. This suggests that the mechanism of action of leucine aminopeptidase may differ from other zinc peptidases.[174]

Several new approaches to more established amide bond surrogates have been developed. Three routes to trans-alkene isosteres have been reported. One route to Tyr-Gly compounds of this type involves the rearrangement of an allylic selenide (Scheme 10), while a more general route used for Tyr-Ala, Phe-Phe, Leu-Phe, and Leu-Leu is convergent and fully stereocontrolled (Scheme 11).[175] The third route (Scheme 12) has been used to prepare Asp-Phe, Leu-Asp, and Gly-Trp analogues, but in this method it has not proved possible to separate the mixture of diastereoisomeric pseudodipeptides.[176]

A short stereochemically defined synthesis of (2S,5S)-Ψ[CH_2O]--dipeptides involving an intermediate 1,4-oxazin-2-one has been developed (Scheme 13). It is not limited to unhindered amino acids; Boc-LeuΨ[CH_2O]-Val-OH has been prepared by this method, although an attempt to prepare Boc-PheΨ[CH_2O]Phe-OH failed.[177] A simple approach to the generation of the Ψ[$CHOHCH_2$] isostere, applicable to a wide range of substituents, has also been reported (Scheme 14). The product, however, is a mixture of diastereoisomers.[178] Reduction of N-protected dipeptide t-butyl esters with diborane has been found a simple and general method of preparing Ψ[CH_2NH] peptides; in this way [PheΨ[CH_2NH]$Leu^{4,5}$]-enkephalin has been synthesised.[179] Other papers concerning peptide bond analogues are listed in Table 5.

Table 5 Studies on pseudopeptides		Ref.
Z-GlyΨ[CSNH]Gly-OBzl	X-ray	180
Z-GlyΨ[CSNH]GlyΨ[CSNH]-Gly-OBzl	X-ray	180

3.5 γ-Glutamyl Peptides- A dipeptide has been isolated from Basidiomycetous mushrooms such as Marismius Alliaceus which are known for their garlic-like odour. This has now been identified as γ-glutamylmarasmine (73) and its structure proved by synthesis. In aqueous solution it gradually decomposes with the formation of the typical odour of the parent mushroom.[192] Angiosperms can be selected for the ability to grow in the presence of normally toxic concentrations of certain trace metal ions. Addition of Cd or Cu to Cd-resistant Datura innoxia cell cultures results in the rapid synthesis and accumulation of sulphur-rich, metal-binding polypeptides. These have now been shown to be poly(γ-glutamylcysteinyl)glycines. Greater than 80% of the cellular Cd is bound to the bis- and tris-forms in Cd-resistant cells.[193]

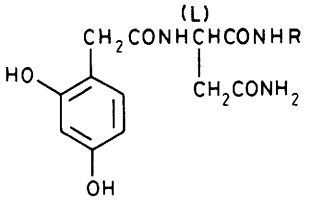
A strain of E. coli enriched in its content of γ-glutamyl-cysteine synthetase and glutathione synthetase by recombinant DNA techniques has been immobilised in a carrageenan matrix and used for the synthesis of the glutathione analogues H-γ-Glu-(β-Cl)--Ala-Gly-OH, H-(R,S)-4F-DL-γ-Glu-Cys-Gly-OH, and H-γ-Glu-HomoCys--Gly-OH.[194] The crystal structure of glutathione has been redetermined at 120 K. The overall geometry agrees with the first report, but several large differences in bond lengths and angles are pointed out. The thiol group is involved in a weak H-bond.

Clear evidence of sulphur as an H-bond donor has only previously been seen in N-acetyl-cysteine.[195]

3.6. Peptides Containing Modified Protein Constituents- A novel way of N-methylating dipeptides features a room temperature retro Diels-Alder reaction of an N-substituted 2-azanorbornene with trapping of the incipient immonium ion with triethylsilane/trifluoroacetic acid (Scheme 15).[196] The difficultly accessible tetrapeptide Z-Ala-MeLeu-MeLeu-MeVal-OBu^t, sequence 8-11 of cyclosporin, has been synthesised by a new method in 61% yield free of racemisation. Couplings were effected with bis(3-cyano-4,6-dimethyl-2-pyridinyl)disulphide and triphenylphosphine.[197] A non-classical method of peptide bond formation based on an oxaziridine-amide isomerisation has been exemplified by the synthesis of the aspartame precursor (74) (Scheme 16).[198]

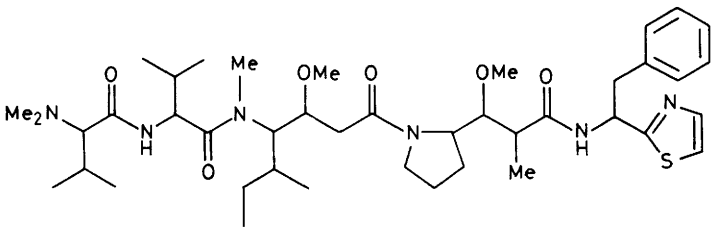
Tyrosine residues in di- and tripeptides have been converted to 4-phosphonophenylalanine by a novel two-step sequence. After conversion of the peptide to its O-triflate with N-phenyltriflimide, treatment with a mixture of diethyl phosphite, N-methylmorpholine, and $Pd(PPh_3)_4$ in CH_3CN gave the diethylarylphosphonate in ca. 80% yield.[199] Phosphoserylphosphoserine, a partial analogue of the human salivary protein statherin, has been prepared. It possesses substantial activity in assays for inhibition of both spontaneous and seeded precipitation of calcium phosphate, but is not as active as statherin or its N-terminal tryptic hexapeptide.[200] The synthesis of Ac-Leu-Arg_2-Ala-Ser(OPO_3H_2)-Leu-Gly-OMe or NHMe, a substrate for c-AMP-dependent protein kinase, has been examined with a view to large-scale preparation. Generation of the O-phosphoserine residue by phosphoric acid treatment of an aziridine residue, however, only gave 10% of the desired product.[201] New evidence now suggests the existence of a novel post-translational reaction for the conversion of L- and D-Ala in the biosynthesis of dermorphin, Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2, in frog skin.[202] Other papers concerning peptides with modified protein constituents are listed in Table 6.

Table 6 Peptides containing modified protein amino acids	Refs.
[D-Ala^2,Phe(4D)^4]-Leu enkephalin	203
Tripeptides containing p-guanido-Phe	204
N-Ac-[D-Phe(pCl)^{1,2},D-Trp^3,D-Arg^6,D-Ala^{10}]-LH-RH	205

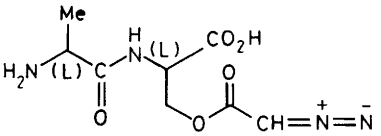


a; R = —$(CH_2)_5NHCO(CH_2)_2NH(CH_2)_4NH(CH_2)_3NH_2$
b; R = —$(CH_2)_5NH_2$
c; R = —$(CH_2)_5NHCO(CH_2)_2NH(CH_2)_3NH(CH_2)_3NH_2$
d; R = —$(CH_2)_3$—$NH(CH_2)_4$—$NH(CH_2)_3$—NH_2
e; R = —$(CH_2)_5NHCO(CH_2)_2NH(CH_2)_4NH$—Arg—H

(75)



(76)



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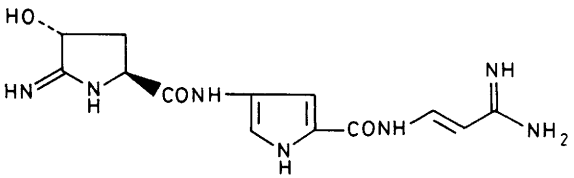

(78)

[(3'-^{125}I)D-Tyr^{0},(4'-N_3)Phe^{8},Nle^{11}]-substance P 206

[D-Ala^{2},p-N_3-Phe^{4},Met^{5}]-enkephalin 207

[4-Br-Phe^{4},Met^{5}]-enkephalin 208

H-Val-Tyr(PO_3H_2)-Phe-OH and H-Arg-Tyr(PO_3H_2)-Val-Phe-OH 209

Boc-Phe-Tyr(SO_3H)NHNHPh and Boc-Tyr(SO_3H)Leu or MetNHNHPh 210

Boc-Tyr(PO_3H_2)-Leu-NHNHPh 210

[D-Ala^{2},Met^{5}]-enkephalinamides containing m-Tyr,β-Me-m-Tyr, N-Phenethyl-m-Tyr, and α,β-diMeTyr 211

[Deamino^{1},Lys(N^{E}-4-azidobenzoyl)^{8}]-vasotocin 212

H-Gly-Glu-Gla_2-Leu-Gln-Gla-Azn-Gln-Gla-Leu-Ile-Arg-Gla-Lys--Ser-Asn-NH_2 (Conotoxin GV. Gla = γ-carboxy Glu) 213

H-Lys-Trp(X)-OMe (X = PhS, 4-$NO_2C_6H_4S$-, 2-$MeCONHC_6H_4S$-, Me_3CS-, or $MeO_2CCH_2CH_2S$-) 214

pGlu-Gln-Phe-MePhe-MeGlu-Leu-Met-NH_2 214a

Complete series of mono-N-methyl derivatives of H-Leu-Arg_2--Ala-Ser-Leu-Gly-OH 214b

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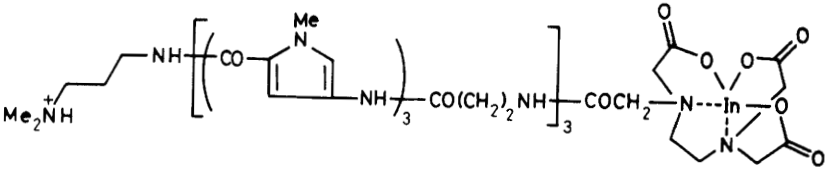
3.7 Peptides Containing β- and Higher Amino Acids- Of a series of analogues of CCK (30-33) of the type Boc-Trp-NH-$(CH_2)_n$COPhe-NH_2 there is modest affinity for CCK central receptors when n = 2 or 3, but when n = 4 the peptide is inactive.[215] A series of dipeptides containing C-terminal γ-amino-γ-hydroxybutyric acid, β-alanine, and ε-aminocaproic acid have been prepared. They all show immunocrossreactivity with antiserum against GABA.[216] A toxin from the venom of the Jaro spider Nephila Clavata, JSTX-3 (75a), has been synthesised, together with some analogues (75b to d). The toxin is of interest because it specifically inhibits the glutamate receptor. The essential structure for this action seems to be the 2,4-dihydroxyphenylacetyl-Asn-cadaverino moiety, the long polyamine chain causing increased affinity for the receptor.[217] A closely related neurotoxin NSTY-3 (75e) from the Papua New Guinea spider Nephila maculata, which contains an N-terminal arginine residue, has also been synthesised.[218] In agreement with earlier calculations, X-ray work shows that both molecules in the asymmetric unit of the crystal of Boc-L-aminosuccinyl-Gly-Ala-OMe adopt a type II' β-turn configuration with an intramolecular 4 → 1 H-bond.[219]

3.8 Peptides Containing Other Unusual Amino Acids- From the Indian ocean sea hare Dolabella Auricularia, a previous source of a number of peptides, has now been isolated dolastatin 10 (76) the



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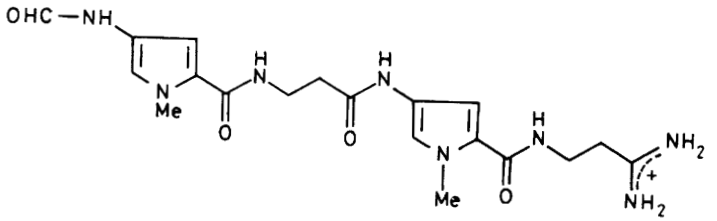

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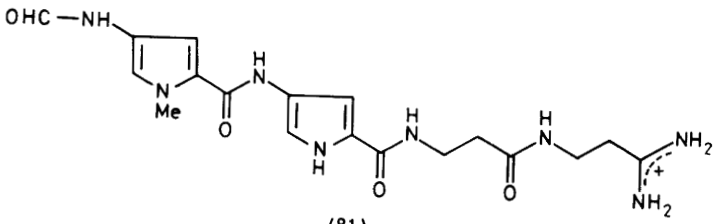

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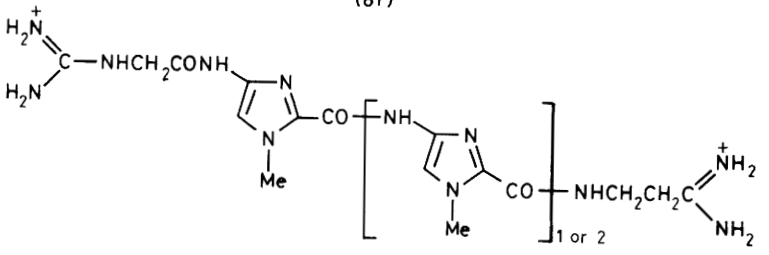
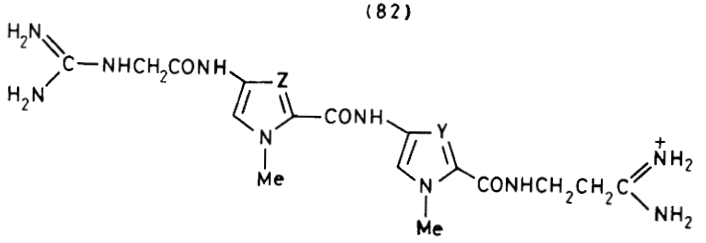


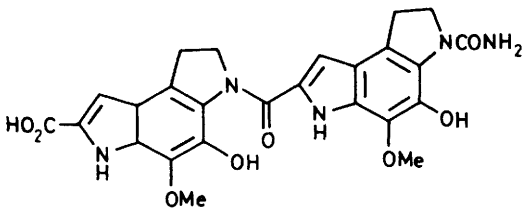
(82)



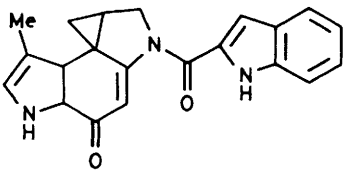
a; Y = CH, Z = N

b; Y = N, Z = CH

(83)



(84)



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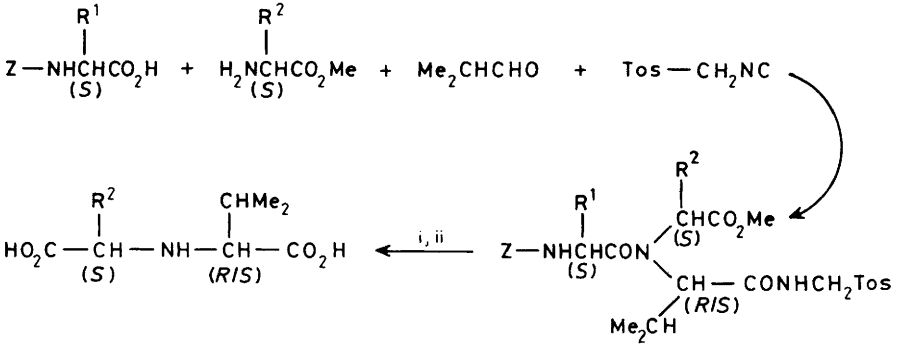
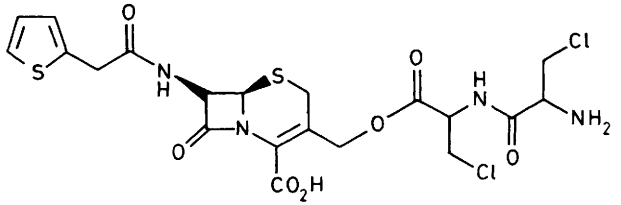


Reagents: i, 6M HCl, 110°C, 20h; ii, amberlite CG—120

Scheme 17



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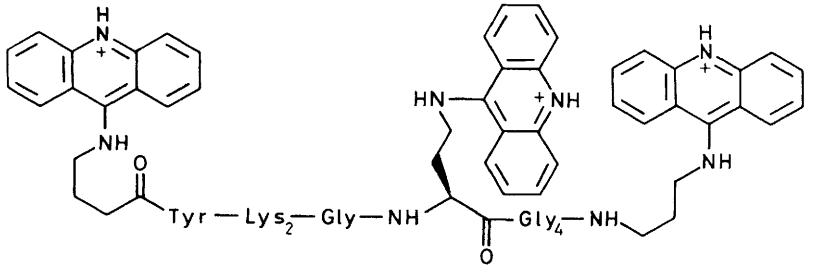
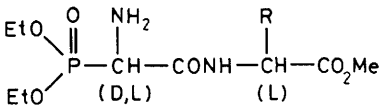


(87)

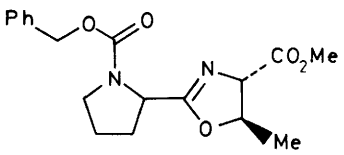
most active antineoplastic substance presently known. It is present naturally only in very low concentrations (total peptides 1 mg/100kg). The stereochemistry is not yet fully known.[220] Three new naturally-occurring dipeptide antibiotics have been reported. The first, from Glycomyces harbinensis obtained from a soil sample from Harbin, China, has structure (77). It shows greater antibacterial action than azaserine, with which it co-occurs.[221] Nitropeptin, from Streptomyces xanthochromogenus collected in Japan, has been identified as N-L-leucyl-β-nitro-glutamic acid. In base it exists as the aci-nitro derivative.[222] Streptomyces idiomorphus, from Formosa, has yielded a new pyrrole-amidine antibiotic TAN-868A (78). It has cytoxic activity against marine tumour cells. DNA thermal denaturation studies suggest that it preferentially reacts with AT-rich regions of double-stranded DNA. Also isolated was the known compound kikumycin A, which lacks the hydroxyl of TAN-868A.[223]

There have been several synthetic studies on pyrrole-containing peptides. A trimer of tetra-N-methylpyrrolecarboxamide coupled head to tail by β-alanine (79) has been prepared. Attachment of EDTA to the N-terminus allows the use of the affinity cleaving method to determine the binding site size, sequence, orientation preference, and groove location. The peptide proved capable of binding 16 base pairs of contiguous A,T-DNA in the minor groove.[224] Two synthetic analogues of distamycin containing β-alanine in place of N-methylpyrrole, (80) and (81), bind to poly(dA-dT) in a similar way to distamycin, but the complexes are less stable. This indicates that the extended conjugation of adjacent pyrrole units imparts stability to the complexes but does not play a significant role in the AT-specific nature of the interaction.[225] New netropsin analogues (82) and (83) bearing one or more imidazole rings in place of pyrrole have been prepared, but their DNA specificity has not been reported yet. During the synthesis, reduction of nitroimidazole derivatives with stannous chloride was found to cause a novel chlorination of the imidazole nucleus.[226]

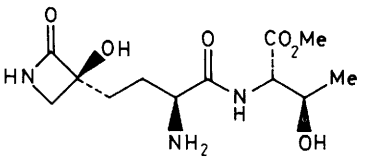
The complete centre and RH section of the anti-tumour antibiotic CC-1065, known as PDE-I dimer (84), has been synthesised.[227] A model (85) of the same part of the molecule has been independently prepared.[228] Peptides containing α,α'-iminocarboxylic acids have been approached by the Ugi reaction (Scheme 17). All products were obtained as a mixture of two diastereoisomers.[229]



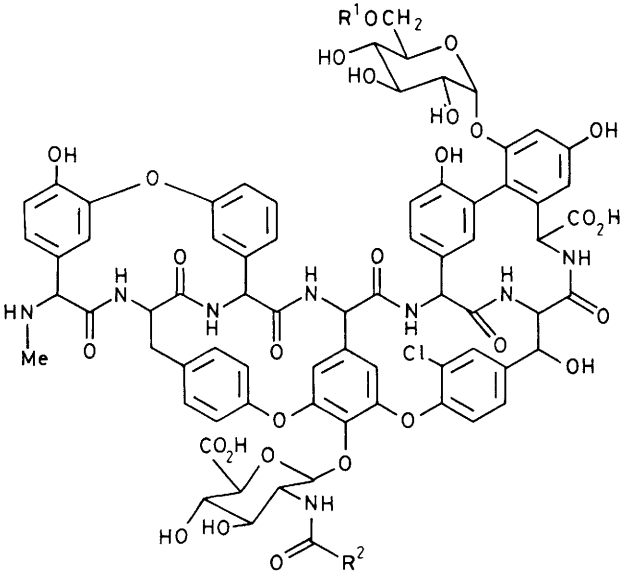
(88)



(89)



(90)



	R^1	R^2
A	H	$-nC_9H_{19}$
B_1	H	$-(CH_2)_7CHMe_2$
B_2	H	$-nC_{10}H_{21}$
C_1	H	$-(CH_2)_8CHMe_2$
C_2	H	$-nC_{11}H_{23}$
C_3	COMe	$-(CH_2)_8CHMe_2$
C_4	COMe	$-nC_{11}H_{23}$

(91)

In an attempt to capitalise upon the lactamase-initiated fragmentation of cephalosporins, a peptide C_{10}-ester of deacetylcephalothin with an antibacterial dipeptide has been constructed (86). The product does display good antibacterial activity, but at high concentrations (>10 mM) it is unstable, undergoing intramolecular aminolysis; this constrains its potential therapeutic utility.[230]

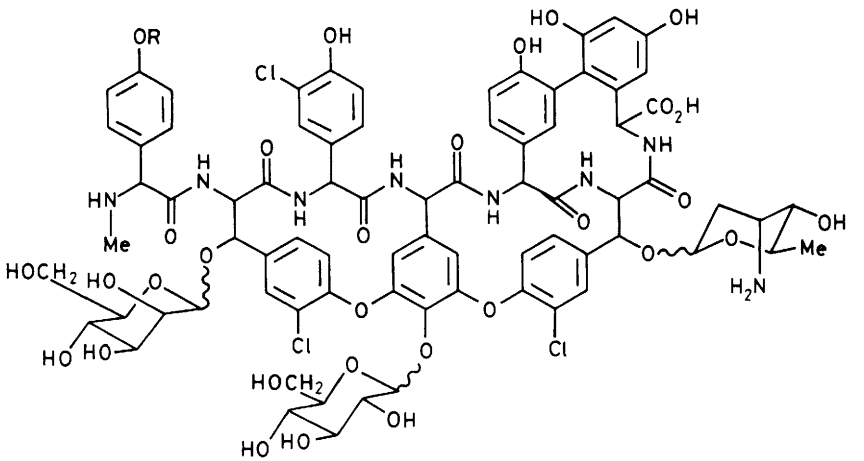
A novel peptide-linked triacridine (87) has been prepared for use in investigating intercalation into DNA, but no binding details are given.[231] [HCy^{11}]-Substance P (Hcy = homocysteine) has been made and converted with [^{11}C]-MeI into [S-methyl-^{11}C]-substance P. The total time of preparation from the start of the synthesis of [^{11}C]-MeI was 45-60 minutes.[232] N^3-(4-Methoxyfumaryl)-L-2,3-diaminopropionic acid (FMDP) was earlier shown to be an irreversible inhibitor of glucosamine-6-phosphate synthetase from bacteria and fungi. A series of 40 dipeptides incorporating FMDP have now been prepared. The most active compounds were those with FMDP C-terminal, the N-terminal amino acid bearing an aliphatic side chain. The highest activity was seen with norvaline.[233]

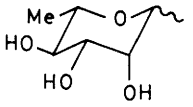
A number of other interesting dipeptides have been made for a variety of reasons. A series of dipeptide triesters (88) has been reported (R = side chain of hydrophobic protein amino acids). The Gly-Val analogue was hydrolysed to the free dipeptide with bromotrimethylsilane, but the product could not be obtained pure.[234] The modified threonine dipeptide (89) has been shown by ^{1}H-n.m.r. to exist at 20 °C as a mixture of *cis* and *trans* isomers of the Pro peptide bond.[235] 2-Bromo-N-benzylisobutyramide reacts with N-protected amino acids in the presence of silver oxide to give depsipeptide derivatives in high yields in 3 h at 20 °C without racemisation.[236] Labelling studies have shown that tabtoxin (90) is built up from Thr, Asp, and a C_2-unit derived from the C_3-pool with the Me-group of Met providing the carbonyl C-atom of the ring. These findings represent a novel pathway in β-lactam biosynthesis.[237]

Other work in this field is listed in Table 7.

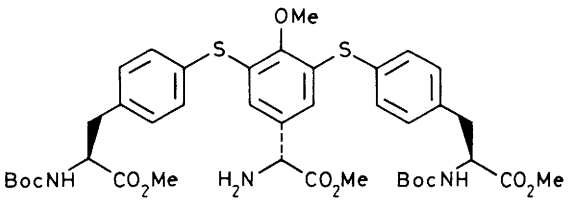
Table 7 Peptides Containing Other Unusual Amino Acids

[Arg^3,D-Phenylglycine^6]-, [Arg^2,D-Homoglutamine^6](3-10) - [Arg^2,D-Homoglutamic acid^6]-(2-10)-neurokinin B	238

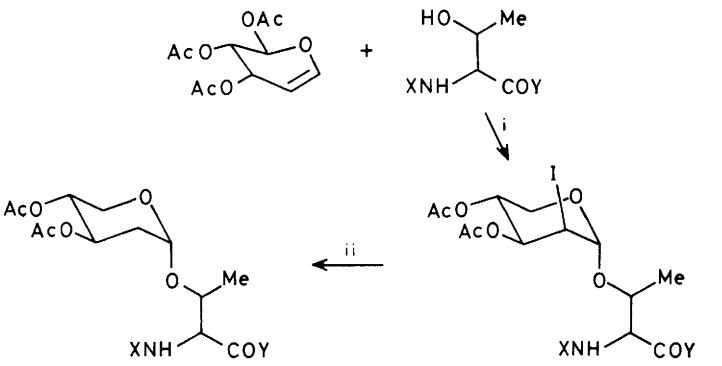


B; R =  C; R = H

(92)



(93)



Reagents: i, *N*-iodosuccinimide, MeCN; ii, Bu^n_3SnH, azobisisobutyronitrile

Scheme 18

4 Conjugate Peptides

4.1 Glycopeptide Antibiotics- The increasing importance of vancomycin for the treatment of methicillin-resistant staphylococcal infections has stimulated the search for novel members of the glycopeptide antibiotics. A new species Actinomadura parvostata has yielded the parvocidins (91), active against a range of Gram-positive bacteria. The most active, parvocidin C, produces a high serum level in vivo and has the potential for a long duration of action. The O-acetyl functionality present in two of the series is a structural feature unique among the known members of this class of antibiotic.[251] Four new members of the chloropolysporins from Faenia interjecta have been identified. Two minor components, D and E, are the epimers of the major components B and C (92). These compounds have the same aglycone as β-avoparcin, and their structures are closely related.[252]

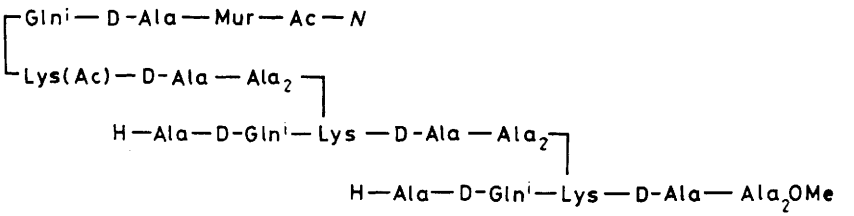
Two other novel glycopeptide antibiotics have been obtained from the Actinomadura strain ATCC 39726. These compounds, factors A and B (whose structures are based on the skeleton of (91)), are the first members of the vancomycin family to possess activity against the Gram-negative coccus responsible for gonorrhoeae.[253] A 2D-n.m.r. study has now given the complete structures of both actinoidin A and actinoidin A$_2$. Hitherto the location of the chlorine atom, the placement of the sugars, and the configurations of the anomeric linkages were unknown.[254] An

unidentified Nocardia sp. has yielded actinoidin A and a novel analogue actinoidin A_3. This latter analogue was isolated on a specific glycopeptide affinity column Affigel-10-D-Ala-D-Ala-OH. It differs from actinoidin A in containing rhamnose instead of acosamine, and has a somewhat lower antibiotic activity.[255]

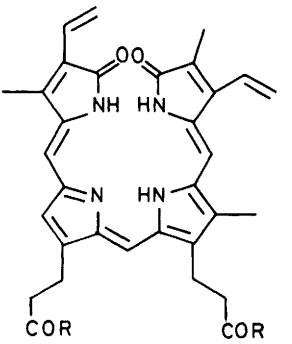
Catalytic hydrogenation of the aridicin aglycone progressively removes the halogen atoms, giving five compounds containing from one to four chlorine atoms whose structures were determined by FAB-MS and 2D ^{1}H-n.m.r. The antibiotic activity decreased as chlorine was replaced by hydrogen. It is thought that the halogen atoms provide a certain degree of conformational rigidity to the glycopeptide framework which is beneficial to the binding interaction.[256] A procedure has been developed for the selective removal by Edman degradation of the N-terminal residue of vancomycin. The resulting hexapeptide should be amenable to systematic modification at its N-terminus. Analogues are of great potential as bacteria do not appear to develop efficient resistance mechanisms to vancomycin.[257]

Hydrolysis of teicoplanin in homogeneous solution gives two diastereoisomeric compounds as a result of an amide bond fission as well as sugar removal. In a biphasic hydroalcoholic medium, however, a single aglycone is produced, the peptide bonds remaining intact.[258] Derivatives of p-mercaptophenylalanine and iodotyrosine or iodohydroxyphenylglycine are efficiently converted to thioethers by a photoinitiated $S_{RN}1$ reaction in ammonia. Products such as (93) were prepared by this route. This compound is the thioether analogue of the bisdiphenyl ether substructure of ristocetin.[259]

A novel bleomycin analogue, lumibleomycin, which bears a new ring system thiazolylisothiazole as the DNA binding site, has been prepared by 302 nm irradiation of a 1:5 peplomycin:Cu(II) system in 30% yield. Binding studies indicate that the bithiazole moiety of bleomycin participates in, but does not wholly determine,the site recognised along the strand.[260] New side chain modified bleomycins have been prepared by the reaction of demethylbleomycin A_2 with α-bromoacetamides. The modified compounds are as effective as bleomycin itself towards B16 mouse melanoma cells in vitro. Three of the compounds were tested for pulmonary toxicity, but gave rise to fibrosis of the same order as the parent.[261]

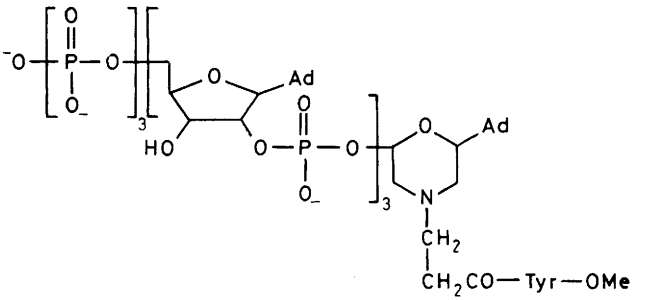


(94)

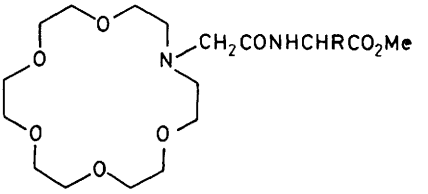


R = dipeptide or tripeptide chain containing Gly, Ala, and Val residues

(95)



(96)



(97)

4.2 Other Glycopeptides- The synthesis of glycopeptides has been reviewed with particular reference to amino acid protecting groups.[262] A procedure using N-iodosuccinimide and glycols to prepare glycosidated peptides (Scheme 18) gave good yields of products even from pentapeptides with nearly complete diastereoselectivity. Boc-Gly-Phe-Leu-Ser-Gly-OMe, for example, was glycosidated in >50% yield.[263] Some modified rigins in which either D-gluconic acid or 2-amino-2-deoxy-β-D-glycopyranose is linked to the parent molecule through amide bonds have been prepared. Of the compounds made, H-Thr-Lys-Pro-Arg-NH-Glc and N^{α}-glycosyl-Gly-Glu-Pro-Arg-OH slightly enhanced phagocytosis (mouse peritoneal macrophages), while H-Thr[(α+β)-O-glucosyl]--Lys-Pro-Arg-OH was found to displace ^{3}H-tuftsin even better than tuftsin but lacked the ability to stimulate phagocytosis.[264]

^{1}H-N.m.r. studies on Ac-Gal-NAc derivatives of Z-Thr-OMe, Z-Thr-Ala-OMe, and Z-Ala-Thr-OMe indicate the possibility of a hydrogen bond between the ThrNH and the carbohydrate N-AcCO. This implies a conformation in which the sugar moiety is restricted to an orientation with its plane roughly perpendicular to the peptide backbone. In such an orientation, steric problems will be minimised in the case of clustered O-glycosidically-linked Thr(Ser) residues as found in human erythrocyte glycophorin.[265] A glycopeptide comprising the sequence 43 to 48 of asialoglycophorin A has been made,[266] and H-Tyr(β-D-Glc)-Gly-Gly-Phe-Leu-OH prepared. The latter is inactive in the GPI assay, but a full agonist in the MVD assay, albeit 1000 times less potent than Leu-enkephalin.[267]

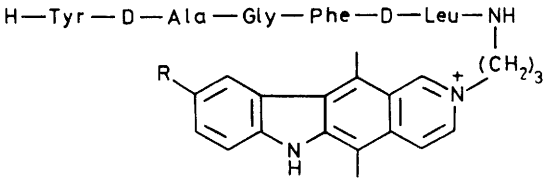
Two glycopeptides associating LH-RH with MDP have been prepared, using lysine and lysine amide as linkers. Both compounds are able to induce anti LH-RH antibodies and immunological castration; they retain the immunoadjuvant activity of MDP.[268] To clarify the relationship of pyrogenic and immunoadjuvant effects of lysine-containing peptides of bacterial cell walls, a series of glycopeptides, the largest of which was (94), have been synthesised and their activities examined. The biological effects of linear and branched peptides clearly differ, and overall the results indicate ways to separate the two types of activity.[269]

N-Ac-Mur-Ala-D-iGln-Phe(3'-iodo-4'-azido)-OMe has been made for photolabelling of receptors. It appears to have a similar biological activity to MDP.[270] New synthetic immuno-

modulators in which a 4-O-phosphono-D-glucosamine derivative related to bacterial lipid A is combined with 1-deoxy-N-acetyl-muramyl dipeptide show promising activity in preliminary studies.[271] MDP and its analogue murabutamide (N-AcMur-Ala-D--Gln-OnBu) have been studied by 2D ^{1}H-n.m.r. at 500 MHz. The results suggest the presence in MDP of two successive turns, whereas only one turn exists in murabutamide. This latter turn mimics the type II β-turn found in L-D depsipeptides, whereas the other is a typical type II β-turn for L-D peptides.[272]

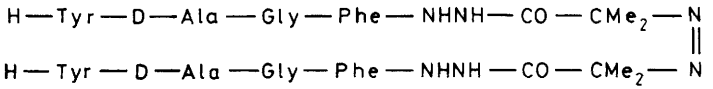
4.3 Non-Carbohydrate Conjugate Peptides- Di- and tripeptides have been attached to biliverdin (95). A spectroscopic investigation of these conjugates shows that the chiral discrimination of the helical, optically labile, bilatriene backbone increases with the number of amide groups per side chain. The preferred helicity of the bilatriene is primarily determined by the presence of chiral centres between hydrogen bonding amide groups.[273] A β-alanyltyrosine derivative of 2',5'-tetraadenylate--5'-phosphate (96) has been prepared. It binds tightly to the 2',5'-oligoadenylate-dependent endoribonuclease in rabbit reticulocyte lysate or mouse L cell extract and inhibits protein synthesis of mouse L cells more effectively than the unmodified 2',5'-tetraadenylate 5'-phosphate.[274] The macrocycles aza-18--crown-6 and 4,13-diaza-18-crown-6 have been alkylated with N-chloroacetyl amino acid esters to give one and two-armed lariat ether peptide derivatives, eg. (97). A substantial selectivity for K^{+} over Na^{+} is observed for the single armed compounds but not for the 4,13-diaza-18-crown-6 derivatives.[275]

Two fluorescent analogues of thymopentin (H-Arg-Lys-Asp--Val-Tyr-OH) have been prepared by reaction of the pentapeptide with fluorescein isothiocyanate and stilbene isothiocyanate. Both derivatives still induce the expression of Thy 1·2 surface marker on nude mouse prothymocytes both *in vivo* and *in vitro*. Amino acid analysis after 6M HCl hydrolysis indicates that the isothiocyanates have reacted with the α-amino group of the arginine.[276] H-Lys-Pro-Val-NH_2 is the C-terminal sequence of α-melanotropin, and both the α-mono and α,ε-di(2-chloroethyl)-nitrosocarbamoyl derivatives have been found to be potent anti-neoplastic agents.[277] Of a series of tripeptides containing an N- (1-methoxycarbonylalkyl)carbamoyl (Mcc) group and a C-terminal α-azanorvaline residue, the most potent inhibitor of human

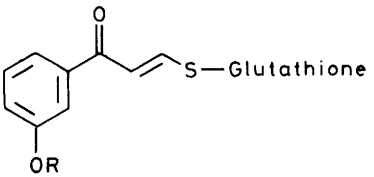


R = H or OH

(98)

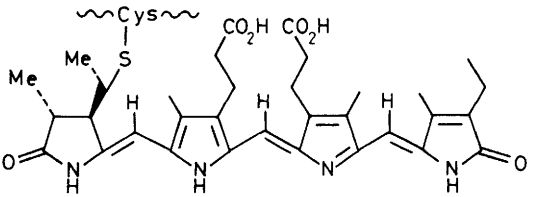


(99)

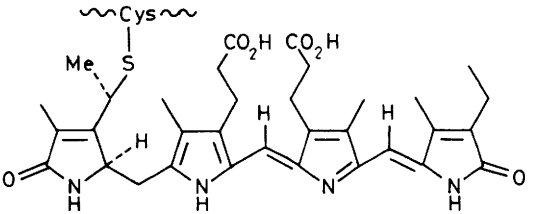


R = n-C_6H_{13} or n-C_8H_{17}

(100)



(101)

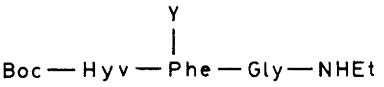


(102)

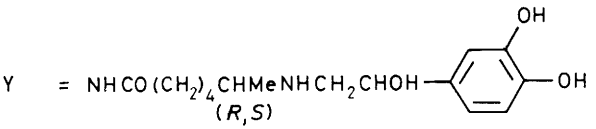
leucocyte elastase was Mcc-Val-Pro-azaNva-OPh (IC_{50} 0.28 μM, K_i 0.02 μM).[278] A series of N-acyl analogues of the bovine atria peptide H-Gly-Pro-Hyp-Gly-Ala-Gly-OH, which shows a protective effect against experimental drug-induced arrhythmia in the hearts of mammals, have been prepared. The most potent anti-arrythmic activity was shown by N-3-(4-hydroxyphenyl)-propionyl-Pro-Hyp-Gly-Ala-Gly-OH, which was ten times as potent as the natural material.[279]

The fluorescent dye Rhodamine 110 linked to a peptide to give $(Z\text{-Ile-Pro-Arg-NH})_2$Rh110 has been shown to bind to porphyrins either as the free bases or when chelated to iron ions such as in cytochrome C. The results may help to explain the alteration of vital cellular functions by rhodamines.[280] In order to specifically direct cytotoxic agents against tumour cells bearing δ-opioid receptors, the DNA intercalating agents ellipticine and 9-hydroxyellipticine have been coupled by quaternisation to an enkephalin analogue to give the conjugate (98). The ellipticine ring of the adduct still intercalates effectively into DNA, and the compounds bind to the opioid receptor from rat brain and NG 108-15 cells with an affinity constant close to 10^{-8} M^{-1}.[281] A hydrazide derivative of an enkephalin analogue (99) has been copolymerised with N-vinyl-imidazole. On central administration the copolymer has a similar analgetic activity to (99), but on systemic administration the polymer conjugate was virtually inactive.[282] As an analogue of the N-terminal part of the lipoprotein from the outer membrane of E. coli, S-[2,3-bis(palmitoyloxy)-[2-R,S]-propyl]-N-palmitoyl--Cys-αMeSer$_2$-Asn-Ala-OH has been prepared. It induces enhanced cell proliferation of spleen lymphocytes like its parent pentapeptide.[283]

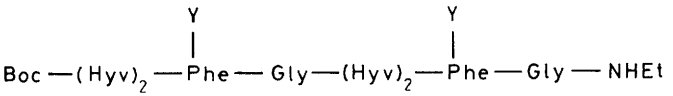
Two stable glutathione derivatives (100) have been made as reference compounds for the reverse phase hplc of leukotrienes The natural peptide derivatives have instability, storage, availability, and cost problems associated with them.[284] S-Oxalylglutathione has been found to be a very effective inhibitor of chicken liver malic enzyme, and is thought to be an important *in vivo* regulator of this enzyme. Oxalyl thiol-esters could be functioning as part of the intracellular messenger system for insulin.[285] Proteolytic digestion of the α-subunit of *Anabaena variabilis* phycoerythrocyanin has given three distinct bilipeptides, H-Asp-Gln-Ala-Ala-Cys(PCB)-Ile-



Hyv = L-α-amino-δ-hydroxyvaleric acid



(103)



(Y as in 103)

(104)

-Arg-OH, H-Gly-Asp-Cys-(PCB)-Ser-GlN-OH (where PCB = phycocyanobilin), and H-Cys(PXB)-Val-Arg-OH (where PXB - phycobiliviolinoid), the thioester links to cysteine being to ring A of PCB (101) and PXB (102).[286]

A series of novel catecholamine conjugates of peptides have been prepared in which isoproterenol has been attached to the aromatic amino group of p-aminoPhe. Their β-adrenergic activities were evaluated *in vitro* by measuring the intracellular accumulation of cyclic AMP in 549 mouse lymphoma cells. The most potent compound was (103). Multiple substitution of the pharmacophore on the same carrier, e.g. (104), did not increase the potency, in contrast to recent studies of the opioid receptor.[287]

References

1. K. Nozawa, S. Udagawa, S. Nakajima, and K. Kawai, Chem. Pharm. Bull., 1978, 35, 3460.
2. P. Waring, R.D. Eichner, U. Tiwari-Palni, and A. Mullbacher, Austral. J. Chem., 1987, 40, 991.
3. K. Sakata, M. Maruyama, J. Uzawa, A. Sakurai, H.S.M. Lu, and J. Clardy, Tetrahedron Lett., 1987, 28, 5607.
4. A. Kubo, N. Saito, R. Yamauchi, and S-I. Sakai, Chem. Pharm. Bull., 1987, 35, 2158; A. Kubo, N. Saito, M. Nakamura, K. Ogata, and S-I. Sakai, Heterocycles, 1987, 26, 1765.
5. A. Kubo, N. Saito, M. Yamato, and Y. Kawakami, Chem. Pharm. Bull., 1987, 35, 2525.
6. R. Plate, P.H.H. Hermkens, H. Behm, and H.C.J. Ottenheijm, J. Org. Chem., 1987, 52, 560.
7. G.J. O'Malley and M.P. Cava, Tetrahedron Lett., 1987, 28, 1131.
8. R.M. Harak and R. Vleggaar, J. Chem. Soc., Chem. Commun., 1987, 1568.
9. R. Plate, M.A.J. Akkerman, H.C.J. Ottenheijm, and J.M.M. Smits, J. Chem. Soc. Perkin I, 1987, 2481.
10. R. Plate. R.J.F. Nivard, and H.C.J. Ottenheijm, J. Chem. Soc. Perkin I, 1987, 2473.
11. R.K.A. Giger, H.R. Loosli, M.D. Walkinshaw, B.J. Clark, and J.M. Vigouret, Experientia, 1987, 43, 1125.
12. A. Lieberknecht and H. Griesser, Tetrahedron Lett., 1987, 37, 4275.
13. N. Shimazaki, I. Shima, K. Hemmi, and M. Hoshimoto, J. Med. Chem., 1987, 30, 1709.
14. N. Shimazaki, I. Shima, K. Hemmi, Y. Tsurumi, and M. Hashimoto, Chem. Pharm. Bull., 1987, 35, 3527.
15. C. Shin, Y. Yonezawa, and H. Ohno, Agri. Biol. Chem., 1987, 51, 2033.
16. R.M. Williams, K. Tanizawa, R.W. Armstrong, and J-S. Dung, J. Amer. Chem. Soc., 1987, 109, 4028.
17. D. Crois and H. Lehner, J. Chem. Soc. Perkin II, 1987, 1523.
18. R. Jankowska and J. Ciarkowski, Int. J. Pept. Protein Res., 1987, 30, 61.
19. M. Gdaniec, B. Liberek, A.S. Kolodziejczyk, R. Jankowska, and J. Ciarkowski, Int. J. Pept. Protein Res., 1987, 30, 79.
20. C.H. Gorbitz, Acta. Chem. Scand., 1987, B41, 83.
21. V.M. Padmanabhan and V.S. Jakkal, Acta. Crystallogr., 1987, C43, 349.
22. M. Gdaniec and B. Liberek, Acta. Crystallogr., 1987, C43, 982.
23. C. Shin, Y. Yonezawa, and K. Ogawa, Heterocycles, 1987, 26, 8.
24. I.A. Natchev, Synthesis, 1987, 1079.
25. G.G. Smith and R. Baum, J. Org. Chem., 1987, 52, 2248.
26. J. Tritt-Goc, R. Goc, E. Pannusamy, and D. Fiat, Int. J. Pept. Protein Res., 1987, 29, 406.
27. J. Pospisek and K. Bláha, Coll. Czech. Chem. Commun., 1987, 52, 514.
28. F. Pinnen, A. Di Muro, G. Zanotti, and G. Lucente, Int. J. Pept. Protein Res., 1987, 30, 388.
29. U. Schöllkopf, S. Grüttner, R. Anderskewitz, E. Egert, and M. Dyrbusch, Angew. Chem. Intl. Edn, 1987, 26, 683.
30. U. Schollkopf, R. Hinrichs, and R. Lonsky, Angew. Chem. Intl. Edn., 1987, 26, 143.
31. U. Schöllkopf, W. Kühnle, E. Egert, and M. Dyrbusch, Angew. Chem. Intl. Edn., 1987, 26, 480; R.R. Schmidt and A. Koln, ibid., 482.
32. N. Manesis, M. Hassan, R. Glaser, and M. Goodman, Biopolymers, 1987, 25, 597; N. Manesis and M. Goodman, J. Org. Chem., 1987, 52, 5331.
33. N. Manesis and M. Goodman, J. Org. Chem., 1987, 52, 5331.
34. C. Ramakrishnan, K. Ramnarayan, and G. Manjula, Int. J. Pept. Protein Res., 1987, 29, 657.
35. B.C. Wilkes and P.W. Schiller, Biopolymers, 1987, 26, 1431.
36. P.W. Schiller, T.M-D. Nguyen, L.A. Maziak, B.C. Wilkes, and C. Lemieux, J. Med. Chem., 1987, 30, 2094.

37. F. Heitz, F. Kaddari, A. Heitz, H. Raniriseheno, and R. Lazaro, Int.J. Pept. Protein. Res., 1987, 30, 801.
38. R. Jacquier, R. Lazaro, H. Raniriseheno, and P. Viallefont, Int. J. Pept. Protein. Res., 1987, 30, 22.
39. R.E. Shute, B. Dunlop, and D.H. Rich, J. Med. Chem., 1987, 30, 71.
40. R.E. Shute and D.H. Rich, Tetrahedron Lett., 1987, 28, 3419.
41. T. Kato, S. Lee, Y. Shimohigashi, A. Tone, Y. Kodera, and N. Izumiya, Int. J. Pept. Protein. Res., 1987, 29, 53.
42. K. Eckart and H. Schwartz, Helv. Chim. Acta., 1987, 70, 489.
43. L. Tomasic and G.P. Lorenzi, Helv. Chim. Acta., 1987, 70, 1012.
44. T.A. Lyle, R.M.F. Freidinger, R.F. Nutt, C.F. Hamnick, R. Saperstein, and D.F. Veber, Int. J. Pept. Protein Res., 1987, 29, 244.
45. D.F. Mierke, P. Lucietto, M. Goodman, and P.W. Schiller, Biopolymers, 1987, 26, 1573.
46. L. Sheh, M. Mokotoff, and D.J. Abraham, Int. J. Pept. Protein Res., 1987, 29, 509.
47. O. Ploux, G. Chassaing, and A. Marquet, Int. J. Pept. Protein Res., 1987, 29, 162.
48. J. Belleney, B.P. Roques, and M-C. Fournie-Zaluskie, Int. J. Pept. Protein Res., 1987, 30, 356.
49. H.I. Mosberg, Int. J. Pept. Protein Res., 1987, 29, 282.
50. B. Charpentier, C. Durieux, I. Menant, and B.P. Roques, J. Med. Chem., 1987, 30, 962.
51. R. Kobayashi, Y. Samejima, S. Nakajima, K. Kawai, and S. Udagawa, Chem. Pharm. Bull., 1987, 35, 1347.
52. D.S. Kemp and J.S. Carter, Tetrahedron Lett., 1987, 28, 4641.
53. D.S. Kemp and J.S. Carter, Tetrahedron Lett., 1987, 28, 4645.
54. J. Lautz, H. Kessler, R. Boclens, R. Kaptein, and W.F. van Gunsteren, Int. J. Pept. Protein Res., 1987, 30, 404.
55. A.N. Stroup, A.L. Rheingold, A.L. Rockwell, and L.M. Gierasch, J. Amer. Chem. Soc., 1987, 109, 7146.
56. R. Kishore, S. Raghothama, and P. Balaram, Int. J. Pept. Protein Res., 1987, 29, 381.
57. T. Inaba, I. Umezawa, M. Yuasa, T. Inoue, S. Mihashi, J. Itokawa, and K. Ogura, J. Org. Chem., 1987, 52, 2958.
58. B.E. Campbell, K.R.K. Easwaran, G.C. Zanotti, M.A. Staples, E.T. Fossell, and E.R. Blout, Biopolymers, 1987, 25, 547.
59. H. Kessler, B. Haupt, M. Frimmer, and K. Ziegler, Int. J. Pept. Protein Res., 1987, 29, 621.
60. S.F. Brady, R.M. Friedinger, W.J. Palaveda, C.D. Colton, C.F. Hamnick, W.L. Whitter, P. Curley, R.F. Nutt, and D.F. Veber, J. Org. Chem., 1987, 52, 564.
61. Y. Saski, W.A. Murphy, M.L. Heiman, V.A. Lance, and D.H. Coy, J. Med. Chem., 1987, 30, 1162.
62. C. Wynants, H.R. Loosli, and G. Van Binst, Int. J. Pept. Protein Res., 1987, 29, 90.
63. C. Wyants, E. Sugg, V.J.Hruby, and V. Van Binst, Int. J. Pept. Protein Res., 1987, 30, 541.
64. R. Kishore, S. Ragothama, and P. Balaram, Biopolymers, 1987, 26, 873.
65. H. Kessler, C. Griesinger, and K. Wagner, J. Amer. Chem. Soc., 1987, 109, 6927.
66. P.K.C. Paul and C. Ramakrishnan, Int. J. Pept. Protein Res., 1987, 29, 433.
67. K. Romanowska and K.D. Kopple, Int. J. Pept. Protein Res., 1987, 30, 289.
68. H. Kessler, S. Stevernagel, D. Gillessen, and T. Kamiyama, Helv. Chim. Acta., 1987, 70, 726.
69. M.A. Bednarek, B.E. Campbell, K.R.K. Easwaran, and E.R. Blout, Biopolymers, 1987, 26, 511.
70. T. Katagi and H. Kataoka, Heterocycles, 1987, 26, 2109.
71. C.M. Deber and L.C. Hsu, Biochem. Biophys. Res. Commun., 1987, 134, 731.
72. G. Zanotti, C. Mohringer, and T. Wieland, Int. J. Pept. Protein Res., 1987, 30, 450.

73. B. Diblasio, E. Benedetti, V. Pavone, C. Pedone, and M. Goodman, Biopolymers, 1987, 26, 2099.
74. I.L. Karle and T. Wieland, Int. J. Pept. Protein Res., 1987, 29, 596.
75. M. Kondo, M. Kimura, K-I. Sato, and M. Harimoto, Bull. Chem. Soc. Japan, 1987, 60, 1391.
76. Y. Shimohigoshi, K. Kodama, S. Imazu, H. Harimoto, K. Sakaguchi, M. Waki, H. Uchida, M. Kondo, T. Kato, and N. Izumiya, FEBS Lett., 1987, 222, 251.
77. R. Traber, H. Hofmann, H-R. Loosli, M.Pouelle, and A. von Wartburg, Helv. Chim. Acta., 1987, 70, 13.
78. I.J. Galpin, A. Karim, A. Mohammed, and A. Patel, Tetrahedron Lett., 1987, 28, 6517.
79. W.R. Chan, W.F. Tinto, P.S. Manchard, and L.J. Todaro, J. Org. Chem., 1987, 52, 3091.
80. M.B. Purvis, D.G.I. Kingston, N. Fujii, and H.G. Floss, J. Chem. Soc. Chem. Commun., 1987, 302.
81. K. Ueda, M. Waki, and N. Izumiya, Int. J. Pept. Protein Res., 1987, 30, 33.
82. H. Aoyagi, H. Mihara, T. Kato, M. Yamada, and T. Ueno, Agric. Biol. Chem., 1987, 51, 1707.
83. A.B. Mauger and O.A. Stuart, Int. J. Pept. Protein. Res., 1987, 30, 481.
84. K.L. Rinehart, V. Kishore, S. Nagarajan, R.J. Lake, J.B. Gloer, F.A. Bozich, K-M. Li, R.E. Maleczka, Jr, W.L. Todsen, M.H.G. Munro, D.W. Sullins, and R. Sakai, J. Amer. Chem. Soc., 1987, 109, 6849.
85. M. Debono, M. Barnhart, C.B. Carrell, J.A. Hoffmann, J.C. Occolowitz, B.J. Abbot, D.S. Fukuda, R.L. Hamill, K. Biemann, and W.C. Herlihy, J. Antibiot., 1987, 40, 761.
86. I. Kitawa, N.K. Lee, M. Kobayoshi, and H. Shibuya, Chem. Pharm. Bull., 1987, 35, 2129.
87. G.R. Pettit, Y. Kamano, C.W. Holzapfel, W.J. Van Zyl, A.A. Tuinman, C.L. Herald, L. Baczynskyj, and J.M. Schmidt, J. Amer. Chem. Soc., 1987, 109, 7581.
88. J.J. Stezowski, H.W. Pohlmann, E. Haslinger, H. Kalchauser, U. Schmidt, and B. Pozelli, Tetrahedron, 1987, 43, 3923.
89. T. Sugiura, Y. Hamada, and T. Shioiri, Tetrahedron Lett., 1987, 28, 2251.
90. T. Ishida, M. Inoue, Y. Hamada, S. Kato, and T. Shiori, J. Chem. Soc. Chem. Commun., 1987, 370.
91. D.R. Monck, L-C.Chen, P.J. Keller, J.M. Beale, and H.G. Floss, J. Amer. Chem. Soc., 1987, 109, 1250.
92. S. Devi, V.B. Pandey, J.P. Singh, and A.H. Shah, Phytochemistry, 1987, 26, 3374.
93. W. Voelter, A.F. Morel, A. Rahman, and N.M. Qureshi, Z. Naturforsch., 1987, 42B, 487.
94. R.S. Bhakuri, Y.N. Shukla, and R.S. Thakur, Phytochemistry, 1987, 26, 324.
95. S.W. Fesik, G. Bolis, H.L. Sham, and E.T. Olejniczak, Biochemistry, 1987, 26, 1851.
96. T. Yasuzawa, K. Shirahata, and S. Sano, J. Antibiot., 1987, 40, 455.
97. R. Kannan, and D.H. Williams, J. Org. Chem., 1987, 52, 5432.
98. G. Zanatti, B. Beijer, and T. Wieland, Int. J. Pept. Protein Res., 1987, 30, 323.
99. M. Feigel, G. Lugert, and C. Heichert, Leibigs. Ann. Chem., 1987, 367.
100. J.S. Davies, E.J. Tremeer, and R.C. Treadgold, J. Chem. Soc. Perkin I, 1987, 1107.
101. S. Bohnert and W-H. Gundel, Z. Naturforsch., 1987, B42, 1159.
102. S. Takahashi, H. Nakamura, T. Kameyama, S. Kurasawa, H. Naganawa, Y. Okami, T. Takeuchi, and H. Umezawa, J. Antibiot., 1987, 40, 1671.
103. P. Van de Elst, E. Van den Berg, H. Pepermans, L. Van der Auwere, R. Zeeuws, D. Tourwe, and G. Van Binst, Int. J. Pept. Protein Res., 1987, 29, 318; P. Van de Elst, D. Gondol, C. Wyants, D. Tourwe, and G. Van Binst, ibid., 331.

104. M. Hollosi, K.E. Kover, S. Holly, L. Radics, an G.D. Fasman, Biopolymers, 1987, 26, 1555.
105. M. Hollosi, K.E. Kover, S. Holly, and G.D. Fasman, Biopolymers 1987, 26, 1527.
106. T. Mukhopadhyay, B.N. Ganguli, H.W. Fehlhaber, H. Koger, and L. Vertesy, J. Antibiot. 1987, 281.
107. D.A. Evans and A.E. Weber, J. Amer. Chem. Soc., 1987, 109, 7151.
108. E.L. Baniek, J.E. Rivier, R.S. Struthers, A.T. Hagler, and L.M. Gierasch, Biochemistry, 1987, 26, 2642.
109. U.P. Malaleyrat, M. Reboud-Ravaux, and M. Wakselman, Int. J. Pept. Protein Res., 1987, 30, 622.
110. G. Bolis, A.K.L. Fung, J. Greer, H.D. Kleinert, P.A. Marcotte, T.J. Perun, J.J. Plattner, and H.H. Stein, J. Med. Chem., 1987, 30, 1729.
111. G.J. Hanson, J.S. Baron, H.S. Lowrie, S.J. Sarussi, P-C. Yang, M. Babler, S.E. Bittner, S.E. Papioannou, and G.M. Walsh, Biochem. Biophys. Res. Commun., 1987, 146, 959.
112. J.R. July, N. Yi, J. Soderquist, H. Stein, J. Cohen, T.J. Perun, and J.J. Plattner, J. Med. Chem., 1987, 30, 1609.
113. H.L. Sham, C.A. Tempel, H. Stein, and J. Cohen, J. Chem. Soc. Chem. Commun., 1987, 863.
114. S.H. Rosenberg, J.J. Plattner, K.W. Woods, H.H. Stein, P.A. Marcotte, J. Cohen, and T.J. Perun, J. Med. Chem., 1987, 30, 1224.
115. S. Thaisrivangs, D.T. Pals, L.T. Kroll, S.R. Turner, and F-S. Hans, J. Med. Chem., 1987, 30, 976.
116. D.J. Kempf, E. De Lara, H.H. Stein, J. Cohen, and J.J. Plattner, J. Med. Chem., 1987, 30, 1978.
117. J.F. Dellaria, M.A. Bopp, J. Cohen, H.D. Kleinert, J.R. July, I. Merits, J.J. Plattner, and H.H. Stein, J. Med. Chem., 1987, 30, 3137.
118. H.L. Sham, H. Stein, C.A. Rempel, J. Cohen, and J.J. Plattner, FEBS Lett., 1987, 220, 299.
119. S. Thaisrivangs, H.J. Schostarez, D.T. Pals, and S.R. Turner, J. Med. Chem., 1987, 30, 1837.
120. H.L. Sham, H. Stein, and J. Cohen, J. Chem. Soc. Chem. Commun., 1987, 1792.
121. S. Thaisrivangs, D.T. Pals, J.A.Laurson,S.R. Turner, and D.W. Harris, J. Med. Chem., 1987, 30, 536.
122. M.G. Bock, R.M. Dipardo, B.E. Evans, K.E. Rittle, J. Boger, M. Poe, B.I. Lamont, R.J. Lynch, E. Hulm, G.P.V. Lasuk, W.J. Greenlee, and D.F. Veber, J. Med. Chem., 1987, 30, 1853.
123. K. Fearon, A. Spaltenstein, P.B. Hopkins, and M.H. Gelb, J. Med. Chem., 1987, 30, 1617.
123a J.R. July, J.J. Plattner, H. Stein, N. Yi, J. Soderquist, P.A. Marcotte, H.D. Kleinert, and T.J. Perun, Biochem. Biophys. Res. Commun., 1987, 143, 44.
124. K.Y. Hui, W.D. Carlson, M.S. Bernatowicz, and E. Haber, J. Med. Chem., 1987, 30, 1287.
125. P. Roy, M. Delepierre, J. Wagnon, D. Nisato, and B.P. Roques, Int. J. Pept. Protein Res., 1987, 30, 44.
126. D. Nisato, J. Wagnon, G. Callet, D. Mettefeu, J-L. Assens, C. Plouzane, B. Tannerre, V. Pliska, and J-L. Fauchere, J. Med. Chem., 1987, 30, 2287.
127. T. Ando, S. Okado, I. Uchida, K. Hemmi, M. Nishikawa, Y. Tsurumi, A. Fujie, K. Yoshida, and M.Okuhara, J. Antibiot., 1987, 468.
128. E.F. Paulus, R. Henning, and H. Uhrbach, Acta Crystallogr. 1987, C43, 941.
129. P. Luger and G. Schnorrenberg, Acta. Crystallogr. 1987, C43, 484.
130. C.J. Blankley, J.S. Kaltenbronn, D.E. DeJohn, A. Werner, L.R. Bennett, G. Bobowski, U. Krolls, D.R. Johnson, W.M. Pearlman, M.L. Hoefle, A.D. Essenburg, D.M. Cohen, and H.R. Kaplan, J. Med. Chem., 1987, 30, 992.
131. H. Yanagisawa, S. Ishihara, A. Ando, T. Kanazaki, S. Miyamato, H. Koike, Y. Iijima, K. Oizumi, Y. Matsushita, and T. Hata, J. Med.

Chem., 1987, 30, 1984.
132. J.D. Godfrey, E.M. Gordon, and D.J. Van Langen, Tetrahedron Lett., 1987, 28, 1603.
133. A. Paldi, M. Mara, S. Bajusz, and L. Graf, Int. J. Pept. Protein Res., 1987, 29, 746.
134. Y. Tsuda, Y. Okada, Y. Nagamatsu, and U. Okamoto, Chem. Pharm. Bull., 1987, 35, 3576.
135. A. Lentini, F. Farchione, B. Ternai, N. Kreva-Ongarjukool, and P. Tovivich, Hoppe-Seylers Biol. Chem., 1987, 368, 369.
136. E. Kasafirek, P. Fric, J. Slaby, and P. Kocna, Coll. Czech. Chem. Comm., 1987, 52, 3034.
137. R. Bone, A.B. Shenvi, C.A. Kettner, and D.A. Agard, Biochemistry, 1987, 26, 7609.
138. K. Suguna, E.A. Padlan, C.W. Smith, W.D. Carlson, and D.R. Davies, Proc. Natl. Acad. Sci. U.S.A., 1987, 84, 7009.
139. J. J. Cooper, S. Foundling, A. Hemmings, T. Blundell, D.M. Jones, A. Hallett, and M. Szelke, Eur. J. Biochem., 1987, 169, 215.
140. T-C.Liang and R.H. Abeles, Biochemistry, 1987, 26, 7603.
141. R.L. Stein and A.M. Strimpler, Biochemistry, 1987, 26, 2611.
142. B. Imperiali and R.H. Abeles, Biochemistry, 1987, 26, 4474.
143 F.G. Salituro, N. Agarwal, T. Hofmann, and D.H. Rich, J. Med. Chem., 1987, 30, 286.
144. S.K. Ner, C.J. Suckling, A.R. Bell, and R. Wrigglesworth, J. Chem. Soc. Chem. Commun., 1987, 480.
145. D.W. Graham, W.T. Ashton, L. Barash, J.E. Brown, R.D. Brown, L.F. Canning, A. Chen, J.P. Springer, and E.F. Rogers, J. Med. Chem., 1987, 30, 1074.
146. R.D. Gray, W.M. Pierce Jr., J.W. Harrod Jnr., and J.M. Rademacher, Arch. Biochem. Biophys., 1987, 256, 692.
147. S. Murao, T. Shin, Y. Katsu, M. Iwahara, and K. Hirayama, Agric. Biol. Chem., 1987, 51, 2029.
148. P.A. Bartlett and C.K. Marlowe, Science, 1987, 235, 569.
149. D.E. Trourud, H.M. Holden, and B.W. Matthews, Science, 1987, 235, 571.
150. T. Kato, H. Hinoo, Y. Terui, J. Nishikawa, Y. Nakagawa, Y. Ikenishi, and J. Shoji, J. Antibiot., 1987, 139.
151. Y. Shimohigashi, T. Costa, A. Pfeiffer, A. Herz, H. Kimura, and C.H. Stammer, FEBS Lett., 1987, 222, 71.
152. M.L. Glowka, G. Gilli, V. Bertolasi, and M. Makowski, Acta. Crystallogr. 1987, C43, 1403.
153. T.P. Singh, M. Haridos, V.S. Chauhan, A. Kumar, and D. Viterbo, Biopolymers, 1987, 26, 819.
154. V.S. Chanchan, A.K. Sharma, K. Uma, P.K.C. Paul, and P. Balaram, Int. J. Pept. Protein Res., 1987, 29, 126.
155. A.C. Boch and L.M. Gierasch, Biopolymers, 1987, 25, 5175.
156. M. ElHajji, S. Rebuffat, D. Lecommandeur, and B. Bodo, Int. J. Pept. Protein Res., 1987, 29, 207.
157. C. Rossi, L. Tuttobello, and M. Ricci, J. Antibiot., 1987, 40, 130.
158. L. Radics, M. Kajtar-Peredy, C.G. Casinovi, C. Rossi, M. Ricci, and L. Tuttobello, J. Antibiot, 1987, 40, 714.
159. D. Obrecht, and H. Heimgartner, Helv. Chim. Acta., 1987, 70, 329; P. Wipf and J. Heimgartner, ibid., 354.
160. T. Li, K-H. Budt, and Y. Kishi, J. Chem. Soc. Chem. Commun., 1987, 1817.
161. C. Mapelli, M.G. Newton, C.E. Ringold, and C.H. Stammer, Int. J. Pept. Protein Res., 1987, 30, 498.
162. R.P. Hummel, C. Toniolo, and G. Jung, Angew. Chem. Intl. Edn., 1987, 26, 1150.
163. P. Cardopatis, A. Michel, J. Matsoukas, and J. Zaven, Gozz. Chim. Ital., 1987, 117, 287.
164. G. Esposito, J.A. Carver, J. Boyd, and I.D. Campbell, Biochemistry, 1987, 26, 1043.
165. H. Vogel, Biochemistry, 1987, 26, 4562.

166. J.R. Cann, R.E. London, C.J. Unkefer, R.J. Vavrek, and J.M. Stewart, Int. J. Pept. Protein Res., 1987, 29, 486.
167. M. Kawai, Y. Batsugan, K. Fukuyama, and T. Taga, Biopolymers, 1987, 26, 83.
168. G. Valle, C. Toniolo, and G. Jung, Gazz. Chim. Ital., 1987, 117, 549.
169. G. Valle, M. Crisma, F. Formaggio, C. Toniolo, and G. Jung, Liebigs. Ann. Chem., 1987, 1055.
170. A.N. Jayashree, C.G. Suresh, M. Vijayan, C. Toniolo, and G.M. Bonora, Acta. Crystallogr., 1987, C43, 1618.
171. I.L. Karle, J. Flippen-Anderson, M. Sukumar, and P. Balaram, Proc. Natl. Acad. Sci. U.S.A., 1987, 84, 5087.
172. G. Grundke, W. Keese, and M. Rimpler, Liebigs. Ann. Chem., 1987, 73 and 729.
173. K.L. Yu and R.L. Johnson, J. Org. Chem., 1987, 52, 2051.
174. P.P. Giannousis and P.A. Bartlett, J. Med. Chem., 1987, 30, 1603.
175. A. Spaltenstein, P.A. Carpino, F. Miyake, and P.B. Hopkins, J. Org. Chem., 1987, 52, 3759.
176. Y-K. Shue, G.M. Carrera Jr., and A.M. Nadzan, Tetrahedron Lett., 1987, 28, 3225.
177. R.E. Tenbrink, J. Org. Chem., 1987, 52, 418.
178. R.J. Arrowsmith, D.E. Davies, Y.C. Fogden, C.J. Harris, and C. Thompson, Tetrahedron Lett., 1987, 28, 5569.
179. H. Oyamada and M. Ueki, Bull Chem. Soc. Japan, 1987, 60, 267.
180. H.A.S. Hansen, K. Clausen, and T.F.M. La Cour, Acta. Crystallogr., 1987, C43, 519 and 522.
181. D.W. Brown, M.M. Campbbell, M.S. Chambers, and C.V. Walker, Tetrahedron Lett., 1987, 28, 2171.
182. T.F.M. La Cour, Int. J. Pept. Protein Res., 1987, 30, 564.
183. V.N. Balaji, S. Profeta Jr., and S.W. Dietrich, Biochem. Biophys. Res. Commun., 1987, 145, 834.
184. J. Couder, P. Van Der Elst, D. Tourwe, and G. Van Binst, Bull. Soc. Chim. Belg., 1987, 96, 629.
185. M. Lebl, E.E. Sugg, G. Van Binst, P. Van Der Elst, D. Tourwe, J. Slavínova, and V.J. Hruby, Int. J. Pept. Protein Res., 1987, 30, 318.
186. A. Aumelas, M. Rodriguez, A. Heitz, B. Castro, and J. Martinez, Int. J. Pept. Protein Res., 1987, 30, 596.
187. M. Rodriguez, M-F Lignon, M-C. Galos, P. Fulcrand, C. Mendre, A. Aumelos, J. Laur, and J. Martinez, J. Med. Chem., 1987, 30, 1366.
188. M.W. Holladay, F.G. Salituro, and D.H. Rich, J. Med. Chem., 1987, 30, 374.
189. T.L. Blundell, J. Cooper, S.I. Foundling, D.M. Jones, B. Atrash, and M. Szelke, Biochemistry, 1987, 26, 5585.
190. M. Rodriguez. P. Dubreuil, J-P. Bali, and J. Martinez, J. Med. Chem., 1987, 30, 758.
191. M. Goodman, J. Coddington, D.F. Mierke, and W.D. Fuller, J. Amer. Chem. Soc., 1987, 109, 4712.
192. L.A.G.M. Van Der Brock, M.L. Breur, R.M.J. Liskamp, and M.C.J. Ottenheijm, J. Org. Chem., 1987, 52, 1511.
193. P. Jackson, C.J. Unkefer, J.A. Doolen, K. Watt, and N.J. Robinson, Proc. Natl. Acad. Sci., 1987, 84, 6619.
194. W.R. Moore and A. Meister, Analyt. Biochem., 1987, 161, 487.
195. C.H. Gorbitz, Acta Chem. Scand., 1987, B41, 362.
196. Y. Trudelle and F. Heitz, Int. J. Pept. Protein Res., 1987, 30, 163.
197. U. Schmidt and B. Potzolli, Liebigs. Ann. Chem., 1987, 935.
198. P. Duhamel, B. Goument, and J-C Plaquevent, Tetrahedron Lett., 1987, 28, 2595.
199. K.S. Petrakis and T.L. Nagabhushan, J. Amer. Chem. Soc., 1987, 109, 2831.
200. D.H. Schlesinger, A. Buku, H.R. Wyssbrod, and D.I. Hay, Int. J. Pept. Protein Res., 1987, 30, 257.
201. T.B. Johnson, and J.K. Coward, J. Org. Chem., 1987, 52, 1771.
202. K. Richter, R. Egger, and G. Kreil, Science, 1987, 238, 133.

203. M.K. Anwer, R.A. Porter, and A.F. Spatola, Int. J. Pept. Protein Res., 1987, 30, 489.
204. H. Tsunematsu, S. Hachiyama, R. Isobe, E. Ishida, M. Kakoi, and M. Yamamoto, Biochem. Biophys. Res. Commun., 1987, 146, 907.
205. A.V. Schally, J.I. Paz-Bouza, J.V. Schlosser, T. Karoshima, L. Debeljuk, B. Gandle, and M. Sampson, Proc. Natl. Acad. Sci. U.S.A., 1987, 84, 851.
206. T-V. Dam, E. Escher, and R. Quirion, Biochem. Biophys. Res. Commun., 1987, 149, 297.
207. C.W.T. Yeung, Arch. Biochem. Biophys., 1987, 254, 81.
208. M. Doi, M. Tanaka, T. Ishida, M. Inoue, T. Fujiwara, K. Tomita, T. Kimura, S. Sakakibara, and G.M. Sheldrick, J. Biochem. Jap., 1987, 101, 485.
209. B.W. Gibson, A.M. Falick, A.L. Burlingame, L. Nadasdi, A.C. Nguyen, and G.L. Kenyon, J. Amer. Chem. Soc., 1987, 109, 5343.
210. V. Cerovsky and K. Jost, Coll. Czech, Chem. Commun., 1987, 52, 1079.
211. A.K. Judd, J.A. Lawson, C.M. Olsen, L.R. Toll, W.E. Polgar, E.T. Uyeno, C.J. Keys, and G.H. Loew, Int. J. Pept. Protein Res., 1987, 30, 299.
212. F. Fahrenholz, P. Eggena, E. Kojro, D. Gazis, and I.L. Schwartz, Int. J. Pept. Protein Res., 1987, 30, 577.
213. J. Rivier, R. Galyean, L. Simon, L.J. Cruz, B.M. Olivera, and W.R. Gray, Biochemistry, 1987, 26, 8508.
214. M.T. Garcia-Lopez, R. Gonzalez-Muniz, M.T. Molinero, J.R. Naranjo, and J. Del Rio, J. Med. Chem., 1987, 30, 1658.
214a B. Calas, R. Michelot, J.P. Le Caer, A. Cave, J. Parello, and B. Potier, Int. J. Pept. Protein Res., 1987, 29, 170.
214b H.N. Bramson, N.E. Thomas, W.T. Miller, D.C. Fry, A.S. Mildvan, and E.T. Kaiser, Biochemistry, 1987, 26, 4466.
215. D.C. Harwell, A. Beeby, C.R. Clarke, and J. Hughes, J. Med. Chem., 1987, 30, 729.
216. K. Ienaga, K. Migashiura, and H. Kimura, Chem. Pharm. Bull., 1987, 35, 1249.
217. Y. Hashimoto, Y. Endo, K. Shudo, Y. Aramaki, N. Kawai, and T. Nakajima, Tetrahedron Lett., 1987, 28, 3511.
218. T. Teshima, T. Wakimiya, Y. Aramaki, T. Nakajima, N. Kawai, and T. Shiba, Tetrahedron Lett., 1987, 28, 3509.
219. S. Capasso, L. Mozzarella, F. Sica, and A. Zagari, Acta. Crystallogr., 1987, C43, 1607.
220. G.R. Pettit, Y. Kamano, C.L. Herald, A.A. Tuinman, F.E. Boettner, H. Kizu, J.M. Schmidt, L. Baczynskyj, K.B. Tomer, and R.J. Bantems, J. Amer. Chem. Soc., 1987, 109, 6883.
221. M.D. Lee, A.A. Fontini, N.A. Kuck, M. Greenstein, R.T. Testa, and D.B. Borders, J. Antibiot., 1987, 40,1657.
222. K. Ohba, H. Nakayama, K. Furihata, A. Shimazu, T. Endo, H. Seto, and N. Otake, J. Antibiot., 1987, 40, 709.
223. M. Takizawa, T. Tsubotani, S. Tanida, S. Harada, and T. Hasegawa, J. Antibiot., 1987, 40, 1220.
224. R.S. Youngquist and P.B. Derva, J. Amer. Chem. Soc., 1987, 109, 7564.
225. D. Dasgupta, P. Parrack, and V. Sasisekharan, Biochemistry, 1987, 26, 6381; P. Parrack, D. Dasgupta, J. Ayyer, and V. Sasisekharan, FEBS Lett., 1987, 212, 297.
226. K. Krowicki, and J.W. Lown, J. Org. Chem., 1987, 52, 3493.
227. R.E. Bolton, C.J. Moody, C.W. Rees, and G. Tojo, Tetrahedron Lett., 1987, 28, 3163.
228. P. Magnus, T. Gallagher, T. Schulz, Y-S. Or, and T.P. Ananthanaryan, J. Amer. Chem. Soc., 1987, 109, 2706.
229. T. Yamado, T. Taniguchi, Y. Kozuta, T. Miyazawa, S. Kuwata, K. Matsumato, and M. Sugiura, Chemistry Lett., 1987, 723.
230. S. Mobashery and M. Johnston, Biochemistry, 1987, 26, 5878.
231. P.O.L. Mack, D.P. Kelly, R.F. Martin, and L.P. Wakelin, Austral. J. Chem., 1987, 40, 97.
232. H.M. Franzen, V. Ragnarsson, K. Nagren, and B. Langstrom, J. Chem. Soc. Perkin I, 1987, 2241.

233. R. Andraszkiewicz, H. Chmara, S. Milewski, and E. Barowski, J. Med. Chem., 1987, 30, 1715.
234. B. Kohler and V. Schollkopf, Liebigs. Ann. Chem., 1987, 267.
235. E. Haslinger and H. Kalchauser, Monatsh., 1987, 118, 607.
236. G. Cavicchioni, Tetrahedron Lett., 1987, 28, 2427.
237. B. Muller, A. Hadener, and C. Tamm, Helv. Chim. Acta., 1987, 70, 412.
238. Y. Uchida, K. Okimura, K. Kurosawa, N. Sakura, K. Hirose, and T. Hashimoto, Bull. Chem. Soc. Japan, 1987, 60, 1561.
239. A. Ljungquist, D-M. Feng, P-F.L. Tan, M. Kubota, T. Okamoto, Y. Zhand, C.Y. Bowers, W.A. Hooks, and K. Folkers, Biochem. Biophys. Res. Commun., 1987, 148, 849.
240. H. Mihara, S. Lee, Y. Shimohigashi, M. Aoyagi, T. Kato, N. Izumiya, and T. Costa, Int. J. Pept. Protein Res., 1987, 30, 605.
241. T. Saino, K. Seya, R. Nishizawa, T. Takita, T. Aoyagi, and H. Umezawa, J. Antibiot., 1987, 40, 1165.
242. S.J. Hocart, M.V. Nekola, and D.H. Coy, J. Med. Chem., 1987, 30, 735 and 739.
243. K. Folkers, C. Bowers, X. Shao-Bo, P-F.L. Tang, M. Kubota, J. Stepinski, and T. Kubiak, Z. Naturforsch., 1987, 42B, 101.
244. K.H. Hsieh, P. Needleman, and G.R. Marshall, J. Med. Chem., 1987, 30, 1097.
245. T. Hashimoto, Y. Uchida, M. Nishijima, N. Sakura, and K. Hirose, Bull. Chem. Soc. Japan, 1987, 60, 1207.
246. M. Rico, F.J. Bermejo, J. Santaro, J.L. Nieto, E. Gallego, J. Herranz, I. Voskuyl-Holtkamp, and C. Shattenkerk, Int. J. Pept. Protein Res., 1987, 29, 193.
247. T.P. Anderson and A. Senning, Liebigs. Ann. Chem., 1987, 59.
248. E. Kojro, I. Willhardt, A. Ranback, Z. Grzonka, and P. Hermann, FEBS Lett., 1987, 212, 83.
249. G. Borin, A. Calderon, P. Ruzza, L. Moroder, W. Gohring, G. Bovermann, and E. Wunsch, Hoppe-Seylers Z. Biol. Chem., 1987, 368, 1363.
250. M.T. Garcia-Lopez, R. Gonzalez-Muniz, R. Herranz, M.T. Molinero, and J. Del Rio, Int. J. Pept. Protein Res., 1987, 29, 613.
251. S.B. Christensen, H.S. Allaudeen, M.R. Burke, S.A. Carr, S.K. Chung, P. De Phillips, J.J. Dingerdissen, M. Dipaulo, A.G. Giovanella, S.L. Heald, L.B. Killner, B.A. Mico, L. Mueller, C.H. Pan, B.L. Pochland, J.B. Rake, G.D. Roberts, M.C. Shearer, R.D. Sitrin, L.J. Nisbet, and P.W. Jeffs, J. Antibiot., 1987, 40, 970.
252. T. Takatsu, S. Takahashi, M. Nakajima, T. Haneishi, T. Nakamura, H. Kuwano, and T. Kinoshita, J. Antibiot., 1987, 40, 933.
253. J.P. Waltho, D.H. Williams, E. Selva, and P. Ferrari, J. Chem. Soc. Perkin I, 1987, 2103
254. S.L. Heald, L. Mueller, and P.W. Jeffs, J. Antibiot., 1987, 40, 630.
255. J.J. Dingderdissen, R.D. Sitrin, P.A. De Phillips, A.J. Giovanella, S.F. Grappel, R.J. Mehta, Y.K. Oh, C.H. Pan, G.D. Roberts, M.C. Shearer, and L.J. Nisbet, J. Antibiot., 1987, 40, 165.
256. S.K. Chung, S.L. Heald, and P.W. Jeffs, J. Org. Chem., 1987, 52, 1606.
257. P.M. Booth, D.J.M. Stone, and D.H. Williams, J. Chem. Soc. Chem. Commun., 1987, 1694.
258. B. Cavelleri, P. Ferrari, A. Malabarba, A. Magni, R. Pallanza, and G.G. Gallo, J. Antibiot., 1987, 40, 49.
259. D.W. Hobbs and W. Clark Still, Tetrahedron Lett., 1987, 28, 2805.
260. T. Marii, I. Saito, T. Matsuura, J. Kuwuhara, and Y. Sugiura, J. Amer. Chem. Soc., 1987, 109, 938.
261. W.J. Vloon, C. Kruk, U.K. Pandit, H.P. Hofs, and J.G. McVie, J. Med. Chem., 1987, 30, 20.
262. H. Kunz, Angew. Chem. Intl. Edn., 1987, 26, 294.
263. H. Kessler, M. Kottenhahn, A. Kling, and C. Kolar, Angew. Chem. Intl. Edn., 1987, 26, 888.
264. R. Rocchi, L. Biondi, F. Cavaggion. F. Filira, M. Gobbo, S. Dagan, and M. Fridkin, Int. J. Pept. Protein Res., 1987, 29, 262.

265. N.J. Maeji, Y. Inoue, and R. Chujo, Int. J. Pept. Protein Res., 1987, 29, 699.
266. H. Paulsen and M. Schulz, Carbohyd. Res., 1987, 159, 37.
267. L. Varga, S. Horvat, C. Lemieux, and P.W. Schiller, Int. J. Pept. Protein Res., 1987, 30, 371.
268. J.M. Bernard, H. Gras-Masse, H. Drobecq, A. Tartar, P. LeFrancier, A. Hosmalin, C. Carelli, and L. Chedid, Int. J. Pept. Protein Res., 1987, 29, 455.
269. J. Jezek, R. Straka, V. Krchnak, M. Ryba, J. Rotta, P. Mayer, and M. Zaoral, Coll. Czech. Chem. Comm., 1987, 52, 1609.
270. J-P. Tenu, A. Adam, V. Souvannavong, G. Barratt, A. Yapo, J-P. Petit, M. Level, M. Clemance, and K. Douglas, FEBS Lett., 1987, 220, 93.
271. Y. Fujishima, K. Kigawa, Y. Ogawa, M. Kiso, A. Hasegawa, H. Ishida, and I. Azuma, Carbohyd. Res., 1987, 167, 317.
272. S. Fermandjian, B. Perly, M. Level, and P. Lefrancier, Carbohyd. Res., 1987, 162, 23.
273. D. Krois, and H. Lehner, J. Chem. Soc. Perkin II, 1987, 219 and 1523.
274. H. Sawai, H. Taira, K. Ishibashi, and M. Itoh, J. Biochem. Japan, 1987, 101, 339.
275. B.D. White, K.A. Arnold, and G.W. Gokel, Tetrahedron Lett., 1987, 28, 1749.
276. A.A. Amoscato, G.F. Babcock, R.M. Sramkoski, B.A. Hynd, and J.W. Alexander, Int. J. Pept. Protein Res., 1987, 29, 177.
277. H. Suli-Vargha, A. Jeney, K. Lapis, and K. Medzihradsky, J. Med. Chem., 1987, 30, 583.
278. A.S. Dutta, M.B. Giles, J.J. Gormley, J.C. Williams, and E.J. Kusner, J. Chem. Soc. Perkin I, 1987, 111.
279. Y. Kohama, N. Okimato, T. Mimura, C. Fukaya, M. Watanabe, and K. Kokoyama, Chem. Pharm. Bull., 1987, 35, 3928.
280. P. Morliere, W.F. Mangel, R. Santos, G. Huppe, J-P. Reyftmann, and E. Kohen, Biochem. Biophys. Res. Commun., 1987, 146, 107.
281. P. Rigaudy, Ch. Garbay-Juureguiberry, A. Jacquemin-Sablon, J-P. Le Peco, and B.P. Roques, Int. J. Pept. Protein Res., 1987, 30, 347.
282. G. Vlason, E. Krasnikova, N. Kozhevnikova, N. Illarionova, and I. Denisov, Biopolymers, 1987, 26, 1489.
283. J. Metzger, A. Olma, M.T. Leplawy, and G. Jung, Z. Naturforschung., 1987, B42, 1195.
284. E.J. Corey, S.W. Wright, and M.M. Mehrotra, Tetrahedron Lett., 1987, 28, 739.
285. R.K. Harris and G.A. Hamilton, Biochemistry, 1987, 26, 1.
286. J.E. Bishop, H. Rapaport, A.V. Klotz, C.F. Chan, A.N. Glazer, P. Fuglistaller, and H. Zuber, J. Amer. Chem. Soc., 1987, 109, 875.
287. M. Fukuda, C.P. Beechon, M. Verlander, M. Goodman, M.M. Khan, and K.L. Melman, Int. J. Pept. Protein Res., 1987, 29, 300.

5
ß-Lactam Antibiotic Chemistry

BY A. V. STACHULSKI

1. Introduction

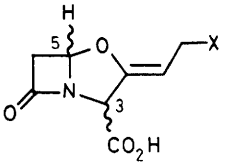
The magic ring continues to cast its spell; there has been another increase in publications in this area during 1987. I have retained the appendix, and the preamble given to this in vol.19 still applies. The division into main sections is essentially unchanged from that in vol.19.

Another continuing trend is the shift away from the classical penicillins and cephalosporins to the newer β-lactams. This is particularly true with regard to the very large section on azetidin-2-ones: much of this work again concerns highly stereoselective syntheses of these molecules, either in their own right or as precursors of bicyclic systems. Moreover, the increasing availability of azetidin-2-ones, often in high isomeric purity, makes them valuable intermediates for other synthetic targets or as guarantors of stereochemistry. I have tried to respond to this trend by introducing a new sub-section.

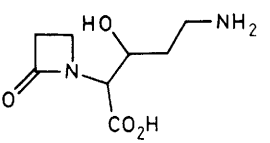
A review of the biosynthesis of sulphur-containing β-lactams[1] includes an account of the availability of the amino-acids involved. The present knowledge of cephalosporin biosynthesis has been reviewed[2] and other more general biosynthetic reviews have appeared.[3,4] A major review of enantioselective syntheses of carbapenem antibiotics has appeared[5] and other synthetic topics reviewed include the use of β-hydroxybutyrates in carbapenem syntheses[6], 2-(substituted methyl)penems[7] and azetidinone-1-sulphonates.[8] Reviews on cycloaddition reactions of imines[9] and the chemistry of chlorosulphonyl isocyanate[10] both contain sections relevant to β-lactam synthesis.

2. New Natural Products

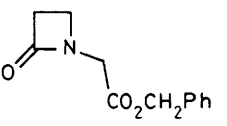
Addition of α-ketoglutarate, iron (II) and oxygen to a reaction mixture containing ultrasonically disrupted S.clavuligerus



(1) 3ß-CO_2H, 5ß-H, X = NH_2
(2) 3α-CO_2H, 5α-H, X = OH



(3)



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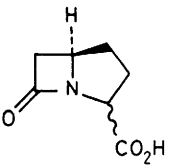
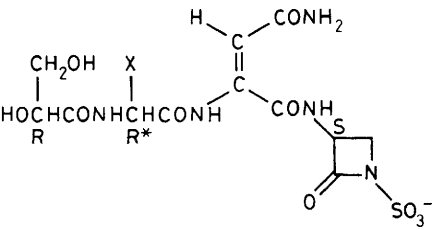
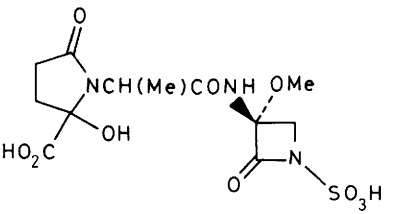


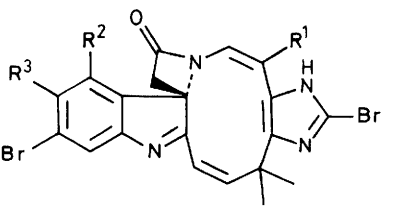
(5) a; 3α-CO_2H
b; 3ß-CO_2H



(6) a; X = CH_3
b; X = CH_2OH
c; X = H
* except for (6c)



(7)



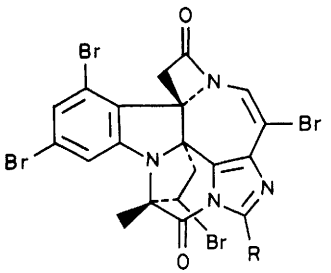
(8) a; R^1 = Cl, R^2 = R^3 = Br
b; R^1 = Cl, R^2 = Br, R^3 = H
c; R^1 = Cl, R^2 = R^3 = H
d; R^1 = OMe, R^2 = R^3 = Br

cells permitted isolation of the novel oxapenam (1), christened clavaminic acid[11] and clearly related in structure to clavulanic acid (2). From the cell extract itself was isolated one isomer of the azetidinone (3), proclavaminic acid, whose structure was confirmed by chemical synthesis[12] from azetidinone (4). The relevance of these compounds to clavulanic acid biosynthesis is explained in section 3. The new carbapenams (5a) and (5b) have been isolated from Serratia and Erwinia species;[13] their biosynthetic role is discussed, again, in section 3.

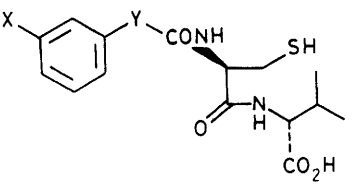
Three new monobactams, PB-5266A, B and C, (6a)→(6c) were isolated from a culture broth of Cytophaga johnsonae;[14] the dehydroasparagine residue makes them unique among natural monobactams. They displayed weak antibacterial activity. The 3-methoxy monobactam (7), related to sulphazecin, was isolated from a strain of Pseudomonas[15] and had weak antibacterial activity but high β-lactamase stability. New representatives of the remarkable fused β-lactam indole alkaloids isolated from the marine bryozoan Chartella papyracea have been described. Chartellines B (8b), C (8c) and methoxydechlorochartelline A (8d)[16] were similar to the known chartelline A (8a). They were not antimicrobially active. More recently two even more complex species, chartellamide A (9a) and B (9b), were isolated from the same organism.[17]

3. Biosynthesis

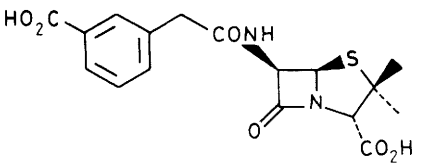
As well as continuing to probe the behaviour of modified peptide substrates when subjected to the isopenicillin N synthetase enzyme (IPNS), in order further to elucidate the possible intermediates and mechanisms involved in penicillin biosynthesis, there has been further study of the enzymes themselves. The IPNS genes from Cephalosporium acremonium were cloned and expressed in a strain of E.coli; the cloned enzyme was isolated and purified.[18] This recombinant protein had the same kinetics for the conversion of the δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine tripeptide (LLD-ACV) to isopenicillin N as the 'natural' enzyme, despite slight differences in processing at its N-terminus. Concerning the enzymic synthesis of LLD-ACV itself, it had been thought that two enzymes might be involved in its synthesis from the component amino-acids. Kinetic evidence has now been presented[19] that a



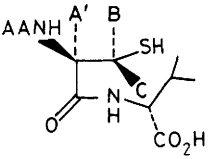
(9) a; R = H
b; R = Br



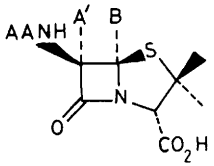
(10) X = CO_2H, Y = CH_2
(12) a; X = $COCH_3$, Y = CH_2
b; X = $COCH_3$, Y = OCH_2
c; R =  , Y = OCH_2



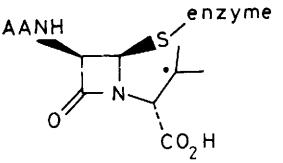
(11)



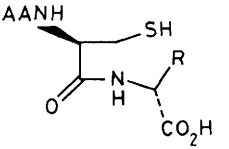
AA = δ-(L-α-aminoadipoyl)
(13) a; A′ = Me, B = C = H
b; A′ = B = H, C = Me
c; A′ = C = H, B = Me



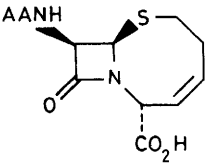
(14) a; A′ = Me, B = H
b; A′ = H, B = Me



(15)



(16) a; R = —CH_2— 
b; R = Me 



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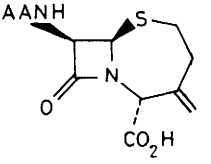
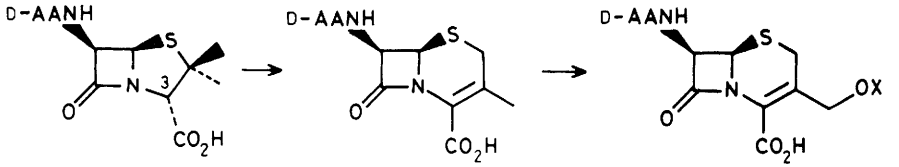


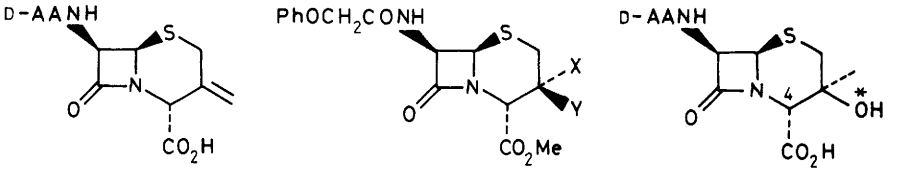
(18)



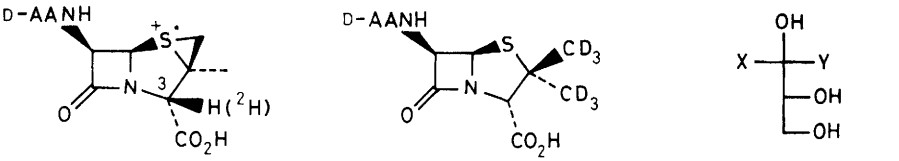
(19) (20) (21) a; X = H
b; X = $COCH_3$

D-AA = δ-(D-α-aminoadipoyl)

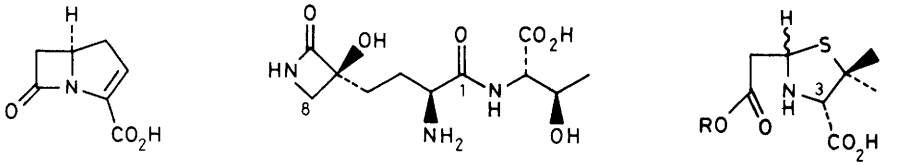
Scheme 1



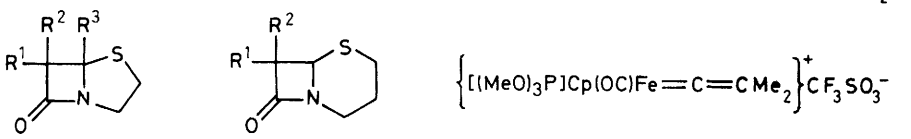
(22) (23) a; X = H, Y = Me
b; X = Me, Y = H (24)



(25) (26) (27) a; X = H, Y = T
b; X = T, Y = H



(28) (29) (30) a; R = Et
b; R = $CHPh_2$



(31) (32) (33) Cp = cyclopentadienyl

single enzyme (ACV synthetase) is involved and that the L→D valine isomerisation occurs at this step.

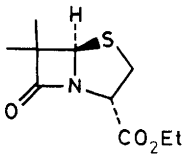
The Oxford group had previously shown that substantial variation in the valine residue of LLD-ACV was possible (see ch.5, vol.19 in this series). Concerning variation of the aminoadipoyl residue, the m-carboxyphenylacetyl dipeptide (10)[20,21] was found to be an excellent IPNS substrate, cyclizing with similar Michaelis constant and maximum velocity parameters to LLD-ACV giving the m-carboxyphenylacetylpenam (11). This is consistent with the previous knowledge that a six-carbon or equivalent chain terminating in a carboxy group was necessary; (10) appears to have a particularly favourable conformation for the active site. In another series of aryl analogues[22], penicillin V-type m-acetylaryl peptides, e.g. (12b) proved better IPNS substrates than penicillin G-type ones, e.g. (12a); p-acetyl analogues were incorporated about five times less efficiently. Consequently the diazirinyl compound (12c) was designed as a possible photoaffinity label for IPNS.

Variation of the cysteine has also been examined; in summary, analogues are tolerated[23] provided a 2R configuration is maintained and a 3-pro-S hydrogen is still available. Thus while the (2R)-2-methylcysteinyl peptide (13a) gave the 6α-methyl isopenicillin N (i.e.(14a)) on IPNS incubation, the corresponding (2S)-2-methyl tripeptide gave no β-lactam product; and the (2R,3S)-3-methyl peptide (13b) gave no β-lactam while the (2R,3R)-analogue (13c) gave the 5α-methyl compound (14b). At an intermediate stage of penicillin biosynthesis, an enzyme-bound radical such as (15) may be present. The LLD-ACV analogues (16a,b) were prepared[24]; on incubation with IPNS they gave respectively (17) (plus a small amount of 2-cyclopropyl penam) and (18). The products are in accord with intermediate cyclopropylcarbinyl radicals which rearrange to but-3-enyl radicals, but labile species with weak iron-carbon bonds are not ruled out.

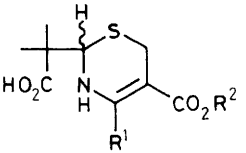
More attention is now being given to the subsequent penicillin N (19) to cephalosporin C (21b) conversion. The first step involves the ring expansion to deacetoxycephalosporin C (DAOC) (20), Scheme 1. The ring expansion activity purified from C.acremonium showed similar behaviour to IPNS in terms of variation of R in Scheme 1,[25,26] viz. a six carbon-N-acyl side chain terminating in a carboxy group was required. However, isopenicillin N [R=δ-(L-α-aminoadipoyl)] gave no DAOC. Subsequent

hydroxylation of DAOC leads to deacetylcephalosporin C (DAC) (21a), the direct precursor of cephalosporin C (21b). The apparently bifunctional enzyme from C.acremonium has, like IPNS, a requirement for α-ketoglutarate, Fe (II) and molecular oxygen; in an atmosphere of $^{18}O_2$, it converted (20) to (21a) labelled with ^{18}O in the hydroxy group.[27] Interestingly, the exomethylene cepham (22) similarly gave ^{18}O labelled (21a), again reminiscent of IPNS. Concerning the ring expansion step, evidence is accumulating for an intermediate penicillin 2β-methyl radical or related species. A chemical model for this step was provided[28] by catalytic reductive debromination of a 2β-bromomethyl penicillin, which gave the same mixture of cephams (23a,b) as a 3β-bromocepham. The 3β-hydroxy-3α-methylcepham (24) is normally a very minor by-product when (19) is incubated with DAOC/DAC synthetase, but [3-^{2}H] (19) gave a roughly 2:1 ratio of (20) + (21): [4-^{2}H] (24).[29] This was interpreted in terms of a deuterium isotope effect on the breakage of the C(3)-^{2}H bond in a bridged species (cation or radical) (25); further support was provided by the isolation of (24), O*=^{18}O, on incubation of [3-^{2}H] (19) in an $^{18}O_2$ atmosphere. On going from (19) to (20) two hydrogens are lost, one from the 2β-methyl group and one from C(3). The hexadeuteriated substrate (26) was converted much more slowly into deuteriated (20) and (21a) than was (19) into (20) and (21a), whereas [3-^{2}H] (19) was converted at essentially the same rate.[30] This is consistent with stepwise hydrogen loss: 2-methyl hydrogen first, then C(3)-H, and also supports an intermediate like (25).

When the aforementioned β-lactams proclavaminic acid (3) and clavaminic acid (1)[11] were isolated, the dioxygenase enzyme responsible for the conversion (3)⟶(1) was also isolated. Proclavaminic acid was prepared in ^{13}C labelled form via the azetidinone (4)[12,31]; both this and enzymically-derived (1) led to labelled clavulanic acid (2) on feeding and therefore appear to be genuine biosynthetic precursors of (2). Glycerol is a known biosynthetic precursor of clavulanic acid; the stereospecifically tritiated glycerols (27a,b) were synthesised from mannitol[32] and fed with [1,3-^{14}C]glycerol to S.clavuligerus cultures. Tritium was largely retained from (27a) but largely lost from (27b), i.e. there is overall retention at the glycerol-derived C(5) in (2). In the carbapenem series, it is thought that the metabolites (5a,b) mentioned earlier[13] may derive from reduction of the carbapenem (28); glutamic acid is a known precursor.



(34)



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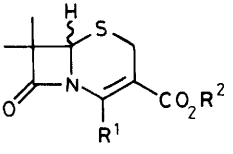

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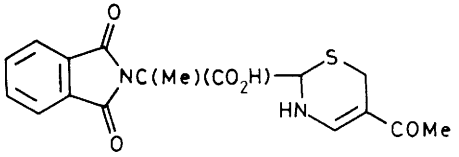

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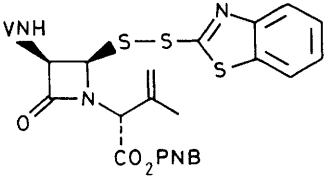
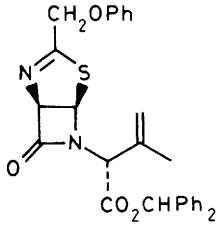


(38) V = $PhOCH_2CO$



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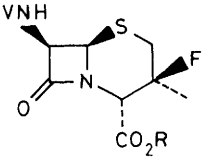

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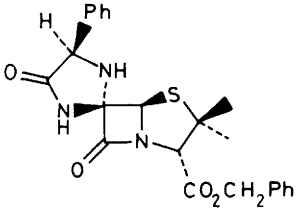

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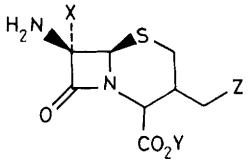
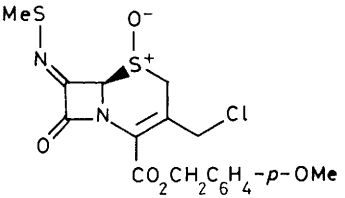
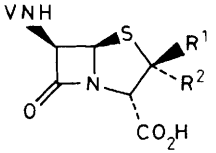


(42) a; X = SMe, Y = Bu^t, Z = OAc
b; X = NHCHO, Y = Bu^t, Z = OAc
c; X = NHCHO, Y = H, Z =



(43)



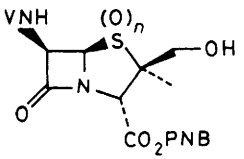
(44) a; $R^1 = CO_2H$, R^2 = Me
b; $R^1, R^2 = CH_2$

Studies on the biosynthesis of tabtoxin (29) are at a relatively early stage. ^{13}C nuclear magnetic resonance data indicated[33] that the β-lactam portion [tabtoxinine β-lactam, C(1)-C (8)] incorporated aspartic acid and pyruvate, while another report[34] concluded from several feeding experiments that threonine, aspartic acid, a glycerol-derived C-2 unit and a methionine S-methyl group were incorporated into (29).

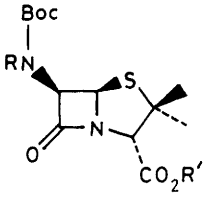
4. Penicillins and Cephalosporins

Three reports have appeared on the synthesis of the penam ring system. Condensation of various formylacetate synthons with D-penicillamine afforded the thiazolidine esters (30a,b).[35] Esterification of (30b) at C-3, formic acid treatment, and cyclization (best achieved using Mukaiyama's two-phase procedure) afforded the methyl (5R,5S)-penicillanates. Japanese workers have used virtually identical methodology to synthesize the parent penam or cepham ring system (31) or (32) carrying alkyl substituents.[36] In these syntheses Mukaiyama-Ohno cyclization (triphenylphosphine-di-2-pyridyl disulphide) was used. The cationic iron vinylidene complex (33)[37] could be made to undergo stepwise cycloadditions to various 2-thiazolines to give bicyclic complexes; oxidation of one of these afforded the ethyl penicillanate (34) of predominantly the 5R-configuration shown (See also ref.129). Two reports on ceph-3-em synthesis proceeded via intermediate 1,3-thiazines: thus Mukaiyama cyclization of the dihydrothiazines (35), ultimately derived from cyanoacetate, afforded ceph-3-em-3-carboxylates (36).[38] The thiazine (37), on the other hand, was cyclized successfully using benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate.[39]

Penicillins and cephalosporins may be interconverted in various ways; in one recent report[40] the penicillin-derived disulphide (38) was converted into the corresponding ceph-3-em ester in very good yield using ammonium acetate in tetrahydrofuran-dimethylsulphoxide, novel conditions for this step. In a similar vein, the bicyclic azetidinone (39) was converted into a 3α-chloro-3β-methylcepham in three steps via a 4-mercaptoazetidinone.[41] Reaction of either an azetidinone disulphide such as (38) or a derived 2β-halomethylpenam with AgF or HgF_2 in dichloromethane[42]



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



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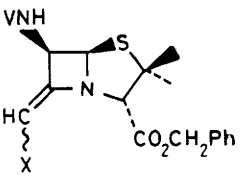

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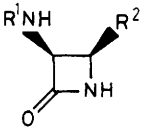
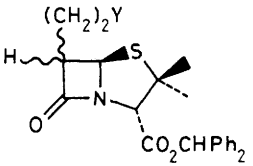
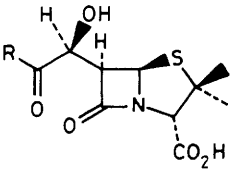


(48) a; $R^1 = Ph_3C$, $R^2 = SO_2Me$
b; $R^1 = Ph_3C$, $R^2 = SBu^t$
c; $R^1 = PhCH_2CO$, $R^2 = SBu^t$



(49) a; α-H, ß-$(CH_2)_2Y$
b; ß-H, α-$(CH_2)_2Y$



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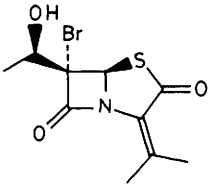

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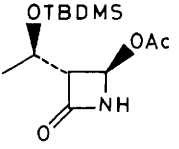
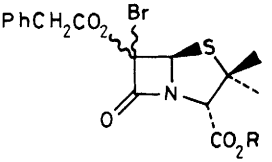


(52) TBDMS = $SiMe_2Bu^t$



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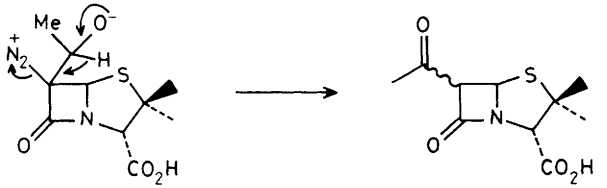


(54)

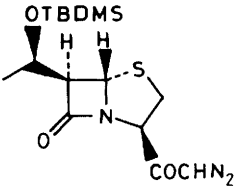
Scheme 2

gave a 3β-fluorocepham, e.g. (40); a proposed intermediate was strikingly similar to postulated biosynthetic intermediate (25)!

A novel aspect of penicillin 6-substitution was illustrated by the preparaton of spirocyclic penicillins such as (41) by mercuration of a 6α-methylthio precursor bearing a suitably placed side-chain amino group.[43] Interestingly, while (41) was the major product and corresponded to α-face attack, a small amount of the isomer corresponding to β-attack was also isolated. A definitive account of 7α-formamido cephalosporins has appeared;[44] the versatile 7β-amino, 7α-formamido intermediate (42b) was obtained from the 7α-methylthio compound (42a) in four steps. For C-3 modification the acetoxy group on the free acid form of (42b) was displaced to give, e.g. the 3-(N-methyl tetrazolylthiomethyl)-cephalosporin (42c). A new procedure for stereoselective 7α-methoxylation of cephalosporins[45] calls for acidic methanolysis of the sulphenimine (43); the α/β ratio is very high, much more favourable than for the unoxidised cephalosporin, probably owing to hydrogen bonding.

Both the penicillin-2-carboxylate (44a) and the 2-exo-methylenepenam (44b) derived from it via oxidative decarboxylation were obtained ultimately from the sulphoxide (45b);[46] (44b) readily isomerised to a penem; (44a,b) both had some antibacterial activity. Full details of a modified synthesis of 2β-hydroxymethyl sulphide (45a) and its chemistry have appeared[47] including conversion to difluoromethyl penicillins. Treatment of 6β-(alkyl-amido) penicillin esters with di-t-butyl dicarbonate/DMAP afforded imides of type (46)[48] but isopropenyl acetate gave degradation; in the cephalosporin series both 'Boc' and acetylimides were obtained. A full paper has appeared on the Wittig-type β-lactam adducts (47) (X=CN,COR) and their chemistry[49] including regeneration of the β-lactam by ozonolysis. The 4-sulphonyl-azetidinone (48a), itself accessible from benzyl 6β-tritylamino-penicillanate, has been converted[50] into the 4-t-butyl thio derivatives (48b), plus its C-4 epimer, and (48c). Penicillin and cephalosporin allyl esters are reported to be smoothly deprotected by pyrrolidine plus a catalytic amount of Pd $(PPh_3)_4$.[51]

There is continuing interest in the preparation and chemistry of penicillins lacking the 6β-amino group. Radical transformation of a 6,6-dibromopenicillanate or either 6-mono-bromoepimer could be achieved stereoselectively to give either



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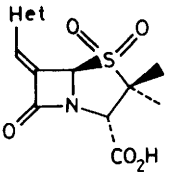

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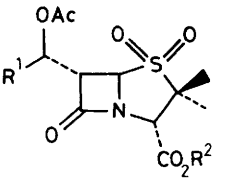

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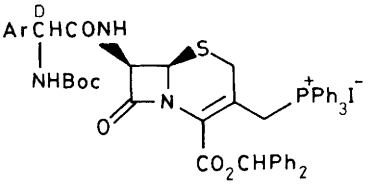

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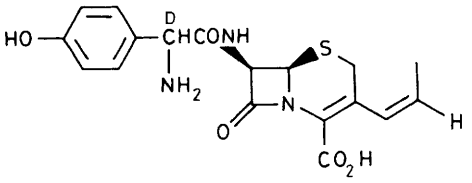

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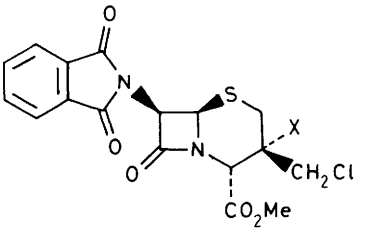
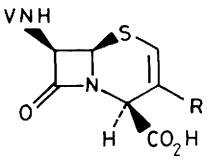
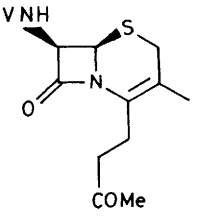


(60) a; X = SePh
b; X = Cl



(61) a; R = H
b; R = OMe



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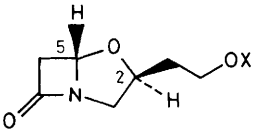
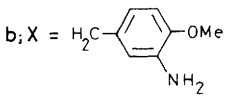


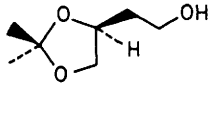
(63) a; X = H

b; X = 

6-alkyl product (49a,b).[52] (Cf.vol.19). Penicillin C-6 Grignard reagents are well known; they have now been reacted with glyoxals to give ketoalcohols of type (50); the one with R=$PhCH_2$ had antibacterial activity.[53] Two reports have appeared on the conversion of the anhydropenicillin (51), itself obtainable from 6,6-dibromopenicillanate, to the valuable azetidinone (52).[54,55] Treatment of penicillin G (salt or benzyl ester) with bromine and nitrous acid gave the bromo-compounds (53).[56] 6α(6β)-Acetylpenicillanic acid (54) was best obtained by treatment of 6-diazopenicillanic acid with acetaldehyde; the mechanism in Scheme 2 was proposed.[57] The bisnorpenicillin diazoketone (55) was obtained from the C(3)-epimer of (52) in five steps[58] together with its C(3)-epimer; for further chemistry of (55) see section 9. Fuller details have been published[59] on the synthesis of the 6-(heterocyclyl) methylenepenam sulphones (56) which are β-lactamase inhibitors (see vol.19); an alternative synthesis involved elimination from acetates of type (57).

There is increasing interest in C(3)-vinylcephalosporins in view of their oral absorption and good antibacterial activity. Wittig reagents (58) were prepared in three steps from a 3-(chloromethyl)ceph-3-em ester[60]; further manipulation gave cephalosporins such as (59), BMY-28100, after isomer seperation. In the synthesis of a series of 3-(methylthio)ceph-3-ems[61] the use of diphenyl chlorophosphate gave a cleaner conversion of alcohol to thioether with no Δ^2-isomer formed. Modification of the C(3)-acetoxymethyl group to C(3)-arylmethyl or-amidomethyl has been achieved using boron trifluoride.[62] Addition of phenylselenyl chloride to a 3-(exomethylene)cepham gave mainly the seleno compound (60a) at room temperature but the dichloro compound (60b) at 80°C.[63] Interestingly, selenoxide elimination from (60a) gave a ceph-2-em while base treatment of (60b) gave a ceph-3-em.

The known antibacterial inactivity of ceph-2-em-4α-carboxylates, it was postulated, might be due to an unfavourable orientation of the 4α-group. However, both the 3H-ceph-2-em-4β-carboxylic acid (61a) and its 3-methoxy analogue (61b) were synthesised[64] and proved to be inactive. Novel 4-substituted ceph-3-ems of the type (62) were available from the corresponding 3-methyl-ceph-3-em ester in a four-step sequence initiated by Michael addition.[65] A mild deoxygenation procedure of ceph-2-em sulphoxides using zinc in DMF-acetic acid was discovered during



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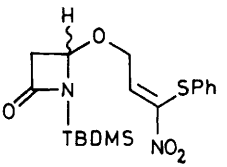

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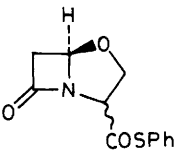

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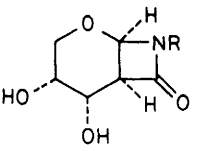

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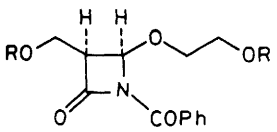
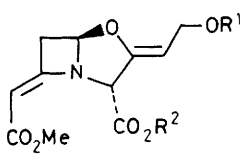


(68)



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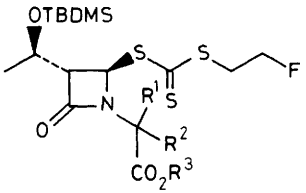
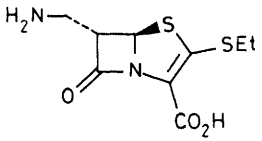
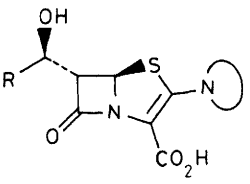


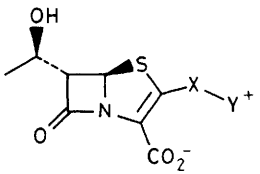
(70) a; R^1 = H, R^2 = Cl, R^3 = PNB
b; R^1, R^2 = O,
R^3 = PNB or $CH_2CH{=}CH_2$



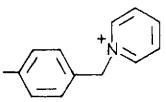
(71)



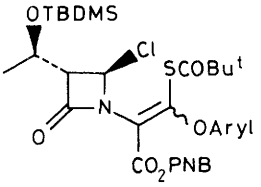
(72) R = H or Me



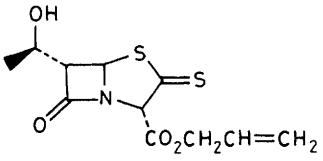
(73) X = various, Y^+ =



(74) $X = CH_2$, Y^+ =



(75)



(76)

this work. 1-Methanesulphonyloxy-6-trifluoromethylbenzotriazole has been recommended[66] as an efficient acylation catalyst for the preparation of semisynthetic cephalosporins.

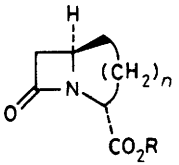
5. Clavulanic acid and Oxapenams

Enantioselective syntheses of the fungicide (-)-2-(2-hydroxyethyl)clavam (63a) and its (5R)-epimer have been reported, starting from 4-acetoxyazetidin-2-one and the resolved alcohol (64).[67] Removal of the protecting group from (63b) was critical and was achieved with DDQ. (Phenylthio)nitromethane has proved to be a valuable synthon for β-lactam annulation; for example, condensation with the appropriate aldehyde[68] provided the nitro-alkene (65) which cyclized with tetrabutylammonium fluoride and ozone to the separable oxapenams (66). The carbohydrate-derived β-lactams (67) were transformed by periodate oxidation, then boro-hydride reduction, to intermediates for oxapenam and oxacephem synthesis e.g. (68).[69] The 'formylacetate synthon' mentioned earlier[35] could be transformed to an oxazolidine-2-acetate but this has not yet been cyclized to an oxapenam.

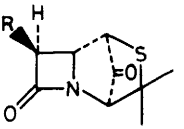
The Wittig-type condensation mentioned earlier[49] could be applied to clavulanates; here only the (methoxycarbonyl)methylene ylid reacted to give the Z-olefins (69).

6. Penems

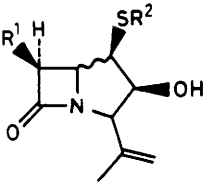
In most penem syntheses the key step is the construction of the C(2)-C(3) double bond; two recent reports[70,71] feature the use of trithiocarbonates for this step. In the first, azetidinone (52) was converted to either of the intermediates (70a,b) which were cyclized using respectively the Wittig or the triethyl phosphite-mediated oxalimide route, giving on deprotection a 2-(2-fluoroethyl) penem. In the second the oxalimide cyclization was used to give after hydrogenolysis the 6-(aminomethyl)-2-(ethyl-thio) penem (71) which had weak antibacterial activity. The Wittig cyclization was applied successfully to a series of azolyldithio-carbamates to afford the penems (72)[72] bearing 2-(1-azolyl) sub-stituents, imidazolyl ones giving the best activity. Various



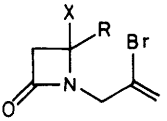
(77) n = 2 or 3



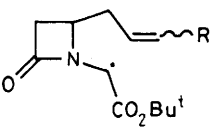
(78)



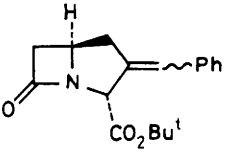
(79)



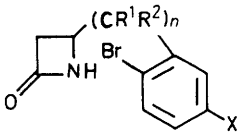
(80) a; X = H, R = $CH_2CH{=}CH_2$
b; X = H or Me, R = $CH{=}CH_2$



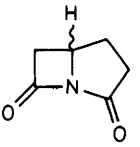
(81) a; R = H
b; R = Ph



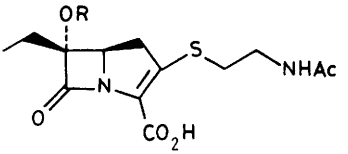
(82)



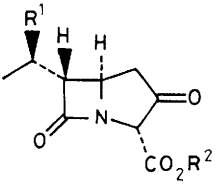
(83) n = 1 or 2



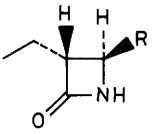
(84)



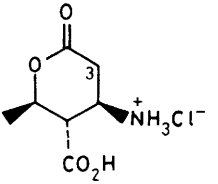
(85) a; R = Me
b; R = H



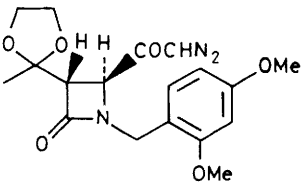
(86) R^1 = H, R^2 = PNB
(89) R^1 = O, R^2 = Me



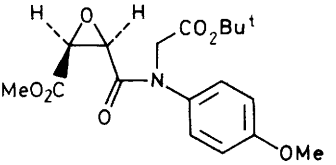
(87) R = E-CH=CHPh
(88) R = C≡CSiMe$_3$



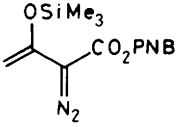
(90)



(91)



(92)



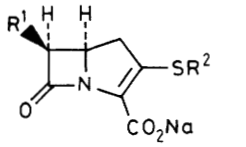
(93)

procedures were used to prepare a series of 2-[(pyridinio)methyl]-phenyl penems[73] with different 'spacers' between the phenyl group and C(2) (73), including intramolecular displacement of a 4-chloro-azetidinone by sulphur via intermediate (75). Their activity was comparable to the known (74).

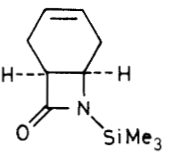
Various oxidative transformations of 2-alkylthiopenems have been reported,[74] in particular into 2-oxopenams, 2-unsubstituted penems and the thione (76), a known versatile intermediate for 2-thiopenems. A series of substituted 2-aminopenems were prepared via displacement of 2-phenoxypenems,[75] bearing *p*-electron withdrawing groups (cyano or nitro). 2-(Phenylamino)-penems, however, had to be prepared through an intermediate similar to (75).

7. Carbapenems and Related Systems

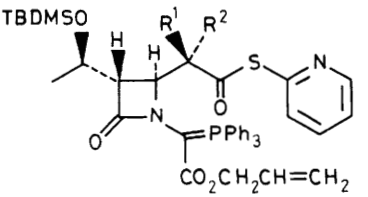
For the scope of this section, see vol.19; a number of references in section 8 are also relevant. Many syntheses of the carbapenem ring system are well established; newer approaches to such systems will be highlighted initially. Barrett has employed the (phenylthio)nitromethane synthon[68] in the synthesis of carbapenams and carbacephams of type (77). Penicillin-derived diazoketones (78) were transformed by ketone reduction, oxidation and sulphoxide rearrangement into a series of carbapenams (79).[76] Full details have been published (*cf.*vol.19) of the conversion of bromides (80a,b) into exomethylene carbapenams or carbacephams by thermal or photochemical radical procedures.[77] Similar methodology has been reported by Bachi[78,79] who noted that the terminal alkene-bearing radical (81a) cyclised to carbacepham products (*endo* mode), whereas the vicinally disubstituted double bond in (81b) cyclised to carbapenams (*exo* mode). In a related process the (E,Z)-2-benzylidenecarbapenam carboxylates (82) were accessible from an acetylenic radical (again the *exo* mode). A full report has appeared on the copper-catalysed intramolecular nucleophilic displacement of bromo-aryl compounds (83) generating benzo-carbapenams and -cephams.[80] The β-lactam fused γ-lactam (84) was prepared in three steps from 4-allylazetidin-2-one by iodine-mediated cyclization, elimination of HI and ozonolysis; it was an analogue of pyrrolidinones used in Alzheimer's disease therapy.[81]



(94) R^1 = *e.g.* $MeSO_2$–(OH)



(95)



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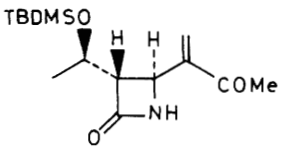

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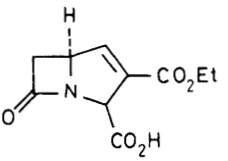
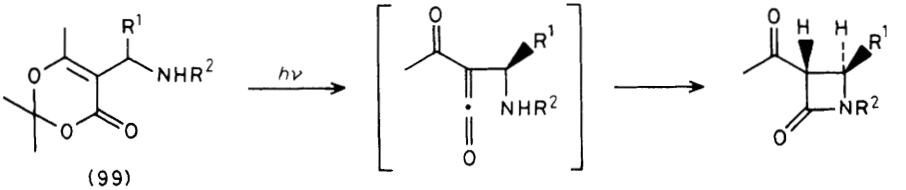
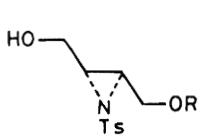


(98)

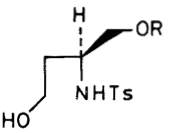


(99)

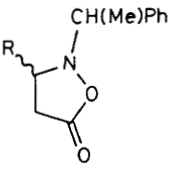
Scheme 3



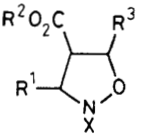
(100) Ts = 4-toluenesulphonyl



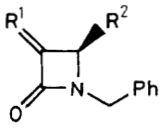
(101)



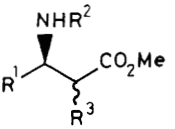
(102)



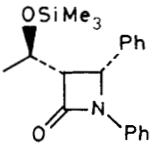
(103)



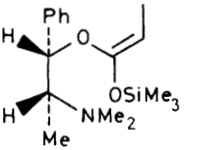
(104) a; R^1 = O
b; R^1 = α-H, ß-MeCONH



(105)



(106)



(107)

Carbapenems of the PS-series, bearing an unsubstituted alkyl group at C(6), have been the subject of three reports. A full account has appeared (cf.vol.19) of the syntheses of 6-methoxy-epi-PS-5 (85a) and the 6-hydroxy compound (85b), by the same strategy.[82] Two formal total syntheses of PS-5 reached the carbapenam-2-one (86) by different routes. In the first[83] the azetidinone (87) was obtained via enolate-imine condensation (see Section 8 for further examples); in the second[84] the known silyl alkyne (88), obtained as a single enantiomer from ethyl (S)-3-hydroxybutanoate, was employed. Both syntheses were completed by elaborating the C(4)-substituents to diazoketoesters followed by the Merck rhodium-catalysed carbene insertion. The former strategy was also applied for the C(6)-isopropyl compound, PS-6.

Meyers has described an elegant cycloaddition route to the thienamycin series [C(6)-hydroxyethyl side chain].[85] Thus the azetidinone (52) was heated with zinc chloride and a 2-silyl-oxydiene to give a carbacephem which was transformed in three steps to the ketone (89). Melillo's lactone (90) is still a popular thienamycin precursor; it has now been synthesized from methyl (R)-(-)-3-hydroxybutyrate in enantioselective fashion, commencing with an enolate addition to an N-acylaldimine.[86] Wolff rearrangement of the diazoketone (91) led to a 4-oxoazetidin-2-yl acetate which was cyclized by the intramolecular Wittig route to a thienamycin analogue bearing a 6-(2-methyl-1,3-dioxolan-2-yl) side chain.[87] An analogue bearing a 6-methyl hydroxyacetate side chain[88] has been reported; the epoxide (92) was closed with base to an azetidinone (see Section 8 for further examples) and the bicyclic synthesis was completed using the carbene insertion procedure. Displacement of 4-acetoxyazetidinone was performed with the in situ generated silylenolate (93). A detailed report has appeared on the synthesis of 5,6-cis-carbapenems (94) bearing a sulphonyl group in the C-6 side-chain.[89] The 5,6-cis-stereo-chemistry could be assured by going through the olefin (95); again carbene insertion was used to complete the bicyclic system. Carbapenems (94) showed good activity.

Following the demonstration of good activity and improved enzymic stability in 1β-methylcarbapenems, there have been a number of reports on various 1-substituted analogues. A detailed structure-activity paper on 2-arylcarbapenems[90] included several 1-methyl analogues; here the 2-aryl substituent did not improve the

biological properties. Addition of aryl Grignards to appropriate 2-pyridyl thioesters [e.g. (96)] followed by Wittig closure was employed. The olefinic ketone (97)[91] was cyclopropanated in up to 70% yield by a modified Simmons-Smith procedure, but the derived spirocyclopropane-1,1'-carbapenem proved more labile to dehydropeptidase than its 1β-methyl analogue. Treatment of intermediate (52) with the silyl enolate of chlorodifluoroacetophenone followed eventually by Wittig closure led to a 1,1-difluoro-2-phenylcarbapenem which proved chemically very labile.[92] In this synthesis N-methylthio protection, removable with 2-mercaptopyridine, was employed. 1-Acyloxycarbapenems, designed to mimic the 3-acetoxymethylcephems, were prepared[93] in both the 5,6-cis and trans series, ultimately by allylic oxidation of a 4-allylazetidinone or by radical benzoyloxylation of a species very like (95).

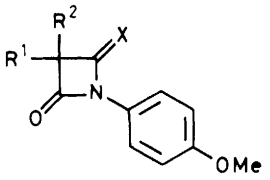
Stoodley has reported[94] the preparation of the 2-ethoxycarbonylcarbapen-1-em (98) by Wittig methodology, eventually from a 4-vinylazetidinone. Hydrogenation of (98) gave a carbapenem and cycloaddition (diazomethane) a tricyclic system which could be transformed to a 1-methylcarbapen-1-em. The palladium (0)-pyrrolidine deprotection of allyl esters[51] is applicable to carbapenems.

8. Azetidinones

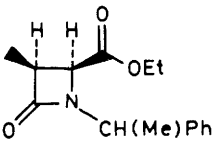
Synthetic methods are grouped according to the bond(s) created in the ring-forming step.

1-2 bond forming reactions.- This sub-section includes 'two-step' ketene+imine[2+2]cycloadditions where the intermediate β-amino-acid or-ester was isolated. Bis(5'-nitro-2'-pyridyl)2,2,2,-trichloroethyl phosphate is a new reagent for the cyclisation of β-amino-acids to β-lactams in up to 90% yield.[95] Irradiation of 1,3-dioxin-4-ones of type (99) leads to β-lactams[96] via, it is thought, a ketene intermediate (Scheme 3).

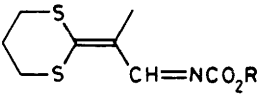
Another novel approach involved the aziridine (100) obtained as a single enantiomer from a 'Sharpless' epoxy-alcohol.[97] Reductive ring opening using Red-Al afforded (101) with high regioselectivity; transformation to the β-amino-acid was followed by Mukaiyama-Ohno cyclization to the β-lactam. Isoxazole inter-



(108) X = O or S



(109)



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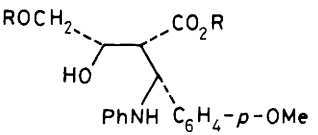
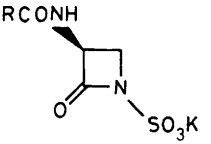
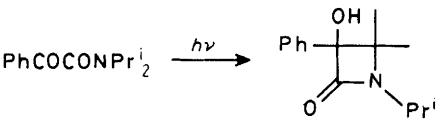


(111) R = TBDMS

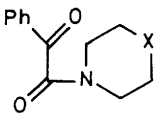


(112)

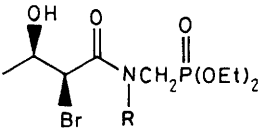


(113)

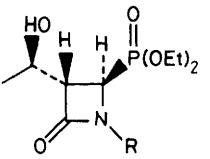
Scheme 4



(114) a; X = CH_2
b; X = O
c; X = $(CH_2)_2$



(115)



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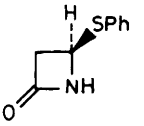
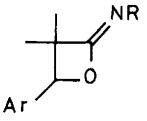


(117)



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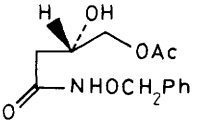

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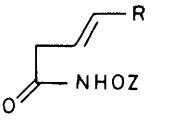
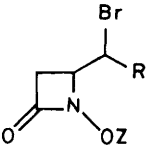


(120) Z = $PhCH_2OCO$



(121)

mediates, available from acrylates, continue to be employed. Thus the isoxazolidinones (102), prepared by addition of N-(α-methylbenzyl) hydroxylamine to E-acrylate esters, were separated and transformed in three steps to optically pure 4-substituted azetidinones[98] using two-phase β-amino-acid cyclization. Three reports featured [3+2] dipolar cycloaddition of nitrones to acrylates, generating isoxazolidines of type (103). Hydrogenolysis produced β-amino-acids or esters, which were closed by standard methods to the β-lactams, bearing 3-(hydroxyethyl) side chains. In two cases[99,100] these were progressed to intermediates for bicyclic synthesis; in a third[101] the 3-substituent was further manipulated *via* the dione (104a) to the 3β-acylamino compound (104b) (nocardicin series). (See 'Chemistry of Azetidinones').

The β-amino-esters resulting from addition of ketenes or ester enolates to imines may be cyclized in various ways to azetidinones. Addition of silyl ketene acetals to imines catalysed by trimethylsilyl trifluoromethanesulphonate afforded esters (105) with up to 100% *anti*-diastereoselectivity;[102] closure was effected on some *anti*-esters in 70-80% yield using lithium hexamethyldisilazide. A similar procedure was used to prepare the azetidinone (106)[103] as a single diastereoisomer. In another report enantioselection was achieved using the (1S,2R)-N-methylephedrine-O-propionate (107); imine addition catalysed by $TiCl_4$ and base closure gave a 3,4-*trans*-β-lactam with 95% enantiomeric excess.[104] The use of isocyanates or isothiocyanates rather than imines, followed by cyclization with triethyl aluminium, afforded 4-oxo or-thioxoazetidin-2-ones (108)[105] which could be further modified to 4-thioacetoxy derivatives. Use of a boron thioester enolate in addition to an appropriate imine bearing a chiral auxiliary (*cf*.vol.19) gave high *anti*-selectivity[106] and asymmetric induction; after base closure, an N-substituted version of the PS-5 precursor (88) resulted. A thioester tin enolate was used also in addition to an α-iminoester; here the 3,4-*cis* β-lactam (109) resulted with high selectivity after mercury-catalysed ring-closure.[107] In a related approach, imines (110) were condensed with R-(-)-3-hydroxybutyrate dianion, giving after further manipulation a 3β-methyl version of Melillo's lactone (90) and thence on β-lactam cyclisation a 1-β-methyl carbapenem precursor.[108]

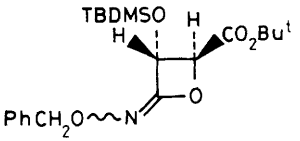
The carbohydrate-derived[109] β-amino silyl ester (111) was cyclized to a β-lactam using 2-chloro-1-methyl pyridinium iodide. Another stereoselective synthesis of 3,4-cis azetidin-2-ones has been achieved by remarkably stereoselective cyanoborohydride reduction of β-oximinoesters followed by hydrolysis and cyclization.[110] 4-Unsubstitued monobactams of type (112) were prepared from aspartic acid via a monoacylated 2,3-diaminopropionic acid.[111]

3-4 bond forming reactions.- Photochemical methods continue to be the most commonly employed for this mode of closure. A chiral version has appeared of the well-known cyclization of α-oxoamides to 3-hydroxyazetidinones, the Norrish Type II products. Though the starting material (Scheme 4) is inherently achiral, crystals separate from benzene with either chirality and give on irradiation either (+)- or (-)-(113).[112] Photocyclizations of the oxoamides (114a-c) in inclusion complexes with various host molecules have been studied;[113] high yields of β-lactams were obtained (up to 80%), and with substantial enantiomeric excess when a chiral host was employed. Similarly, inclusion complexes of N,N-dialkylpyruvamides with deoxycholic acid or cyclodextrin gave 3-hydroxy-3-methylazetidin-2-ones on irradiation with some asymmetric induction.[114]

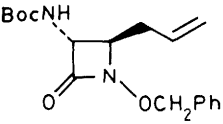
Treatment of the (2S, 3R)-bromohydrins (115) with lithium hexamethyldisilazide afforded optically active 4-diethoxyphosphinylazetidin-2-ones (116) of 3,4-trans stereochemistry[115] presumably via an epoxide intermediate.

4-1 bond forming reactions.- The known Pummerer cyclization of 3-(phenylsulphinyl)propionamides has now been applied in an enantioselective mode; the (+)-sulphoxide gave (4S)-β-lactam (117) in 67% enantiomeric excess.[116] An interesting report has appeared[117] on the lanthanide shift reagent-induced rearrangement of the iminooxetane cycloadduct (118) to a 4-arylazetidin-2-one.

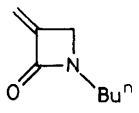
Intramolecular alkylation or Mitsunobu reactions continue to be employed for this mode; for example Mitsunobu closure of the (3R)-hydroxyhydroxamate (119) derived by stereoselective reduction gave a (3S)-4-acetoxymethyl β-lactam.[118] Miller has published two



(122)



(123)



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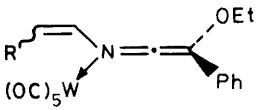

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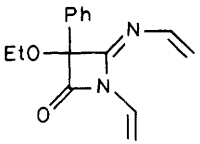

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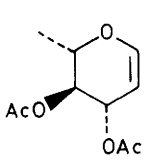

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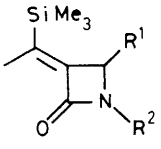

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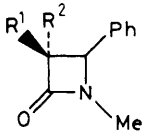
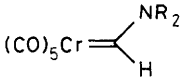
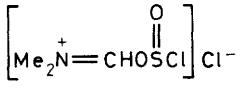


(129) $R^1 = R^2 = Me$

(131) $R^1 = H$, $R^2 = NMe_2$



(130)



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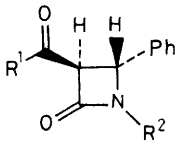
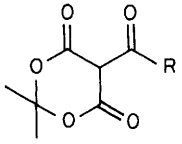


(133)



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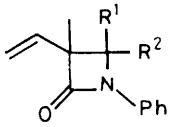

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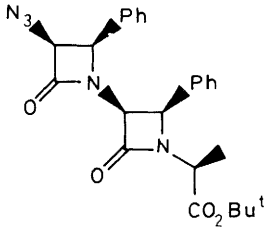

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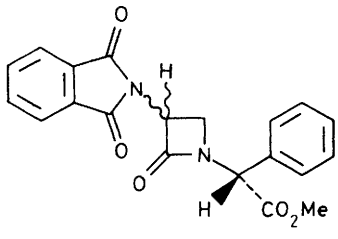


(137)

further reports on the bromine-induced cyclization of O-acyl-β,γ-unsaturated hydroxamates to β-lactams. Thus a variety of γ,δ-substituted olefins (120) gave β-lactams (121).[119] Where an α-substituent was present, it was found[120] that 3α-alkyl groups gave mainly 3,4-trans β-lactams while 3α-urethanes gave mainly cis products. Tartaric acid has been used as a precursor of optically active 3-hydroxy-4-alkoxycarbonyl β-lactams via Mitsunobu closure or (better) mesylate displacement.[121] Interestingly, some of the O-alkylated product (122) was obtained under Mitsunobu conditions. The 3α-aminoazetidinone derivative (123) was obtained from an oxazolidinone via ring opening, conversion to a hydroxamate and Mitsunobu cyclization.[122]

Reactions in which two bonds are formed

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

[3+1] additions:

1-2 and 4-1 bond formation.- A further report has appeared[123] on the cyclization of β-halo acid chlorides to azetidinones using thiosemicarbazides. An organometallic procedure has been used also: an (N-butyl)methyleneaziridine underwent ring expansion on treatment with carbon monoxide and a palladium catalyst, giving β-lactam (124).[124]

3-4 and 4-1 bond formation.- A formal example of this class is the reaction of the carbene complex (125) with an alkenyl isocyanide, giving (among other products) an 'azetidinylidene' complex which may be oxidised to the β-lactam (126)[125]; a similar iron complex gives (126) more selectively.

[2+2] additions:

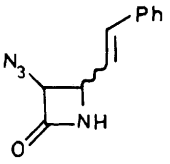
2-3 and 4-1 bond formation.- Three reports have featured alkene plus isocyanate cycloaddition. The use of a pressure bottle gave

excellent yields of β-lactams from volatile olefins and chlorosulphonyl isocyanate.[126] A detailed examination of the addition of trichloroacetyl isocyanate to the rhamnal derivative (127) showed that the reaction was reversible and that better stereoselectivity was observed in the β-lactam adducts at normal pressure.[127] The isocyanate-derived β-lactams (128) were converted by chlorination and elimination to α-vinylidene-β-lactams[128] which proved relatively stable; the vinylidene substituent could be modified in various ways.

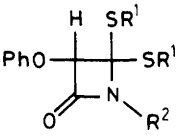
1-2 and 3-4 bond formation.- As in vol.19, detailed reference to ketene-imine and enolate-imine condensations will only be made where there were new chemical features. Organometallic complexes have again been used, e.g. addition of (33)[37] to N-methylbenzaldimine followed by heating afforded a product oxidisable to (129). (Dialkylamino)carbene complexes (130) underwent photolytic [2+2] addition to imines giving, for example, mainly the cis-isomer (131) in fair yield.[129] As with (33), (130) was applied to the synthesis of penams, cephams and oxapenams in fair to excellent yield.

New reagents continue to be employed for the conversion of carboxylic acids to ketene precursors. The 'Vilsmeier type' N-chlorosulphinylmethylene derivative (132) was used to activate acetic acids followed by treatment with triethylamine and an imine to give several 1,2,4-trisubstituted azetidinones.[130] Similarly N, N-dimethylphosphoramidic dichloride was employed in the synthesis of a number of azetidinones and azetidin-2,3-diones.[131] In a novel approach, a number of β-lactams of type (133) were prepared by heating an HCl-saturated benzene solution of the acyl Meldrum's acids (134) with imines;[132] acylketenes were likely intermediates (cf.Scheme 3). The addition of certain vinylketenes to imines affored 3-vinyl-β-lactams (135) in a low yield.[133]

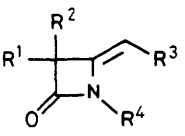
Highly stereoselective addition of azidoketene to chiral 3-imino-β-lactams, themselves available by [2+2] cycloaddition, generated bis-β-lactams of type (136).[134] Nocardicin analogues were accessible through enantioselective addition of a phthalimido ketene to a chiral formaldimine trimer, generating the separable 4-unsubstituted azetidin-2-ones (137).[135] Other 3-aminoazetidin-2-ones and 4-styryl monobactams (viz. the N-sulphonic acids) were accessible through azidoketene cycloaddition;[136] only the N-4,4'-



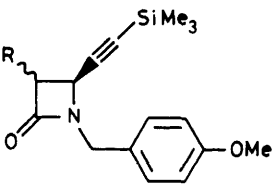
(138)



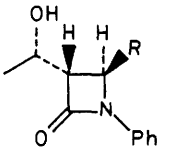
(139)



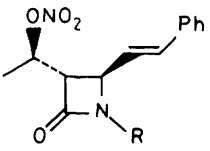
(140)



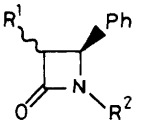
(141) a; α – R
b; β – R



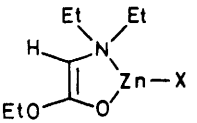
(142)



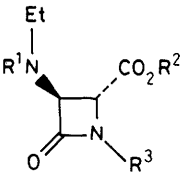
(143)



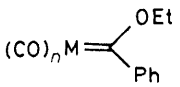
(144) a; R^1 = β-Ph, R^2 = Ph
b; R^1 = α-alkyl, R^2 = Ph
(145) R^1 = α-NEt_2, R^2 = Me



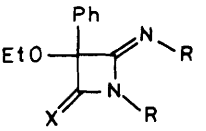
(146)



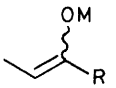
(147)

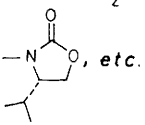


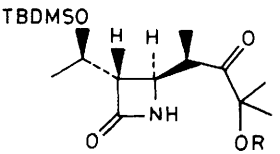
(148) M = Fe, n = 4
(150) M = W, n = 5



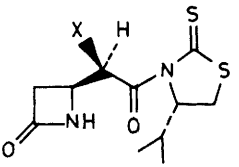
(149) a; X = $(CO)_4$Fe
b; X = O



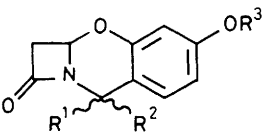
(151) M = Zr complex, $B(OR)_2$, SnBr
R = SBu^t,  , *etc.*



(152)



(153) X = OR, SR, or NHR



(154)

dimethoxybenzhydryl compounds were successfully transformed to NH compounds, e.g. (138); the final monobactams had low activity. Further β-lactams synthesized by the ketene plus imine route were a series of novel 3-(carboxymethylene)azetidinones[137], starting from 4,4'-bis(thioethoxy)-3-butenoic acid, 4-(dithioalkyl)azetidin-2-ones of type (139) from dithiocarbonimidates,[138] 4-(2-furyl)-azetidin-2-ones,[139] 3,3-diphenylazetidin-2-ones[140] (ketene via azidoketone), and other 3,4-disubstituted analogues.[141]

Concerning the ester enolate plus imine [2+2] mode, two reports have demonstrated that enolizable imines may be used. Thus a series of 3,4-dialkyl and 3,3,4-trialkyl β-lactams were prepared[142] when lithium ester enolates and enolizable aldimines reacted in the presence of dimethylaluminium chloride, in good to excellent yield, and N-trimethylsilylimines reacted without catalyst to give 4-unsubstituted β-lactams[143] including some 3-amino derivatives; cis-trans mixtures resulted. Using a ketenimine component led to 4-alkylidene compounds of type (140)[144] having almost wholly Z-geometry at the double bond. The relative 3,4-stereochemistry in the 4-alkynyl β-lactams (141a,b) was found to be determined by the enolate; a diethylaluminium enolate of an S-t-butylthioester gave largely the anti-product (141a) while a complex zirconium enolate gave the syn-β-amino thioester corresponding to (141b).[145] Full details have been published of the condensation of the dianion of (S)-3-hydroxybutanoates with appropriate aldimines, generating 3,4-trans compounds such as (142) and, after further transformations, the (+)-thienamycin precursor (52).[146] Inversion of the hydroxyethyl side-chain in such adducts to the required R configuration is usually achieved via the Mitsunobu reaction, but conversion to a nitrate ester of type (143) via a mesylate displacement has been reported;[147] hydrogenolysis regenerates the alcohol. Condensation of lithium enolates with N-benzylideneaniline in the presence of a trialkylaluminium is reported to give only cis-β-lactams.[148] Addition of zinc enolates to the same imine with ultrasound gave 88% of the trans-azetidin-2-one (144a) when R=Ph but mainly cis-adducts (144b) when R=alkyl.[149] Another report[150] on the use of zinc enolates disclosed the formation of exclusively trans-azetidin-2-ones, e.g. (145) when an α-amino-acid ester enolate was used. The geometry of the zinc enolate is thought to be as in (146); the same intermediate was postulated in the diethyl zinc-mediated dimerisation of

α-iminoesters to trans-β-lactams (147).[151] In another variant, addition of an acetylide salt to an oxime was used to prepare some 2-phenyl-3-arylazetidin-2-ones.[152]

Reactions in which three bonds are formed

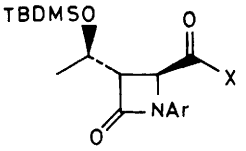
[1+1+2] additions

This class is represented by the combination of the iron complex (148) with two moles of an isocyanide;[153] azetidinylidene complexes (149a) resulted and were hydrolysed to β-lactams (149b) using permanganate. (Cf.ref.125). Ketenimine complexes are intermediates. The tungsten complex (150), similarly, reacted with an alkyne-isocyanide mixture[154] giving a mixture of 3,4-regioisomeric (4-alkylidene)azetidinylidene complexes.

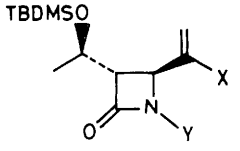
Other syntheses reported were a seven-step conversion of a dihydropyranone to 4-(2-methoxyethyl)azetidinone[155] and a synthesis of tetra-n-butylammonium 4-benzoyl-2-oxoazetidinyl-1-sulphonate.[156]

Chemistry of azetidinones

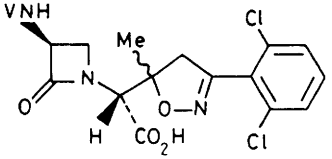
A number of reports have appeared on the displacement of a leaving group at C(4), particularly for the generation of carbapenem precursors (see also section 7). Normally the acetate (52) is employed; the corresponding 4-chloro compound, prepared from the 4-t-butylthio derivative by chlorination, was also successfully displaced by a silyl enolate like (93) or by various metal enolates (151);[157] t-butyl thioates gave the best stereoselection. Excellent β:α diastereoselection was found in the preparation of 1β-methylcarbapenem precursor (152)[158] when a tin enolate was used; various 2-heterosubstituted tin enolates, similarly, gave at least 96:4 diastereoselection on a 3-unsubstituted 4-acetoxyazetidinone[159] giving 1β-heteroatom carbapenem precursors (153). Other stereoselective displacements on (52) were achieved using a zinc enolate derived from a 2-(bromopropionyl)-2-oxazolidone[160] and a 4-methyl version of silyl enolate (93)[161], both for 1β-methyl-



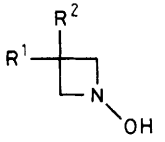
(155) a; X = N(Me)OMe
b; X = H, Me, C≡CR, *etc.*



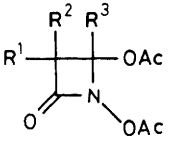
(156)



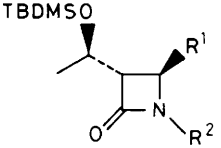
(157)



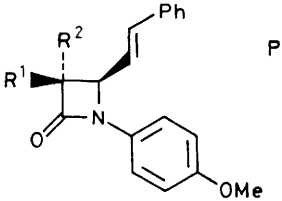
(158)



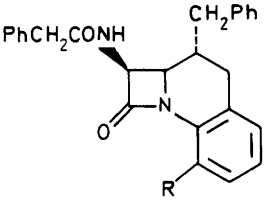
(159) R^3 = H or OAc



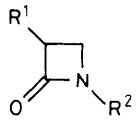
(160) a; R^1 = (*R*)-CH(OH)Me, R^2 = $CH(C_6H_4\text{-}p\text{-}OMe)_2$
b; R^1 = CH_2CO_2H, R^2 = H



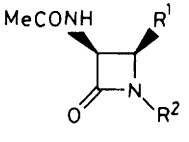
(161) a; R^1 = H, R^2 = $(CH_2)_2CO_2Me$
b; R^1 = $(CH_2)_2CO_2Me$, R^2 = H



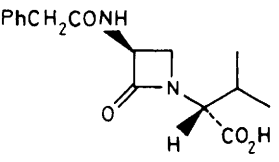
(162)



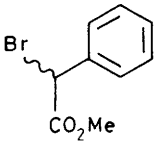
(163) a; R^1 = $CH{=}CH_2$, R^2 = CH_2Ph
b; R^1 = $HO_2CCH(NH_2)CH_2CH_2$, R^2 = H



(164)



(165)



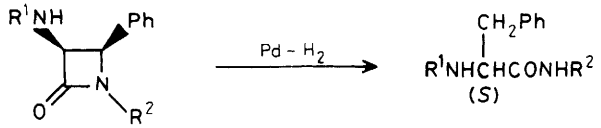
(166)

carbapenem precursors. Displacement of a 4-acetoxy group with a 2-hydroxybenzaldehyde or ketone afforded 2,3-benzo-1-oxaoctems (154).[162]

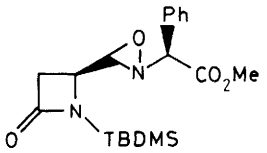
Other modifications of 4-substituted azetidin-2-ones include the reaction of hydroxamates (155a) with various nucleophiles, giving 4-acyl compounds (155b) which may be transformed to 1β-methylcarbapenem precursors via complexed propargyl cationic intermediates.[163] Stereoselective hydrogenation of olefinic azetidinones of type (156) has also been employed as a route to 1β-methylcarbapenem precursors, using either Raney nickel catalysis[164] on a bicyclic system, a bis-(diarylphosphino)-dinaphthyl catalyst[165] or simply an N-silylated intermediate.[161] Good to excellent stereoselection was observed. Other reductions involved Raney nickel desulphurization of 4-(dithioalkyl)β-lactams (139)[138] or treatment of 4-acetoxyazetidinones with a hydrosilane catalysed by trimethylsilyl triflate,[166] both generating 4-unsubstituted species. Another Raney nickel desulphurization was used on a 4-benzothiazole sulphide in the final step of a synthesis of the nocardicin (157).[167]

Dihydroalane reduction of 1-benzyloxy β-lactams followed by debenzylation gave 1-hydroxy azetidines (158); lead tetraacetate oxidation then afforded the acetoxylated compounds (159)[168] but the triacetoxy product was observed only in the 3,3-dimethyl case. Ruthenium tetraoxide oxidation of 1-isopropylazetidin-2-one afforded the N-acetyl and N-unsubstituted products in roughly equal amounts.[169] 4-Acetonyl and-phenacyl β-lactams were available from the corresponding 4-formyl compounds via nitroalkanes in a general modified Nef reaction.[170] The (4R)-1-hydroxyethyl azetidinone (160a), available from S-ethyl lactate, was transformed to the iodide, thence by elimination, N-deprotection, hydroboration, and oxidation to the thienamycin intermediate (160b).[171] Treatment of some 4-benzoyl-β-lactams with sodium hydride and an alkyl halide in dimethylformamide leads to 5-alkoxy-γ-lactams; the mechanism is to be clarified.[172]

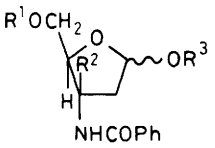
The radical alkylation mentioned earlier[52] was equally applicable to the synthesis of cis-and trans-azetidinones (161a,b). Radical cyclization of 1-(2-bromophenyl)azetidinones bearing C(4) alkenyl or alkynyl substituents led to tricyclic azetidinones by 5-exo or 6-exo cyclization, e.g. (162).[173]



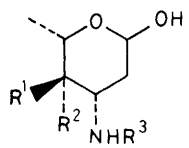
Scheme 5



(167)

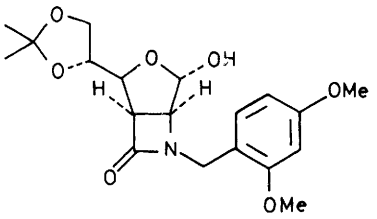


(168) R^2 = H or Me



(169) a; R^1 = OH, R^2 = R^3 = H

b; R^1 = H, R^2 = OH, R^3 = $COCO_2CH_2Ph$



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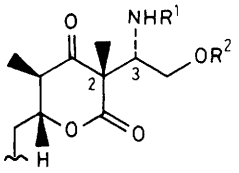

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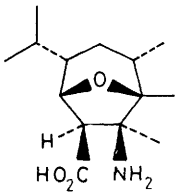
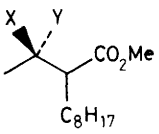
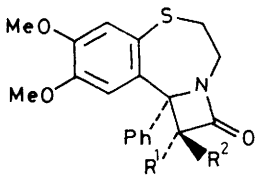


(172)



(173) a; X = H, Y = $NHCH_2Ph$

b; X = $NHCH_2Ph$, Y = H



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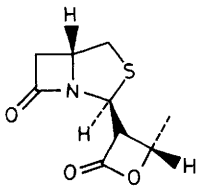

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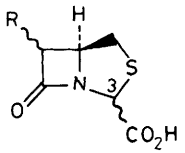


(176) a; R = α-Me, 3α- or 3β-CO_2H

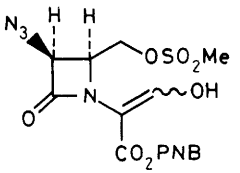
b; R = β-Me, 3α-CO_2H

Modification or substitution at the 3-position was featured in other reports. Thus the known 3-vinylazetidin-2-one (163a) was converted in six steps to deoxytabtoxinine β-lactam (163b)[174] which lacked the glutamine synthetase inhibition of the 3-hydroxy compound [cf. (29)]. Asymmetric alkylation of an N-(1-phenyl)ethylazetidinone was used to prepare the enantiomeric 3-methyl-β-lactams.[175] The further acetylation of some 3-acylamino-azetidinones gave imides as the bicyclic β-lactams mentioned earlier.[48] Oxidative cleavage of silylated 3-(1-hydroxyethyl)-azetidinones using Jones' reagent gave 3-acetyl compounds[176] which by nitrosation and reductive acetylation gave largely or exclusively the 3β-products (164); cf. ref.101 and (104). Cleavage of acid (165), ultimately penicillin derived, to the NH compound, then realkylation with (166) gave separable isomers, one identical to 3-(phenylacetyl)aminonocardicinic acid, proving the 3-configuration to be the same as the penicillin 6-configuration.[177]

Further uses of azetidinones

This new section is designed to illustrate, inevitably selectively, the wider synthetic and structural uses to which monocyclic β-lactams are now being put. Various amino-acid and peptide derivatives are available from β-lactams; thus 3-amino-4-phosphonobutanoic acid[178] was prepared in two steps from 4-iodomethylazetidin-2-one. A series of papers has exploited the reductive cleavage of 4-arylazetidin-2-ones, generating phenylalanine peptides and similar species (Scheme 5).[179,180,181] Independent peptide synthesis has confirmed, by comparison, the extremely high stereoselectivity of [2+2] azidoketene plus imine cyclization,[134,179] and the reports also describe syntheses of optically pure α-alkyl, α-amino acids and a series of dipeptides. The dipeptide sweetener aspartame (Asp-Phe-OMe) has been obtained by UV rearrangement and ring opening of the 4-oxaziridinyl-β-lactam (167).[182]

Azetidinones are also useful in aminoglycoside synthesis; for instance the 2,3-dideoxy-3-aminopentoses (168) were synthesized in six steps from 4-substituted azetidinones.[183] Other examples were the syntheses of acosamine (169a) and a daunosamidine



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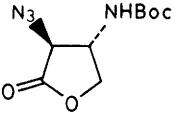
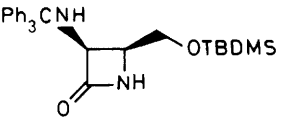
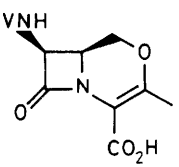


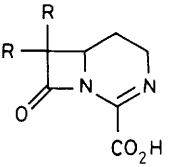
(178)



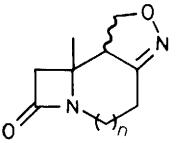
(179)



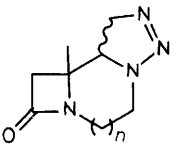
(180)



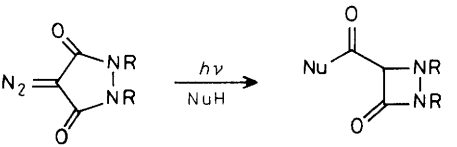
(181)



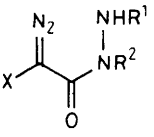
(182) n = 1 or 2



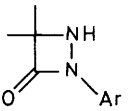
(183) n = 1, 2 or 3



Scheme 6



(184)



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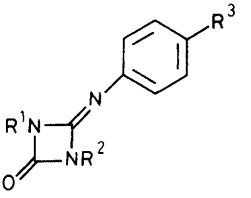
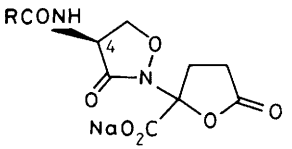
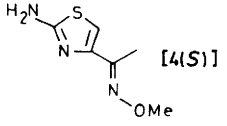
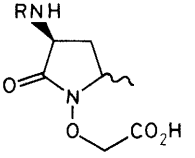


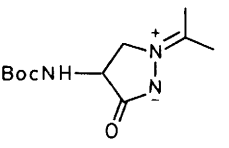
(186)



(187) a; R = Me [4(*S*)]
b; R = $PhCH_2$ [4(*S*), 4(*R*)]
c; R =  [4(*S*)]



(188)



(189)

derivative (169b)[184] and the conversion of the bicyclic (170) into 2-amino-2,3-dideoxy sugar analogues.[185] Thomas defined the 2,3-stereochemistry of the lankicidin fragment (171) using a β-lactam intermediate[186] and the cis β-amino-acid moiety of the helminthosporic acid analogue (172) was β-lactam derived.[187] The bicyclic β-lactams formed on irradiation of 2-pyridones (see vol.19passim) may be converted to various 7-membered heterocycles,[188] and the photochemical rearrangement of certain 4-pyrimidinones to pyrimidine-5-carboxylates also involves bicylic β-lactam intermediates.[189]

The well-defined stereochemistry of azetidin-2-ones (characteristic $J_{3,4}$ coupling, etc.) can help to define other stereochemical points. Thus the β-amino esters (173a,b) were separately cyclized to β-lactams[190] to determine their relative 2,3-stereochemistry. The thiolysis of enantiomers of 3-methyl-2-aziridinecarboxylic acids was found to proceed largely by opening at C(2) with inversion by cyclising the products to 3-substituted β-lactams which were analysed by ^{13}C nuclear magnetic resonance.[191] A detailed spectroscopic study of the configurations and conformations of the β-lactam fused 1,4-benzothiazepines (174) and the derived sulphones has appeared.[192]

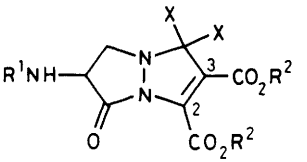
9. Major Structural Variants

See vol.19 for the scope of this section. A fair number of reports on isopenams and isocephems have appeared; thus the penicillin-derived diazoketone (55)[58] was rearranged, presumably via a ketene, to the isopenam β-lactone (175) using rhodium acetate in benzene. The three isopenams (176a,b) have been prepared ultimately from a substituted (benzylamino) malonic acid.[193] The versatile intermediate (177) was obtained as a single enantiomer from aspartic acid in several steps via lactone (178) and converted to both 2-isocephem and 2-iso-oxacephem nuclei.[194] The known chiral azetidinone (179) was converted into a 2-iso-oxacephem in four steps, using Mitsunobu's procedure to close the oxazine ring,[195] and a similar strategy was used to prepare penicillin V analogue (180) via an enol-alcohol intermediate.[196] Such isocephems have appreciable antibacterial activity. The (phenylthio)-nitromethane synthon was also used[68] for oxacephem synthesis.

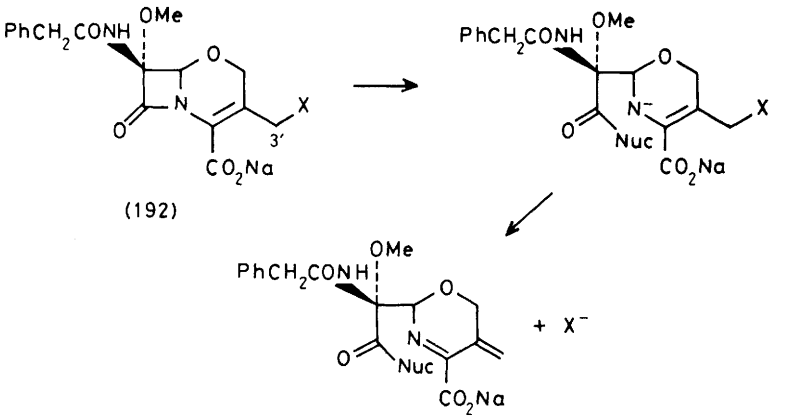
1-Carba-3-azacephems (181) were obtained using either an intramolecular aza-Wittig reaction or by treatment of a 4-(2-aminoethyl)azetidinone with a glyoxylate and further manipulation.[197] Intramolecular olefin-nitrile oxide or azide cycloaddition was employed to generate the novel tricyclic systems (182) and (183);[198] further chemistry of the triazolines, viz. conversion to aziridines and imines, was investigated.

Interest in 1,2-diazetidinones (aza-β-lactams) continues to grow. A full report has appeared[199] on the photochemical Wolff contraction of 4-diazopyrazolidine-3,5-diones to aza-β-lactams (Scheme 6) in the presence of O or N nucleophiles. Some regioselectivity is observed where the two N-substituents differ, N-phenyl in particular showing a higher migratory aptitude than N-alkyl.[200] Rhodium carbenoid mediated cyclization of diazo hydrazides (184) also generates β-lactams.[201] A number of new routes to aza-β-lactams from α-halocarboxylic acids and hydrazine derivatives have appeared,[202] involving either alkylation followed by dehydration or acylhydrazide formation followed by intramolecular alkylation; equivalent to the former strategy was the Grignard-induced cyclization of α-(2-arylhydrazino)-esters to give (185).[203] A 1,2-diazetidinone was a minor product from the reaction of a vinylketene with diethylazodicarboxylate.[133] Addition of alkyl or aryl isocyanates to certain carbodi-imides afforded 1,3-diazetidinones of type (186)[204] and a 1,3-diazetidinone also resulted from the reaction of 5-hydroxytryptophan with alkyl isocyanates in acetone, presumably via intermediate imines.[205]

Other reports have described molecules designed to mimic the biological action of β-lactams. Semisynthetic modifications of lactivicin (187a-c) were prepared from commercially available D-cycloserine;[206] (187b,c) had good antibacterial activity. On the other hand, phenoxyacetyl N-sulphonyl cycloserine[207] was antibacterially inactive despite the apparently activated carbonyl group. Only one report appeared on γ-lactam analogues last year, namely the oxamazin analogues (188) prepared in two ways from γ-nitro-α-amino-acid esters;[208] they were also devoid of biological activity. Much more interesting from the antibacterial point of view were the bicyclic pyrazolidinones described in three reports. Cycloaddition of the pyrazolidinium ylid (189) and an acetylene

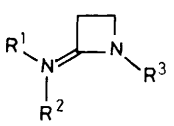


(190) X = Me
(191) X = H



(192)

Scheme 7



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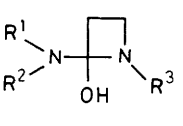
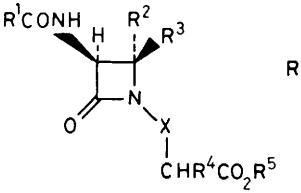
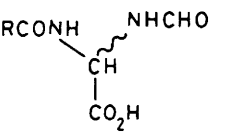


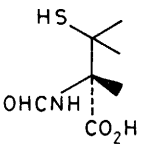
(194)



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



(196)



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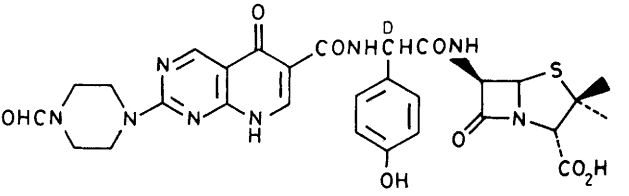


(198)

dicarboxylate[209] (a known reaction in the 4-desamino series) afforded bicyclic molecules (190) which by deprotection and reacylation on nitrogen afforded antimicrobially active products. Another report described the synthesis of the 'bis-nor analogue' (191) by a similar route.[210] Unsymmetrically substituted propiolates may be added and the regioisomers separated;[210,211] the most active compounds have a C-2 carboxy substituent and a C-3 acyl group.

10. Mechanistic Studies on Mode of Action and Degradation

The relationship between intrinsic antibacterial activity, β-lactam chemical reactivity and nature of the 3'-substituent has been extensively studied for cephalosporins; a similar study has now been reported for the 7α-methoxy-1-oxacephems (192).[212] The rate of alkaline β-lactam hydrolysis correlated well with the inductive effect of the C(3')-substituent but the rate showed little correlation with intrinstic activity. Alkaline degradation, as with cephalosporins, produces first a β-lactam-cleaved enamine, then an imine (Scheme 7; cf.vol.19).[213] Overall the observations agree with recent reports that leaving-group ability at C(3') in cephems is not related to β-lactam reactivity. Differing views have been expressed on whether the degree of pyramidal nature of the β-lactam is important for biological activity[212,214] - apparently less important for the oxacephems. Hydrolysis of (azetidin-2-ylidene)ammonium salts (193) was shown to generate both a β-lactam and an aminoamide;[215] this was taken to demonstrate general acid catalysed breakdown of intermediate (194) and non-facile four-membered ring opening, even when R^3 was a good leaving group. The remarkable lack of biological activity of thiamazins (195a) compared to oxamazins (195b) has been discussed[216] in terms of infrared stretching frequencies, hydrolysis rates, pyramidal nature and relative fit at the enzyme active sites, the latter considered most plausible.

Aqueous degradation of BRL 36650, a 6α-formamido (acylureido)penicillin, from ambient pH gave a near-quantitative C(5)-C(6) bond cleavage[217] generating the α-formamido glycine (196) and N-formylpenicillamine (197); similar 6α-H penicillins are known to give negligible (197) under such conditions (cf.vol.19).

A detailed report on the aqueous degradation of the acylated amoxycillin (198)[218] showed formation of the (5R)-and(5S)-penicilloic acids and a deformylated product in base; in acid the (5R,S)-penilloic acids resulted.

Appendix to Chapter 5: β-Lactam Antibiotics Prepared for Structure-Activity Relationships

The β-lactams are arranged in the same sequence as the main sections of the report.

β-Lactam

	Ref
Chlorine-containing catecholic ureidopenicillins	219
Nine N-(2-aryltriazinylcarbonyl)amoxycillin analogues	220
N-(pyrido[2,3-d]pyrimidine-6-carbonyl)ampicillin and amoxycillin analogues	221
N-alkylampicillins and N-heteroarylampicillins and cephalexins	222
New thiazolidine-type ureidopenicillins	223
[(2-Isopropyl-5-methylcyclohexyloxy)-α,α-dialkylmethyl]penicillins	224
Two acid-stable semisynthetic penicillins	225
Novel bis-penicillins, $RCONH(CH_2)_6NHCOR$	226
2β-(Triazolylmethyl)penam-1, 1-dioxides	227
C(3')-Analogues of 7α-formamido ureidocephalosporins	228
7α-Methoxy (pyrimidinyl)ureidocephalosporins	229
Catecholic ureidocephalosporins and - cephamycins	230
2-(Hydrazonoacetamido)cephalosporins	231
7β-(Isoxazolylmethoxyimino)acetamidocephalosporins	232
Novel (aminothiazolylglycyl)cephems bearing hydroxypyridones	233
A 7β-(aminothiazolyl)cephem oral antibiotic	234
Novel fluorine-containing cephalosporins	235
Some cephalexin analogues	236
7β-(7-or8-substituted coumarinyl)cephalosporins	237
Thioglycosyl cephalosporins	238
C(3')-Cefotaxime analogues	239,240,241
C(3')-Tetrazolyl cephalothin analogues	242

References

1 J. Nüesch, J. Heim, and H.-J. Treichler, Ann.Rev.Microbiol., 1987, 41, 51.
2 A.L. Demain and S. Wolfe, Dev.Ind.Microbiol., 1987, 27, 175 (Chem.Abs., 1987, 107, 55364).
3 M. Sakisaka, Gendai Kagaku Zokan, 1987, 9, 73 (Chem.Abs.,1987, 107, 130532).
4 U. Graete and E.J. Bormann, Biol.Zentralbl., 1987, 106, 33 (Chem. Abs., 1987, 107, 150801).
5 T. Nagahara and T. Kametani, Heterocycles, 1987, 25, 729.
6 T. Nakai and T. Chiba, Farumashia, 1986, 22, 612 (Chem.Abs., 1987, 106, 49816).
7 M. Alpegiani, C. Battistini, A. Bedeschi, G. Francheschi, F. Guidici, E. Perrone, C. Scarafile, and F. Zarini, Chim. Ind. (Milan), 1986, 68, 70 (Chem.Abs., 1987, 106, 175971).
8 C.M. Cimarusti, Gazz.Chim.Ital., 1986, 116, 169 (Chem.Abs., 1987, 106, 66944).
9 J.S. Sandhu and B.Sain, Heterocycles, 1987, 26, 777.
10 A.Kamel and P.B. Sattur, Heterocycles, 1987, 26, 1051.
11 S.W. Elson, K.H. Baggaley, J. Gillett, S. Holland, N.H. Nicholson, J.T.Sime, and S.R. Woroniecki, J.Chem.Soc., Chem. Commun., 1987, 1736.
12 K.H. Baggaley, J.T. Sime, N.H. Nicholson, S.W. Elson, J. Gillett, S. Holland, and S.R. Woroniecki, J.Chem.Soc., Chem. Commun., 1987, 1738.
13 B.W. Bycroft, C. Maslen, S.J. Box, A.G. Brown, and J.W. Tyler, J.Chem.Soc., Chem.Commun., 1987, 1623.
14 T. Kato, H. Hinoo, Y. Terui, J. Nishikawa, Y. Nakagawa, Y. Ikenishi, and J. Shoji, J.Antibiot., 1987, 40, 139 and preceding paper.
15 N. Katayama, K. Koyama, Y. Nozaki, S. Harada, and H. Ono, Takeda Kenkyushoho, 1987, 46, 62(Chem.Abs., 1987,107, 214535).
16 U. Anthoni, L. Chevolot, C. Larsen, P.H. Nielsen, and C. Christophersen, J.Org.Chem., 1987, 52, 4709.

17 U. Anthoni, K. Book, L. Chevolot, C. Larsen, P.H. Nielsen, and C. Christophersen, J.Org.Chem., 1987, 52, 5638.
18 J.E. Baldwin, S.J. Killin, A.J. Pratt, J.D. Sutherland, N.J. Turner, M.J.C. Crabbe, E.P. Abraham, and A.C. Willis, J.Antibiot., 1987, 40, 652.
19 G. Banko, A.L. Demain, and S. Wolfe, J.Am.Chem.Soc., 1987, 109, 2858.
20 J.E. Baldwin, R.M. Adlington, M.J.C. Crabbe, G.C. Knight, T. Nomoto, and C.J. Schofield, J.Chem.Soc.,Chem.Commun.,1987, 806.
21 J.E. Baldwin, R.M. Adlington, M.J.C. Crabbe, T. Nomoto, and C.J. Schofield, Tetrahedron, 1987, 43, 4217.
22 J.E. Baldwin, A.J. Pratt, and M.G. Moloney, Tetrahedron, 1987, 43, 2565.
23 J.E. Baldwin, R.M. Adlington, N. Moss, and N.G. Robinson, J. Chem.Soc., Chem.Commun., 1987, 1664.
24 J.E. Baldwin, R.M. Adlington, B.P. Domayne-Hayman, G. Knight, and H.-H. Ting, J.Chem.Soc., Chem.Commun., 1987, 1661.
25 J.E. Baldwin, R.M. Adlington, J.B. Coates, M.J.C. Crabbe, J.W. Keeping, G.C. Knight, T. Nomoto, C.J. Schofield, and H.-H. Ting, J.Chem.Soc., Chem.Commun., 1987, 374.
26 J.E. Baldwin, R.M. Adlington, M.J.C. Crabbe, G. Knight, T. Nomoto, C.J. Schofield, and H.-H. Ting, Tetrahedron, 1987, 43, 3009.
27 J.E. Baldwin, R.M. Adlington, C.J. Schofield, N.P. Crouch, and H.-H. Ting, J.Chem.Soc., Chem.Commun., 1987, 1556.
28 J.E. Baldwin, R.M. Adlington, T.W. Kang, E. Lee, and C.J. Schofield, J.Chem.Soc., Chem.Commun., 1987, 104.
29 J.E. Baldwin, R.M. Adlington, R.T. Aplin, N.P. Crouch, G. Knight, and C.J. Schofield, J.Chem.Soc., Chem.Commun., 1987, 1651.
30 J.E. Baldwin, R.M. Adlington, R.T. Aplin, N.P. Crouch, C.J. Schofield, and H.-H. Ting, J.Chem.Soc., Chem.Commun., 1987, 1654.
31 S.W. Elson, K.H. Baggaley, J. Gillett, S. Holland, N.H. Nicholson, J.T. Sime, and S.R. Woroniecki, J. Chem.Soc., Chem.Commun., 1987, 1739.
32 C.A. Townsend and S.-S. Mao, J.Chem.Soc., Chem.Commun., 1987,86.
33 C.J. Unkefer, R.E. London, R.D. Durbin, T.F. Uchytil, and P.J. Langston-Unkefer, J.Biol.Chem., 1987, 262, 4994 (Chem.Abs., 1987, 106, 192523).
34 B. Müller, A Hädener, and C. Tamm, Helv.Chim.Acta, 1987, 70, 412.
35 S. Wolfe and R.Z. Sterzycki, Can.J.Chem., 1987, 65, 26.
36 T. Chiba, J. Sakaki, T. Takahashi, K. Aoki, A. Kamiyama, C. Kaneko, and M. Sato, J.Chem.Soc., Perkin Trans. 1, 1987, 1845.
37 A.G.M. Barrett and M.A. Sturgess, J.Org.Chem., 1987, 52, 3941.
38 A. Reliquet, F. Reliquet, J.C. Meslin, F. Sharrard, and H. Quiniou, Phosphorus and Sulphur, 1987, 32, 153.
39 J.C. Roze, J.P. Pradere, and H. Quiniou, Sulphur Lett., 1986, 4, 61 (Chem.Abs., 1987, 106, 156101).
40 M. Davis and W.-Y. Wu, Aust.J.Chem., 1987, 40, 1519.
41 K.H. Chung, J.C. Lee, and K.S. Yeo, Bull.Korean Chem.Soc., 1986, 7, 254 (Chem.Abs., 1987, 107, 6969).
42 R.G. Micetich, S. Betageri, R. Singh, and P. Payne, J. Org. Chem., 1987, 52, 915.

43 G. Burton and F.P. Harrington, J.Chem.Soc., Perkin Trans. I, 1987, 635.
44 A.W. Guest, C.L. Branch, S.C. Finch, A.C. Kaura, P.H. Milner, M.J. Pearson, R.J. Ponsford, and T.C. Smale, J.Chem.Soc., Perkin Trans. I, 1987, 45.
45 T. Tsuji, H. Itani, and H. Ishitobi, Tetrahedron Lett., 1987, 28, 2745.
46 J.E. Baldwin, A.K. Forrest, S. Ko, and L.N. Sheppard, J.Chem. Soc., Chem.Commun.,1987, 43, 1003.
47 J.E. Baldwin, J.E. Cobb, and L.N. Sheppard, Tetrahedron, 1987, 43, 1003.
48 D.O. Spry, N.J. Snyder, A.R. Bhala, C.E. Pasini, and J.M. Indelicato, Heterocycles, 1987, 26, 2911.
49 M.L. Gilpin, J.B. Harbridge, and T.T. Howarth, J.Chem.Soc., Perkin Trans. I, 1987, 1369.
50 D.F. Corbett, A.C. Kaura, C.D. Maycock, and R.J. Stoodley, J. Chem.Soc., Perkin Trans. I, 1987, 2009.
51 R. Deziel, Tetrahedron Lett., 1987, 28, 4371.
52 G. Sacripante and G. Just, J.Org.Chem., 1987, 52, 3659.
53 L.A. Reed, D.A. Charleson, and R.A. Volkmann, Tetrahedron Lett., 1987, 28, 3431.
54 A. Martel, J.-P. Daris, C. Bachand, and M. Menard, Can.J. Chem., 1987, 65, 2179.
55 M. Endo, Synth.Commun., 1987, 17, 1029.
56 J.K. Chung, Y.M. Goo, and Y.Y. Lee, Bull.Korean Chem.Soc., 1986, 7, 405 (Chem.Abs., 1987, 107, 58706).
57 Y.Y. Lee, K. Kim, H.H. Kim, Y.M. Goo, Y.B. Lee, and Y.A. Joe, Bull.Korean Chem.Soc., 1987, 8, 130.
58 H. Ludescher, C.-P. Mak, G. Schulz, and H. Fliri, Heterocycles, 1987, 26, 885.
59 Y.L. Chen, C.-W. Chang, K. Hedberg, K. Guarino, W.M. Welch, L. Keissling, J.A. Retsema, S.L. Haskell, M. Anderson, M. Manousos, and J.F. Barrett, J.Antibiot., 1987, 40, 803.
60 T. Naito, H. Hoshi, S. Aburaki, Y. Abe, J. Okumura, K. Tomatsu, and H. Kawaguchi, J.Antibiot., 1987, 40, 991.
61 K. Sakagami, T. Watanabe, S. Fukatsu, H. Nitta, M. Hatanaka, and T. Ishimaru, J.Antibiot., 1987, 40, 1325.
62 H. Sadaki, H. Imaizumi, T. Inaba, T. Kirakawa, Y. Murotani, and I. Saikawa, Yakugaku Zasshi, 1986, 106, 117 (Chem.Abs., 1987, 106, 84235).
63 M. Botta, F. de Angelis, A. Gambacorta, L. de Ferra, G. Montesperelli, and R. Nicoletti, Tetrahedron Lett., 1987, 28, 5539.
64 N.C. Cohen, I. Ernest, H. Fritz, H. Fuhrer, G. Rihs, R. Scartazzini, and P. Wirz, Helv.Chim.Acta, 1987, 70, 1967.
65 D.H. Bremner, N.S. Ringan, and A.D.M. Smith, Tetrahedron Lett., 1987, 28, 6687.
66 C.H. Lee, C.J. Moon, K.S. Kim, J.H. Kim, and D.W. Kim, Bull.Korean Chem.Soc., 1987, 8, 336.
67 D. Hoppe and T. Hilpert, Tetrahedron, 1987, 43, 2467.
68 A.G.M. Barrett, G.G. Graboski, M. Sabat, and S.J. Taylor, J.Org.Chem., 1987, 52, 4693.
69 M. Chmielewski, Z. Kaluza, W. Abramski, and C. Belzecki, Tetrahedron Lett., 1987, 28, 3035.
70 I. Kawamoto, R. Endo, K. Suzuki, and T. Hata, Heterocycles, 1987, 25, 123.
71 W.M. Welch and K.J.C. Guarino, J.Org.Chem., 1987, 52, 4401.
72 M. Lang, P. Schneider, R. Scartazzini, W. Tosch, E.A. Konopk, and O. Zak, J.Antibiot., 1987, 40, 217.

73 E. Perrone, M. Alpegiani, A. Bedeschi, F. Giudici, F. Zarini, G. Francheschi, C. Della Bruna, D. Jabes, and G. Meinardi, J.Antibiot., 1987, 40, 1636.
74 V.M. Girijavallabhan, S.W. McCombie, P. Pinto, S.-I. Lin, and R. Versace, J.Chem.Soc., Chem.Commun., 1987, 691.
75 A.J. Barker, M.R. Teall, and G. Johnson, Tetrahedron Lett., 1987, 28, 2283.
76 C.-P. Mak, G. Schulz, and H. Fliri, Heterocycles, 1987, 26, 1001.
77 J. Knight and P.J. Parsons, J.Chem.Soc., Perkin Trans. I, 1987, 1237.
78 M.D. Bachi, A. de Mesmaeker, and N.S.-de Mesmaeker, Tetrahedron Lett., 1987, 28, 2637.
79 M.D. Bachi, A. de Mesmaeker, and N.S.-de Mesmaeker, Tetrahedron Lett., 1987, 28, 2887.
80 R.Joyeau, L.D.S. Yadav, and M. Wakselman, J.Chem.Soc., Perkin Trans. I, 1987, 1899.
81 J.T. Drummond and G. Johnson, Tetrahedron Lett., 1987, 28, 5245.
82 A. Watanabe, Y. Fukagawa, T. Ishikura, and T. Yoshioka, Bull.Chem.Soc.Jpn., 1987, 60, 2091.
83 D.-C. Ha and D.J. Hart, J.Antibiot., 1987, 40, 309.
84 T. Chiba and T. Nakai, Chem.Lett., 1987, 2187.
85 A.I. Meyers, T.J. Sonin, S. Scholz, and Y. Ueda, Tetrahedron Lett., 1987, 28, 5103.
86 M. Hatanaka and H. Nitta, Tetrahedron Lett., 1987, 28, 69.
87 J. Fetter, K. Lempert, M. Kajtar-Peredy, G. Bujtas, and G. Simig, J.Chem.Res.(S), 1987, 28.
88 D. Häbich and W. Hartwig, Tetrahedron Lett., 1987, 28, 781.
89 N. Tamura, H. Natsugari, Y. Kawano, Y. Matsushita, K. Yoshioka, and M. Ochiai, Chem.Pharm.Bull., 1987, 35, 996.
90 R.N. Guthikonda, L.D. Cama, M. Quesada, M.F. Woods, T.N. Salzmann, and B.G. Christensen, J.Med.Chem., 1987, 30, 871.
91 C.U. Kim, P.F. Misco, and B.Y. Luh, Heterocycles, 1987, 26, 1193.
92 N.V. Shah and L.D. Cama, Heterocycles, 1987, 25, 221.
93 J.H. Bateson, A.M. Quinn, and R. Southgate, Tetrahedron Lett., 1987, 28, 1561.
94 R. Sharma, R.J. Stoodley, and A. Whiting, J.Chem.Soc., Perkin Trans. I, 1987, 2361.
95 S. Kim, S.B. Chang, and P.H. Lee, Tetrahedron Lett., 1987, 28, 2735.
96 M. Sato, H. Ogasawara, K. Takayama, and C. Kaneko, Heterocycles, 1987, 26, 2611.
97 D. Tanner and P. Somfai, Tetrahedron Lett., 1987, 28, 1211.
98 S.W. Baldwin and J. Aubé, Tetrahedron Lett., 1987, 28, 179.
99 Y. Ito, Y. Kimura, and S. Terashima, Bull.Chem.Soc.Jpn., 1987, 60, 3337.
100 T. Kametani, S.-D. Chu, and T. Honda, Heterocycles, 1987, 25, 241.
101 J.J. Tufariello, D.J.P. Pinto, A.S. Milowsky, and D.V. Reinhardt, Tetrahedron Lett., 1987, 28, 5481.
102 G. Guanti, E. Narisano, and L. Banfi, Tetrahedron Lett., 1987, 28, 4331.
103 G. Guanti, E. Narisano, and L. Banfi, Tetrahedron Lett., 1987, 28, 4335.
104 C. Gennari, I. Venturini, G. Gislon, and G. Schimperna, Tetrahedron Lett., 1987, 28, 227.

105 G. Cainelli, D. Giacomini, M. Panunzio, G. Martelli, and G. Spunta, Tetrahedron Lett., 1987, 28, 3593.
106 M. Shibasaki, Y. Ishida, G. Iwasaki, and T. Iimori, J.Org.Chem., 1987, 52, 3489.
107 T. Yamada, H. Suzuki, and T. Mukaiyama, Chem.Lett., 1987, 293.
108 M. Hatanaka, Tetrahedron Lett., 1987, 28, 83.
109 I. Panfil, C. Belzecki, and M. Chmielewski, J.Carbohydrate Chem., 1987, 6, 463.
110 T. Chiba, T. Ishizawa, J.-ichi Sakaki, and C. Kaneko, Chem. Pharm.Bull., 1987, 35, 4672.
111 Q. Dong and D. Liu, Yaoxue Xuebao, 1987, 22, 19 (Chem.Abs., 1987, 107, 236286).
112 F. Toda, M. Yagi, and S.-ichi Soda, J.Chem.Soc., Chem.Commun., 1987, 1413.
113 F. Toda, K. Tanaka, and M. Yagi, Tetrahedron, 1987, 43, 1495.
114 H. Aoyama, K.-ichi Miyazaki, M. Sakamoto, and Y. Omote, Tetrahedron, 1987, 43, 1513.
115 M. Shiozaki and H. Masuko, Bull.Chem.Soc.Jpn., 1987, 60, 645.
116 T. Kaneko, Y. Okamoto, and K. Hatada, J.Chem.Soc.,Chem. Commun., 1987, 1511.
117 G. Barbaro, A. Battaglia, and P. Giorgianni, Tetrahedron Lett., 1987, 28, 2995.
118 H. Yamada, H. Sugiyama, and M. Kajiwara, Heterocycles, 1987, 26, 2841.
119 G. Rajendra and M.J. Miller, Tetrahedron Lett.,1987, 28, 6257.
120 G. Rajendra and M.J. Miller, J.Org.Chem., 1987, 52, 4471.
121 T. Kolasa and M.J. Miller, Tetrahedron Lett., 1987, 28, 1861.
122 T. Ishizuka and T. Kunieda, Tetrahedron Lett., 1987, 28, 4185.
123 T. Okawara, R. Kato, N. Yasuda, T. Yamasaki, and M. Furukawa, J.Chem.Res.(S), 1987, 254.
124 H. Alper and N. Hamel, Tetrahedron Lett., 1987, 28, 3237.
125 R. Aumann, E. Kuckert, C. Krüger, and K. Angermund, Angew. Chem.Int.Ed.Engl., 1987, 26, 563.
126 F.M. Hauser and S.R. Ellenberger, Synthesis, 1987, 324.
127 M. Chmielewski, Z. Kaluka, D. Mostowicz, C. Belzecki, E. Baranowska, J.P. Jacobsen, P. Salanski, and J. Jurczak, Tetrahedron, 1987, 43, 4555.
128 J.D. Bunyak, J. Mathew, M.N. Rao, E. Haley, C. George, and U. Siriwardane, J.Chem.Soc., Chem.Commun., 1987, 735.
129 C. Borel, L.S. Hegedus, J. Krebs, and Y. Satoh, J.Am.Chem.Soc., 1987, 109, 1101.
130 A. Arrieta, B. Lecea, and C. Palomo, J.Chem.Soc., Perkin Trans. I, 1987, 845.
131 F.P. Cossio, I. Ganboa, J.M. Garcia, B. Lecea, and C. Palomo, Tetrahedron Lett., 1987, 28, 1945.
132 Y. Yamamoto and Y. Watanabe, Chem.Pharm.Bull., 1987, 35, 1871.
133 G. Barbaro, A. Battaglia, and P. Giorgianni, J.Org.Chem., 1987, 52, 3289.
134 I. Ojima, K. Nakahashi, S.M. Brandstadter, and N. Hatanaka, J. Am.Chem.Soc., 1987, 109, 1798.
135 O. Nakaguchi, T. Oku, H. Takeno, M. Hashimoto, and T. Kamiya, Chem.Pharm.Bull., 1987, 35, 3985.
136 D. Dugat, G. Just, and S. Sahoo, Can.J.Chem., 1987, 65, 88.
137 W. Hinz and G. Just, Can.J.Chem., 1987, 65, 1503.
138 S.D. Sharma, U. Mehra, J.P.S. Khurana, and S.B. Pandhi, Synthesis, 1987, 990.
139 R. Niwa, N. Katagiri, and T. Kato, Croat.Chem.Acta , 1986, 59, 121 (Chem.Abs., 1987, 106, 156180).

140 G.S. Singh and S.N. Pandeya, J.Chem.Eng.Data, 1987, 32, 278 (Chem.Abs., 1987, 106, 156174).
141 S.D. Sharma, S. Kaur, and U. Mehra, Ind.J.Chem., Sect. B, 1986, 25B, 141 (Chem.Abs., 1987, 106, 4719).
142 M. Wada, H. Aiura, and K.-ya Akiba, Tetrahedron Lett., 1987, 28, 3377.
143 G. Cainelli, D. Giacomini, M. Panunzio, G. Martelli, and G. Spunta, Tetrahedron Lett., 1987, 28, 5369.
144 A. Battaglia, G. Cainelli, D. Giacomini, G. Martelli, and M. Panunzio, Tetrahedron Lett., 1987, 28, 4347.
145 G. Iwasaki and M. Shibasaki, Tetrahedron Lett., 1987, 28, 3257.
146 G. I. Georg, J. Kant, and H.S. Gill, J.Am.Chem.Soc., 1987, 109, 1129.
147 G. Cainelli, M. Panunzio, T. Basile, A. Bongini, D. Giacomini, and G. Martelli, J.Chem.Soc., Perkin Trans. I, 1987, 2637.
148 N. Oguni, S. Tomago, and K. Inami, Chem.Express, 1986, 1, 579 (Chem.Abs., 1987, 106, 156108).
149 N. Oguni, T. Tomago, and N. Nagata, Chem.Express, 1986, 1, 495 (Chem.Abs., 1987, 106, 138174).
150 J.T.B.H. Jastrzebski, F.H. van der Steen, and G. van Koten, Recl.Trav.Chim.Pays-Bas, 1987, 106, 516.
151 M.R.P. van Vliet, J.T.B.H. Jastrzebski, W.J. Klaver, K. Goubitz, and G. van Koten, Recl.Trav.Chim.Pays-Bas, 1987, 106, 132.
152 D.K. Dutta, R.C. Boruah, and J.S. Sandhu, Ind.J.Chem., Sect.B, 1986, 25B, 350 (Chem.Abs., 1987, 106, 4718).
153 R. Aumann and H. Heinen, Chem.Ber., 1987, 120, 1297.
154 R. Aumann and E. Kuckert, Chem.Ber., 1987, 120, 1939.
155 B.Y. Chung and B.Y. Lee, Bull.Korean Chem.Soc., 1986, 7, 148 (Chem.Abs., 1987, 106, 119570).
156 B. Alcaide, G. Dominguez, and J. Plumet An.Quim., Ser.C, 1987, 83, 128 (Chem.Abs., 1987, 107, 217299).
157 M. Endo, Can.J.Chem., 1987, 65, 2140.
158 F. Shirai and T. Nakai, J.Org.Chem., 1987, 52, 5492.
159 Y. Nagao, T. Kumagai, T. Abe, M. Ochiai, T. Taga, K. Machida, and Y. Inoue, J.Chem.Soc., Chem.Commun., 1987, 602.
160 Y. Ito and S. Terashima, Tetrahedron Lett., 1987, 28, 507.
161 C.U. Kim, B. Luh, and R.A. Partyka, Tetrahedron Lett., 1987, 28, 507.
162 A. Arnoldi, L. Merlini, and L. Scaglioni, J.Heterocyclic Chem., 1987, 24, 75.
163 J.S. Prasad and L.S. Liebeskind, Tetrahedron Lett., 1987, 28, 1857.
164 L.M. Fuentes, I. Shinkai, A. King, R. Purick, R.A. Reamer, S.M. Schmitt, L. Cama, and B.G. Christensen, J.Org.Chem., 1987, 52, 2563.
165 T. Ohta, H. Takaya, M. Kitamura, K. Nagai, and R. Noyori, J. Org.Chem., 1987, 52, 3176.
166 F.P. Cossio, B. Lecea, and C. Palomo, J.Chem.Soc., Chem. Commun., 1987, 1743.
167 P.Borowicz, J.Antibiot., 1987, 40, 1204.
168 P.A. van Elburg and D.N. Reinhoudt, Heterocycles, 1987, 26, 437.
169 S.Yoshifuji, Y. Arakawa, and Y. Nitta, Chem.Pharm.Bull., 1987, 35, 357.
170 J.M. Aizpurua, M. Oiarbide, and C. Palomo, Tetrahedron Lett., 1987, 28, 5361.
171 Y. Ito and S. Terashima, Chem.Lett., 1987, 445.

172 B. Alcaide, G. Dominguez, A. Martin-Domenech, and J. Plumet, Heterocycles, 1987, 26, 1461.
173 G. Just and G. Sacripante, Can.J.Chem., 1987, 65, 104.
174 B.B. Snider and M.I. Johnston, Synth.Commun., 1987, 17, 1877.
175 N.N. Romanova, V.A. Budylin, G.V. Grishina, V.M. Potapov, M.L. Demchuk, I. Yu. Sivkova, and Yu. G. Bundel, Khim.Geterotsikl. Soedin., 1986, 607 (Chem.Abs., 1987, 106, 176072).
176 J.J. Tufariello and D.J.P. Pinto, Tetrahedron Lett., 1987, 28, 5485.
177 O. Nakaguchi, K. Hemmi, Y. Shiokawa, M. Hashimoto, and T. Kamiya, Chem.Pharm.Bull., 1987, 35, 3464.
178 T. Oshikawa and M. Yamashita, Chem. and Ind., 1987, 655.
179 I. Ojima, K. Nakahashi, S.M. Brandstadter, and N. Hatakana, J.Am.Chem.Soc., 1987, 109, 1798.
180 I. Ojima and X. Qiu, J.Am.Chem.Soc., 1987, 109, 6537.
181 I. Ojima and H.-J. C. Chen, J.Chem.Soc., Chem. Commun., 1987, 625.
182 P. Duhamel, B. Goument, and J.-C. Plaquevent, Tetrahedron Lett., 1987, 28, 2595.
183 F.M. Hauser, S.R. Ellenberger, and R.P. Rhee, J.Org.Chem., 1987, 52, 5041.
184 D.-C. Ha and D.J. Hart, Tetrahedron Lett., 1987, 28, 4489.
185 M. Shiozaki and N. Ishida, Chem.Lett., 1987, 1403.
186 E.J. Thomas and A.C. Williams, J.Chem.Soc., Chem.Commun., 1987, 992.
187 M.G.B. Drew, J. Mann, H.J. Holland, and T. Lewis, J.Chem.Res. (S), 1987, 389.
188 J. Kurita, T. Yoneda, N. Kakusawa, and T. Tsuchiya, Heterocycles, 1987, 26, 3085.
189 S.-ichi Hirokami, T. Takahashi, M. Nagata, and T. Yamazaki, J.Org.Chem., 1987, 52, 2455.
190 T. Uyehara, N. Asao, and Y. Yamamoto, J.Chem.Soc., Chem.Comm., 1987, 1410.
191 Y. Hata and M. Watanabe, Tetrahedron, 1987, 43, 3881.
192 J. Szabo, G. Bernath, A. Katocs, L. Fodor, and P. Sohar, Can.J.Chem., 1987, 65, 175.
193 F. Bertha, K. Lempert, and M. Kajtar-Peredy, Acta Chim.Hung., 1985, 120, 111 (Chem.Abs., 1987, 106, 175984).
194 H. Nitta, M. Hatanaka, and T. Ishimaru, J.Chem.Soc.,Chem. Commun., 1987, 51
195 H. Mastalerz and V. Vinet, J.Chem.Soc., Chem.Commun., 1987, 1283.
196 M. Hrytsak and T. Durst, Heterocycles, 1987, 26, 2393.
197 H.R. Pfaendler and R. Strasser, Liebigs Ann.Chem., 1987, 911.
198 K.S. Keshava Murthy and A. Hassner, Tetrahedron Lett., 1987, 28, 97.
199 G. Lawton, C.J. Moody, and C.J. Pearson, J.Chem.Soc., Perkin Trans. I, 1987, 877.
200 G. Lawton, C.J. Moody, C.J. Pearson, and D.J. Williams, J. Chem.Soc., Perkin Trans. I, 1987, 885.
201 G. Lawton, C.J. Moody, and C.J. Pearson, J.Chem.Soc., Perkin Trans. I, 1987, 899.
202 E.C. Taylor and J.S. Hinkle, J.Org.Chem., 1987, 52, 4107.
203 J.G. Schantl and M. Decristoforo, Tetrahedron Lett., 1987, 28, 6577.
204 H. Ulrich, R. Richter, and B. Tucker, J.Heterocyclic Chem., 1987, 24, 1121.
205 M.F. Brana, M. Garrido, J.L. Hernando, M.L. Lopez Rodriguez, and M.J. Morcillo, J.Heterocyclic Chem., 1987, 24, 1725.

206 H. Natsugari, Y. Kawano, A. Morimoto, K. Yoshioka, and M. Ochiai, J.Chem.Soc., Chem.Commun., 1987, 62.
207 J.E. Baldwin, S.C. Ng, and A.J. Pratt, Tetrahedron Lett., 1987, 28, 4319.
208 M.J. Crossley, R.L. Crumbie, Y.M. Fung, J.J.Potter, and M.A. Pegler, Tetrahedron Lett., 1987, 28, 2883.
209 L.N. Jungheim, S.K. Sigmund, and J.W. Fischer,Tetrahedron Lett., 1987, 28, 285.
210 L.N. Jungheim, S.K. Sigmund, N.D. Jones, and J.K. Swartzendruber, Tetrahedron Lett., 1987, 28, 289.
211 L.N. Jungheim and S.K. Sigmund, J.Org.Chem., 1987, 52, 4007.
212 M. Narasida, J. Nishikawa, F. Watanabe, and Y. Terui, J.Med. Chem., 1987, 30, 514.
213 J. Nishikawa, F. Watanabe, M. Shudou, Y. Terui, and M. Narasida, J.Med.Chem., 1987, 30, 523.
214 L. Zai-wei and Y. Pin, Chin.J.Antibiot., 1987, 105.
215 M.I. Page, P. Webster, S. Bogdan, B. Tremerie, and L. Ghosez, J.Chem.Soc., Chem.Commun., 1987, 318.
216 D.B. Boyd, C. Eigenbrot, J.M. Indelicato, M.J. Miller, C.E. Pasini, and S.R. Woulfe, J.Med.Chem., 1987, 30, 528.
217 E.A. Cutmore, A.W. Guest, J.D.I. Hatto, T.C. Smale, A.V. Stachulski, and J.W. Tyler, J.Chem.Soc., Chem.Commun., 1987, 21.
218 S. Mishio, T. Hirose, J. Nakano, and J.-ichi Matsumoto, Yakugaku Zasshi, 1987, 107, 634.
219 N. Ohi, B. Aoki, T. Kuroki, M. Matsumoto, K. Kojima, and T. Nehashi, J.Antibiot., 1987, 40, 22.
220 Y.Qing-xiang, W. Yong-fen and L. Ren-yong, Chin.J.Antibiot., 1987, 109.
221 S. Mishio, T. Hirose, J. Nakano, J.-ichi Matsumoto, and S. Minami, Yakugaku Zasshi, 1987, 107, 592.
222 S. Mishio, T. Hirose, J. Nakano, and J.-ichi Matsumoto, Yakugaku Zasshi, 1987, 107, 607.
223 N. Vitezic and M. Japelj, Vestn.Slov.Kem.Drus., 1986, 33, 387 (Chem.Abs., 1987, 107, 58703).
224 Sh.L. Mndzhoyan, N.D. Marukhyan, A.G. Tolmayan, Yu. Z. Ter-Zakharyan, E.V. Kazaryan, and G.S. Sakanyan, Khim.-Farm.Zh., 1986, 20, 60 (Chem.Abs., 1987, 106, 84234).
225 T.K. Chakraborty, S.K. Goswami, and S.K. Mandal, Indian Drugs, 1986, 23, 537 (Chem.Abs., 1987, 107, 58705).
226 J.I. Jin, M.S. Chang, and S.H. Min, Yakhak Hoechi, 1986, 30, 24 and 294 (Chem.Abs., 1987, 106, 196086 and 107, 236287).
227 R.G. Micetich, S.N. Maiti, P.Spevak, T.W. Hall, S. Yamabe, N. Ishida, M. Tanaka, T. Yamazaki, A. Nakai, and K. Ogawa, J.Med. Chem., 1987, 30, 1469.
228 C.L. Branch, M.J. Basker, S.C. Finch, A.W. Guest, F.P. Harrington, A.C. Kaura, S.J. Knott, P.H. Milner, and M.J. Pearson, J. Antibiot., 1987, 40, 646.
229 R. Maier, B. Wetzel, E. Woitun, W. Reuter, U. Lechner, and K.R. Appel, Arzneim.-Forsch., 1986, 36, 1297 (Chem.Abs., 1987, 106, 18208).
230 N. Ohi, B. Aoki, T. Shinozaki, K. Moro, T. Kuroki, T. Noto, T. Nehashi, M. Matsumoto, H. Okazaki, and I. Matsunaga, Chem. Pharm.Bull., 1987, 35, 1903.
231 A. Brandt, M. Cerquetti, G.B. Corsi, G. Pascucchi, A. Simeoni, P. Martelli, and U. Valdavi, J.Med.Chem., 1987, 30, 764.
232 A. Sala, D. Chiarino, M. Napoletano, E. Albini, A. Carenzi, and D.D. Bella, J.Antibiot. 1987, 40, 1555.

233 K. Mochida, Y. Ono, M. Yamasaki, C. Shiraki, and T. Hirata, J. Antibiot., 1987, 40, 182.
234 Y. Hamashima, T. Kubota, K. Minami, K. Ishikura, T. Konoike, M. Yoshiota, T. Yoshida, H. Nakashimizu, and K. Motokawa, J. Antibiot., 1987, 40, 1468.
235 K. Nishide, T. Kobori, D. Tunemoto, and K. Kondo, Heterocycles, 1987, 26, 633.
236 H. Sadaki, H. Imaizumi, T. Inaba, T. Hirakawa, Y. Murotani, Y. Watanabe, S. Minami, and I. Saikawa, Yakugaku Zasshi, 1986, 106, 123 (Chem.Abs., 1987, 106, 156100).
237 W. Xia, T. Duan, and M. Li, Yaoxue Xuebao, 1986, 21, 816 (Chem.Abs., 1987, 106, 152851).
238 F. Sztaricskai, P. Herczegh, R. Bognar, I. Miskolczi, and I. Koczka, Magy.Kem.Foly, 1986, 92, 454 (Chem.Abs., 1987, 107, 58704).
239 J. Blümbach, W. Dürckheimer, E. Ehlers, K. Fleischmann, N. Klesel, M. Limbert, B. Mencke, J. Reden, K.H. Scheunemann, E. Schrinner, G. Seibert, M. Wieduwilt, and M. Worm, J. Antibiot., 1987, 40, 29.
240 A. Ejima, T. Hayano, T. Ebata, T. Nagahara, H. Koda, H. Tagawa, and M. Furukawa, J.Antibiot., 1987, 40, 43.
241 H. Sadaki, H. Imaizumi, T. Inaba, T. Hirakawa, Y. Murotani, Y. Watanabe, S. Minami, and I. Saikawa, Yakugaku Zasshi, 1986, 106, 129 (Chem.Abs., 1987, 106, 84236).
242 D. Koh, S.-W. Park, and Y. Kim, Bull.Korean Chem.Soc., 1987, 8, 189.
243 N. Nagano, K. Nakano, T. Shibanuma, Y. Murakami, and R. Hara, J. Antibiot., 1987, 40, 173.
244 H. Sadaki, H. Imaizumi, T. Inaba, T. Hirakawa, Y. Murotani, Y. Watanabe, S. Minami, and I. Saikawa, Yakugaku Zasshi, 1986, 106, 147 (Chem.Abs., 1987, 106, 138120).
245 M. Sato, M. Takemura, S. Atarashi, K. Higashi, H. Fujiwara, T. Nagahara, M. Furukawa, T. Ikeuchi, S. Ozawa, N. Nishizawa, and Y. Osada, J. Antibiot., 1987, 40, 483.
246 M. Sato, M. Takemura, S. Atarashi, K. Higashi, T. Nagahara, M. Furukawa, T. Ikeuchi, and Y. Osada, J. Antibiot., 1987, 40, 1292.
247 C.U. Kim, P.F. Misco, B.Y. Luh, and M.J.M. Hitchcock, J. Antibiot., 1987, 40, 1707.
248 K. Mochida, C. Shiraki, M. Yamasaki, and T. Hirata, J. Antibiot., 1987, 40, 14.
249 O. Nakaguchi, T. Oku, M. Hashimoto, and T. Kamiya, Chem.Pharm.Bull., 1987, 35, 3979.
250 H. Yamashita, N. Minami, K. Sakakibara, S. Kobayashi, M. Ohno, M. Hamada, and H. Umezawa, J. Antibiot., 1987, 40, 1716.
251 R.E. Mewshaw and T.J. Commons, J. Antibiot., 1987, 40, 1563.
252 K.S. Kim and J.H. Chambers, J.Antibiot., 1987, 40, 124.
253 S. Shen, S. Su, and X. Lei, Yiyao Gongye, 1987, 18, 119 (Chem. Abs., 1987, 107, 4121).

6
Metal Complexes of Amino Acids and Peptides

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

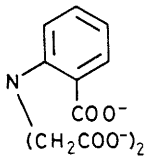
1 Introduction

This chapter deals with the synthesis, structures, reactions and applications of metal-amino acid and metal peptide complexes and with a few exceptions covers material published during 1987. Two reviews on cobalt(III)-amino acid complexes have appeared. In one of these the cobalt(III)-promoted hydrolysis of amino acid esters and peptides and the synthesis of small peptides are covered,[1] while in the other steric and electronic effects in the amino acid side chain on the formation of mono, bis and tris cobalt(III)-amino acid chelates from $[Co(NH_3)_6]^{3+}$ are reviewed.[2] Mixed-ligand chelates of scandium(III) and yttrium(III) in which HEDTA or NTA are the primary ligands and β-diketones, phenols or amino acids are the secondary ligands are the subject of another review.[3] The interaction of zinc(II) and cadmium(II) with naturally occurring amino acids as models for complex formation in gelatin emulsions is discussed in the context of metal ions in photographic silver halide systems.[4] The use of ^1H n.m.r. spectroscopy to study amino acid coordination to chromium(III) is the subject of a dissertation.[5]

2 Amino Acids

2.1 Solid-State Studies. - The 1987 literature contains many reports on the synthesis and characterisation of new complexes, and the greater accessibility to X-ray diffraction techniques has resulted in an increased number of crystal structures being reported for metal-amino acid complexes.

First-Row d-Block Metal Ion Complexes. - A chromium(III) complex of N-(2-carboxyphenyl)iminodiacetate,(L,1), $K[CrL(Gly)].2H_2O$, has been synthesised and characterised.[6] Three complexes of iron(III) with 2-hydroxy-5-methyl-1,3-xylylenebis(N-carboxymethylglycine), (2), H_5-HXTA, have been structurally characterised in the solid state by X-ray crystallography and in solution by visible,



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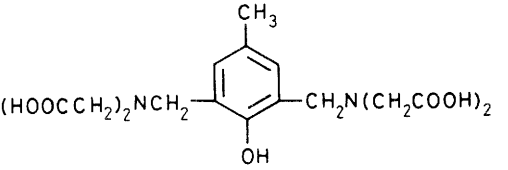

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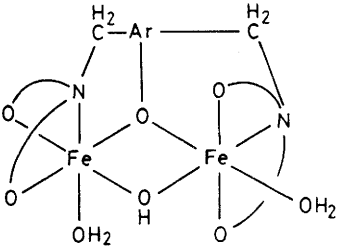

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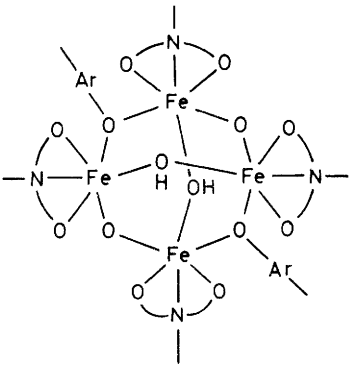

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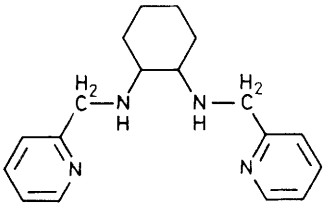

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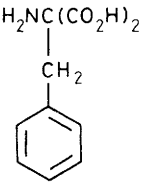

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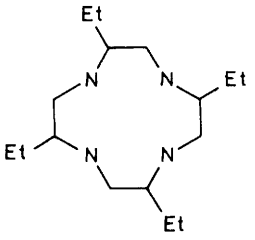

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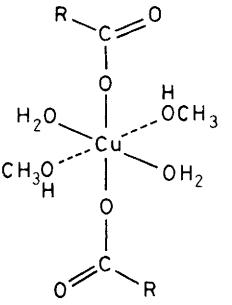

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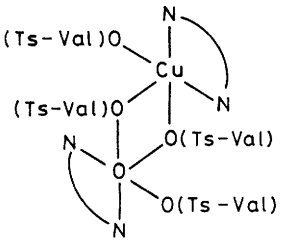
resonance Raman, n.m.r. and Mössbauer spectroscopy.[7] At low pH the binuclear complex $Fe_2(HXTA)OH(H_2O)_2$, (3), containing two six-coordinate but differently ligated iron(III) centres bridged by hydroxy and phenolate groups crystallises from solution. At intermediate pH this complex dimerises to a tetranuclear species which was characterised in solution and was formed as a result of the replacement of two terminal aquo ligands, one from each complex, by a bridging oxo ligand. At high pH and in the presence of pyrrolidene (pyrr) the tetranuclear complex $(pyrrH)_4[Fe_4(HXTA)_2(O)_2(OH)_2]$ is obtained. This complex, (4), contains a tetrahedron of iron(III) centres bridged by two oxo, two hydroxo and two phenolate groups. On the basis of spectroscopic evidence the complexes formed at low and high pH retain their solid-state structures in solution. The complex $Me_4N[Fe_2(HXTA)(OAc)_2]$, which possesses a μ-acetato diferric core, undergoes a $1e^-$ reduction to give a mixed-valence iron(II)-iron(III) complex.[8] Electronic absorption and Mössbauer spectroscopy confirm the mixed-valence formulation, and the n.m.r. spectrum shows that the complex retains the triply bridged diiron structure during and following reduction.

The iron(III) complexes $Fe(Gly)_2Cl_2(OH)$, $Fe(Gly)_3(NO_3)_2(OH)$, $Fe(Val)_3Cl_3$, $Fe(Met)_3Cl_3$, $Fe(Val)_3Cl_2(OH)$ and $Fe(H\text{-}Phe)_2(Phe)(NO_3)_2$, some of which are hydrated, have been synthesised and characterised.[9] Spectroscopic and variable-temperature magnetic data for the complexes Fe(AA)(OH)Cl.2MeOH (AA=Met, Leu, His) are consistent with the existence of an equilibrium between the S=1/2 and S=3/2 spin states.[10] The trinuclear complexes $[Cr_nFe_{3-n}(\mu_3\text{-}O)(Gly)_6(H_2O)_3](NO_3)_7.xH_2O$ (n=0-3, x=2,3) and $[Fe^{II}Fe^{III}_2(\mu_3\text{-}O)(Gly)_6(H_2O)_3]Cl_6$ have been synthesised.[11] Magnetic data down to liquid-nitrogen temperatures and electrochemical data for all the complexes are discussed.

The structure of Λ-δ$_1$-$[Co(R_1R\text{-}picchxn)(R\text{-}ABMA)]ClO_4.1.5H_2O$ containing the ligands N,N'-di-(2-picolyl)-1R,2R-diaminocyclohexane, (R,R-picchxn,5), and 2-amino-2-benzylpropanedicarboxylate, (ABMA,6), has been determined.[12] Decarboxylation of the dicarboxylate ligand gives the corresponding diastereoisomeric amino acid complex in high optical yield. Reaction of the complex $[CoBr(H_2O)L]Br_2$ containing the macrocycle (2R,5R,8R,11R)-2,5,8,11-tetraethyl-1,4,7,10-tetraazacyclododecane, (7), with amino acids, HAA, occurs under mild temperature and pH conditions to give the products $[CoL(AA)]^{2+}$.[13] The crystal structures of two

of these, i.e. $[CoL(AA)]Br(ClO_4).H_2O$ (AA = S- or R-Ala), show cis octahedral configurations about the metal ion with the macrocycle coordinated in a folded manner and the chiralities of the four nitrogen atoms from the starting material retained. A number of amino acid complexes of the type β-$[Co(R\text{-picpn})AA]^{2+}$ [R-picpn = R-methyl-1,6-di(2-pyridyl)-2,5-diazahexane; AA = R- or S-Val, Phe, Trp or Pro] have been prepared from Λ-α-$[Co(R\text{-picpn})Cl_2]^+$ and the amino acids in aqueous solution.[14] Steric effects in the amino acid side chain have little effect on the geometry adopted by the tetradentate ligand. The crystal and molecular structure of $[Co(R\text{-picpn})S\text{-Pro}](ClO_4)_2$ confirms the geometry previously deduced by n.m.r. methods with both N atoms of the tetradentate ligand having an S configuration. An anionic cobalt(III) complex containing the EDTA-type ligand 1,2-diaminoethane-N-ethanoic acid-N,N',N'-trispropanoic acid has been prepared, resolved and characterised.[15]

The isomers cis(O)- and trans(O)-$[Co(tn)(Gly)_2]Cl$ (tn = 1,3-propanediamine) have been synthesised and studied in solution by electronic absorption, ^1H n.m.r. and c.d. spectroscopy.[16] The three geometric isomers of $[Co(\beta\text{-Ala})_2tn]Cl$ (trans-O, C_1 and C_2 cis-O) have been prepared, and the Raman spectra of these and other complexes including $[Co(Gly)tn_2]Cl_2$, mer and fac- $Co(\beta\text{-Ala})_3$, $[Co(\beta\text{-Ala})en_2]Cl_2$ and trans(O)-$[Co(\beta\text{-Ala})_2en]Cl$ have been reported.[17] The ligand field spectra of the complexes $Co(Gly)_{3-x}(\beta\text{-Ala})_x$ (x=0-3) have been analysed and angular overlap calculations have been used to explain differences in the splitting of the $^1T_{1g}(O_h)$ state between the meridional isomers.[18] A number of cobalt(III) complexes containing the ligands L-Cys, S-Me-L-Cys and D- and L- Semet (Semet = selenomethionine) have been prepared.[19] Two of the isomers of $[Co(L\text{-Cys})dien]ClO_4$ have been isolated and the crystal structure of one of these has been determined. This isomer contains three crystallographically independent complex cations in an asymmetric unit all of which have trans (N_iS) configurations and L-Cys coordinated facially as a tridentate N,O,S ligand. Methylation with dimethyl sulfate gives the analogous S-methyl-L-cysteinate complexes for which electronic absorption, c.d., ^1H and ^{13}C n.m.r. spectra are reported. Five isomeric forms of the complexes $[Co(L\text{- or }L\text{-Semet})_2]^+$ and $[Co(D\text{-Semet})(L\text{-Semet}]^+$ have been characterised spectroscopically in solution and the trans(O), trans(N) and cis,cis,cis isomers have been obtained crystalline and optically resolved.[20]



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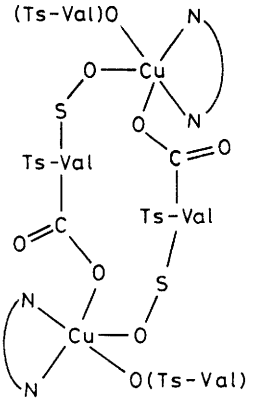

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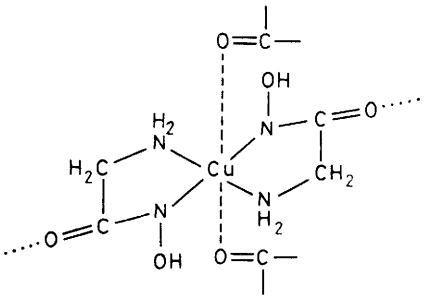

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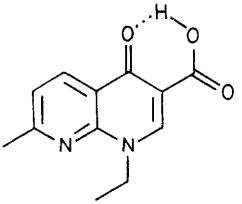

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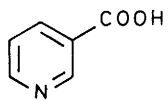

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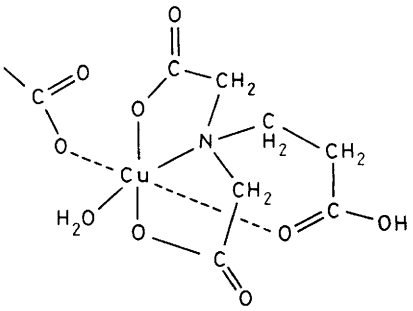


(14)

Reaction of $(+)_{589}$- and $(-)_{589}$-$[Co(en)_2Gly](O_3SCF_3)_2$ with $POCl_3$ in DMF solution affords the corresponding isomers of $[Co(en)_2(C\text{-Formyl-Gly})]Cl_2.HCl.2H_2O$.[21] Treatment of the $(+)_{589}$ isomer with $NaBH_4$ at pH 7 gave a 1:1 mixture of the (R) and (S) forms of the serine complex $(+)_{589}$-$[Co(en)_2Ser]^{2+}$, each of which is optically pure, thus confirming retention of stereochemistry during the formylation reaction. The addition of (S)-penicillamine to $(-)_{589}$-$[Co(en)_2(C\text{-Formyl-Gly})]^{2+}$ in aqueous pyridine -HCl buffer solutions gave the penicilloato (Pen) complex isomers Δ-$[Co(en)_2(2S,4S,\alpha R)\text{-Pen}]ClO_4.2H_2O$ and Δ-$[Co(en)_2(2S,4S,\alpha S)\text{-Pen}]ClO_4.3H_2O$, for which crystal and molecular structures are reported. When the condensation reaction was carried out in DMSO or DMF all four possible isomeric (4S) penicilloato complexes were observed.

A number of complexes of the type $[Co_2L_2(O_2)OH]$ [H_2L = $(HO_2CCHRNHCH_2\text{-})_2$, R = H, Me, Et, Me_2CH, Me_2CHCH_2, $PhCH_2$; H_2L = $(HO_2CCHRNR'CH_2\text{-})_2$, RR' = $CH_2CH_2CH_2$] and $Co_2L_2L'_2(O_2)$ (L' = imidazole or substituted imidazole) have been prepared and characterised.[22] The binuclear complexes $[LCo(\mu\text{-}OH)_2Co(NH_3)_4]^{2+}$ (L = EDDA, $(Gly)_2$ or NTA) have been prepared and their structures assigned on the basis of absorption, c.d. and ^1H n.m.r. spectroscopy.[23]

Complexes of N-protected amino acids with copper(II) and their applications as models for copper(II)-peptide interactions continue to attract interest and the structures of a number of such complexes have been determined by X-ray diffraction methods. The structure of the complex $Cu(Ts\text{-}Val)_2(H_2O)_2(CH_3OH)_2$, (8), is made up of discrete units in which the metal ion is centrosymmetrically surrounded by two carboxylate and two water ligands with two methanol molecules completing the tetragonal bipyramidal coordination.[24] The complex $[Cu(Ts\text{-}Val)_2(bipy)]_2$ consists of two crystallographically independent dimeric units. In both dimers the coordination is tetragonal pyramidal with the bipy ligand and two carboxylate groups in the equatorial plane. In one of the dimers, (9), the apex of the pyramid is occupied by an oxygen of a carboxylate group which is basal to the other copper. In the other dimer, (10), the metal ions are linked by tosylvalinate ions, each bridging via monodentate carboxylate and sulphonate groups. The dimers interact by ring stacking between bipy ligands. Spectroscopic and magnetic properties point to a

dimeric structure with strong antiferromagnetic exchange in $[Cu(Ts\text{-}Val)_2(H_2O)]_2.4H_2O$. In the complex $Cu(Ts\text{-}\beta\text{-}Ala)_2(Pip)_2$ the metal ion lies on a symmetry centre coordinating two piperidine (Pip) and two carboxylate oxygen ligands.[25] The interaction of N-acetyl-L-glutamine with copper(II) chloride in water and water-methanol solutions produces the complexes $Cu(Ac\text{-}Glu).2H_2O$ and $Cu(Ac\text{-}Glu)_2(Melm)_2.2H_2O$ (Melm = N-methylimidazole).[26] The structure of the latter consists of discrete $Cu(Ac\text{-}Glu)_2(Melm)_2$ units in which the metal ion lies in a square-planar N_2O_2 environment composed of symmetry related carboxylate oxygens from Ac-Glu and nitrogens from Melm. Spectroscopic and thermal analysis are consistent with this structure and indicate that $Cu(Ac\text{-}Glu)_2.2H_2O$ contains a CuO_4 chromophore.

The crystal and molecular structure of the complex $[Cu(L\text{-}Trp)phen]ClO_4.2.5H_2O$ has been determined.[27] In this complex the metal ion is five-coordinate with the amino acid (NH_2,COO^-) and phenanthroline (N,N) ligands occupying the equatorial sites and a carboxylate oxygen atom from a symmetry-related neighbouring molecule in the axial position. This gives rise to a one-dimensional, polymeric, spiral structure from which indole and phenanthroline rings project outwards and stack on each other. The structure of the complex $CuCl(OH\text{-}Pro)H_2O$ consists of a one-dimensional polymer chain in which one carboxylate oxygen acts as a bridge between a pair of metal ions and the other is coordinated to one of these ions.[28] The chains are linked by hydrogen bonding. The geometry around each copper ion is distorted octahedral and is composed of three carboxylato oxygen atoms, a water ligand, a pyrrolidine nitrogen and a chloride ligand.

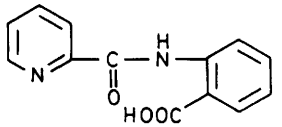
Adjustment of a solution containing $Cu(ClO_4)_2.6H_2O$ and glycinehydroxamic acid (HL) to pH 8 produced the complex $CuL_2.2H_2O$ for which the crystal structure is reported.[29] In this complex, (11), the metal ion has a distorted octahedral geometry in which the four nitrogen atoms of the glycinehydroxamate ligands and two carbonyl oxygen atoms from neighbouring molecules account for the coordination. The electron spin resonance spectrum of the complex (powder and dilute solution) has also been reported. The structure of a copper(II) complex with nalidixic acid (H-Nal, 12), a drug used to control several gram-negative bacterial infections, has been reported.[30] In the complex, $[Cu(phen)(Nal)H_2O]NO_3$, the Nal ligand is coordinated to the metal via the carboxylate and oxo groups. Complexes of this ligand with Mg, Ca, Cr, Mn, Fe, Ni,

Cu, Zn, Cd, Hg and Pd have previously been reported.[31] The crystal structure of a polymeric 2:1 complex of copper(I) chloride with nicotinic acid, (13), has been determined.[32]

The crystal structures of an aminotriacetate complex of copper(II) and of a bimetallic amino tetra-acetate complex involving copper(II) and nickel(II) have been determined. In the complex $Cu(Hnpda)H_2O$ [$H_3npda = N(CH_2COOH)_2CH_2CH_2COOH$], (14), the tetradentate ligand forms three short, nearly coplanar bonds with the metal ion and a fourth longer bond which gives a six-membered ring structure.[33] A short in-plane bond from an aquo ligand and a long apical bond from an acetate ligand of a neighbouring molecule complete the tetragonal coordination around the metal ion. The solution chemistry, spectroscopic and magnetic properties of the complex are discussed. The molecular structure of the complex $[(H_2O)_4Cu(Cdta)Ni].3H_2O$ (Cdta = trans-cyclohexane-1,2-diamine-N,N,N',N'-tetra-acetate) consists of heterobimetallic units in which copper(II) occupies a hydrated octahedral site and nickel(II) a chelated one.[34] The metal ions are linked through a bridging carboxylate group. The molecular structure of $[(H_2O)_5Ni(Cdta)Cu]H_2O$ is also described. While antiferromagnetic coupling occurs in the first of these complexes, no exchange is observed in the second even at temperatures of 4K.

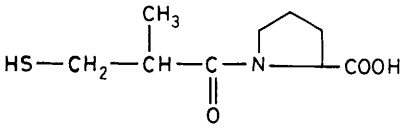
A number of copper(II)-amino acid complexes have been prepared and characterised by spectroscopic methods. These include the amino acid-dithiocarbamate complexes $Cu(S_2CNHCH(R)CO_2H)_2$,[35] and also the following: [CuL(AA)]X (L = 1,10-phenanthroline, 5-nitro-1,10-phenanthroline : AA = Phe, Tyr, L-Dopa : X = BPh_4, OAc),[36] $CuL(AA)H_2O$ (L = thiosalicylate, AA = Gly, Ala, Val),[37] $Cu(H\text{-}Asp)Asp(Glu).H_2O$, Cu(Asp)Met, Cu(Glu)Met,[38] Cu(Thr)His(H_2O) and Cu(Thr)AA (AA = His, Val, Ala, Ser, Gly).[39] The irreversible reduction of the last two complexes, which have distorted octahedral structures, at the dropping mercury electrode in 0.1M $NaClO_4$ solution has been studied. The complexes $Cu(IDA)AA(H_2O)_2$ (IDA = iminodiacetate, AA = Gly, Ala, Ser, Val, Thr) have been synthesised and characterised and their reduction at the dropping mercury electrode has also been investigated.[40]

The complexes $M(AA)_2.nH_2O$ [M = Mn(II), Zn(II); AA = Asp, Glu, Met ; n = 0,2,4],[38] $ML(AA)H_2O$ [M = Co(II), Zn(II) : HL = thiosalicylate, AA = Gly, Ala, Val],[37] and the octahedral oxovanadium(IV) complex $VO(L)AA(H_2O)$ (L = 5-sulfosalicylate, AA =



(15)

(16) R = H, Me, Et, or Pr



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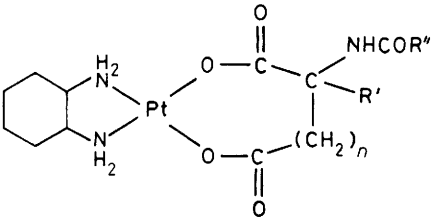
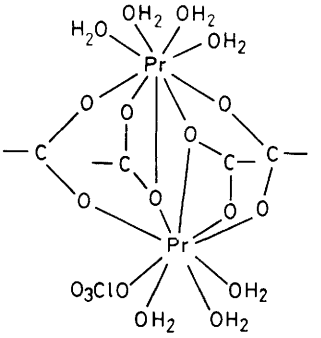


(18) R′ = H or alkyl

R″ = H, halogen, alkyl, aryl or aralkyloxy



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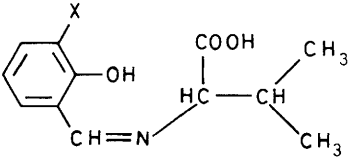


(20) X = H or OCH_3

Gly, Ala, Phe) [41] have been synthesised and characterised. A range of complexes of the ligand N-(2-carboxyphenyl)pyridine-2'-carboxamide (H_2L, 15) have been synthesised.[42] These include $M(HL)_2.nH_2O$ (M = Co, Ni, Cu, Zn : n = 2, 2.5, 3), $Cu_2(HL)_2L.3H_2O$, $Cu_3(HL)_3L(NO_3)$, all of which are polymeric, as well as $M_2(HL)_2(OH)_2.nH_2O$ (M = Co, Ni, Cu, Zn : n = 2,4) and CuL, which appear to be dimeric. Other reported complexes of this ligand include $PdL(H_2O)$, $Pt_3(HL)_4L.7H_2O$, $M(H_2L)_2Cl_2.EtOH$ (M = Co, Cu), $Ni(H_2L)_2Cl_2.EtOH.H_2O$, $M(H_2L)_2X_2$ (M = Co, Ni ; X = NCS, NO_3), $Zn(H_2L)(HL)Cl$, $Zn(H_2L)_2(NO_3)_2$ and $Cu(H_2L)(HL)NO_3$. Only Pd(II) and Cu(II) give complexes having deprotonated amide ligands.

A series of nickel(II) complexes with 1,4-diazacycloheptane-N,N'-dicarboxylate ligands (L, 16) have been prepared.[43] These complexes are either octahedral $NiL(H_2O)_2$ (R = H), square planar NiL(R=Me,Et,Pr), or square pyramidal NiL(py) (R = Me, py=pyridine). The stability constants of these complexes show that the R groups play an important role in determining the stability.

The mercaptoamido acid, Captopril, (17), is an antihypertensive agent which acts by inhibiting the zinc(II) containing angiotensin converting enzyme.[44] The interaction of zinc(II) with this drug has been investigated and a new 1:1 complex has been isolated. On the basis of infrared and Raman spectroscopy it appears that Captopril coordinates to the metal ion through its carboxylate and carbonyl oxygen atoms as well as through its thiolate group. The complexes $M(SR)_2(1\text{-MeIm})_2$ [M =Co, Zn, Cd;SR = 2,4,6-$Pr^i_3C_6H_2$(S) or 2,3,5,6-Me_4C_6H(S)] have been synthesised as models for the postulated $Zn(Cys)_2(His)_2$ site in the gene transcription protein TFIIIA.[45]

Other Metal Complexes.- The biological activity of metal-amino acid complexes continues to be an area of active research. A number of Cisplatin analogues of the type $Cis\text{-}PtCl_2(NH_2CMe_3)AA$ (AA = L-Ser, L-Leu, L-Phe, L-Met, L-Asn) have been synthesised and tested against leukemic L 1210 cells, and it appears that the inclusion of amino acid ligands contributes neither to the cytotoxicity nor to the preferential uptake of these drugs by the test cells.[46] A series of Carboplatin analogues such as (18) have been synthesised and screened for antitumour activity.[47] Seven platinum(II) complexes of the type $[Pt(bipy)AA]^{n+}$ (AA = L-Val, L-Ile, L-Asp, L-Glu, L-Gln, L-Pro, S-Me-L-Cys) were synthesised and investigated for inhibition of growth of P-388 cells but were

found to be inferior in this respect to those complexes containing L-Ala, L-Leu, L-Met and L-Asn ligands.[48] In contrast to Cisplatin, which causes base pair substitution mutagenesis, these complexes are non-mutagenic on TA 100 and TA 98 bacterial strains. The ternary complexes cis-$[M(L)_2AA]Cl$ (M = Pt, L = Inosine ; M = Pd, L = guanosine ; AA = Gly, Ile, Val, Pro, Ala, Phe) have been synthesised.[49] In 0.1M HCl solution protonation and ring opening of the chelates occur to give the complexes cis-$[ML_2(H\text{-}AA)Cl]Cl$. A complex of $RhCl_3$ with oxalylhomocysteine thiolactone has been shown to inhibit growth of sarcoma in mice.[50]

Ruthenocene derivatives are finding increasing applications as metallopharmaceuticals, and radiolabelled ruthenocenylalanine has been evaluated as a pancreatic imaging agent. Several ruthenocene derivatives have been synthesised for possible use as radiopharmaceuticals (^{97}Ru, ^{103}Ru, ^{106}Ru), in metalloimmunoassay and in the synthesis of neuropeptides containing terminal tyrosine and alanine residues.[51] These derivatives are thermally stable crystalline solids of the form $[CpRuL]PF_6$ (L = Ac-TyrOEt, Ac-PheOEt, Ac-TrpOEt, Ac-Tryptamine and Tryptophol) and were obtained by the reaction of $[CpRu(MeCN)_3]PF_6$ with the ligands in dichloromethane at 40-50°. The photolabile complexes rac-$[Ru(bipy)_2AA]ClO_4.H_2O$ and rac-$[Ru(phen)_2AA]ClO_4.H_2O$ (AA = Gly, N-MeGly, N-PhGly) have been synthesised and their structures in solution analysed using ^1H n.m.r. spectroscopy.[52] The crystal structure of the complex rac-$[Ru(bipy)_2Gly]ClO_4.2H_2O$ has been determined. In this complex the Ru-N(imine) bond trans to the oxygen donor atom is significantly shorter than the other three Ru-N(imine) bonds. The mercury(II) complexes $Hg(en)(ClO_4)_2$, $Hg(en)Lys(ClO_4)$, $Hg(en)His(ClO_4)$ and Hg(en)Cys (en = 1,2-diaminoethane) have been synthesised and their activities against acetylcholinesterase investigated.[53] A number of organosilicon and organotin complexes of phenylglycyl hydrazones have been synthesised and tested successfully as antibacterials.[54]

Reaction of $K_3[IrCl_6]$ with Ala in aqueous solution at 110°C affords the octahedral complex $K_2[Ir(Ala)Cl_4].2H_2O$ for which the crystal and molecular structure has been reported.[55] Crystal and molecular structures are also reported for the binuclear lanthanide complexes $Pr_2(L\text{-}Glu)_2(ClO_4)_4.11H_2O$ [56] and $[Er(Gly)_2(H_2O)_4]_2(ClO_4)_6.4$ dioxane.[57] In these complexes the metal ions are nine and eight coordinate respectively and are interconnected by four carboxylate bridging ligands, (19).

Schiff Base Complexes.- A number of papers describing metal complexes of Schiff base ligands derived from amino acids have appeared. Chromium(III) complexes of the type $K[Cr(Sal\text{-}AA)_2]H_2O$ containing tridentate salicylideneamino acid ligands derived from the amino acids Gly, Ser, Met, Leu, Phe, Trp or Ala were prepared and characterised.[58] The high-spin, five- or six-coordinate iron(III) complexes Fe(Sal-AA)Cl (AA = L-Ala, Val, Phe, His) and Fe(Sal-Histamine) have been synthesised and studied by a range of spectroscopic techniques.[59] The spectral properties of these complexes and their adducts with catecholate anions have been discussed in relation to those of iron tyrosinate proteins, particularly the catechol dioxygenases. In solution monomeric and dimeric species co-exist in equilibrium, and these have been studied by nuclear magnetic and electron spin resonance methods.

Several salicylideneglycinatocopper(II) complexes of composition $Cu(Sal\text{-}Gly)L(H_2O)_x$ (L = imidazole, 2-propylimidazole, pyridine, pyrazole, 3,5-dimethylpyrazole : x = 0,1) have been synthesised.[60] The e.s.r. and electronic spectra of these complexes are consistent with square-pyramidal coordination around the metal ion similar to that in N-salicylideneglycinato(thiourea)-copper(II). A number of other salicylideneamino acid complexes of copper(II) of composition $Cu(Sal\text{-}AA).xH_2O$ (AA = Gly, Ala, β-Ala, Ser, Tyr, Orn, Arg, His : x = 1, 1.5, 2) have also been investigated by e.s.r. spectroscopy.[61]

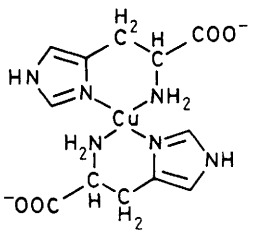
A number of 1:1 and 2:1 complexes of zinc(II) with salicylideneamino acids derived from Gly, Ala, Val, Leu, Ile, Ahx, Ser, Asp and Glu have also been prepared and their formation constants reported.[62] Complexes of the Schiff base ligand N-(2-hydroxy-1-naphthylidene)glycine (H_2L) have been synthesised and studied by spectroscopic methods.[63] The complexes have formulae $ML(H_2O)$ (M = Cu, Fe, Co, Ni, Mn, Zn), $M'[ML_2].H_2O$ (M' = Na, K ; M= Fe, Co, Mn), $VO(L)H_2O$, $ThL_2.H_2O$ and $UO_2L.H_2O$. A number of square-planar nickel(II) complexes with Schiff base ligands derived from 2-hydroxy-1-naphthaldehyde and the amino acids Gly, Ala, Val, Leu, Ile, Ahx, Ser and Asp have been prepared.[64] Dioxouranium(VI) complexes of N-salicylidene-L-valine and N-(orthovanillidene)-L-valine, (20, X = OCH_3), have been synthesised and characterised and their conformations determined by ^1H n.m.r. and by c.d. studies.[65]

2.2 Solution Studies.- This section summarises the literature on the structures, reactions and equilibria of metal-amino acid complexes in solution.

Structures in Solution.- Frozen DMSO solutions of the oxovanadium(IV) complexes $VO(AA)_2.H_2O$ (AA = Gly, Ala, β-Ala, Leu, Bz-Gly, Cys) have been studied by electron spin resonance spectroscopy and shown to contain the O-bonded amino acid complexes $VO(AA)_2(DMSO)_3$.[66] Other complexes identified include $VO(Cys)(DMSO)_3$ and $VO(AA)_2DMSO$ (AA = Gln, Asn) in which the amino acids are bidentate. Solutions of the complexes in water, methanol and pyridine have also been investigated.[67]

Complex formation of glycine,[68] and L-proline,[69] with Co(II), Cu(II) and Mn(II) in aqueous solution at various pH values, temperatures and metal-ion concentrations was investigated by ^{17}O and ^{14}N paramagnetic chemical shifts and relaxation rates. The species $[M(Gly)]^+$, $[M(Gly)_2]$ and $[M(Gly)_3]^-$, containing bidentate glycinate, and $[M(H\text{-}Gly)]^+$ and $[M(H\text{-}Gly)_2]^{2+}$, which contain monodentate glycinate, have been identified. In the case of Cu(II) at pH >12 the species $[Cu(Gly)OH]^-$ and $[Cu(Gly)_2(OH)_2]^{2-}$ also exist. The prolinate complexes $Co(Pro)_2$, and $[Co(Pro)_3]^-$ have been detected in basic solution. The ^{17}O chemical shifts of Pro in these complexes are shifted by 700 and 390 ppm respectively downfield from the free ligand.

The use of ^1H n.m.r. spectroscopy to determine the absolute configurations of complexes is described.[70] Chiral C-deuteroiminodiacetic acid, $HOOCCH_2NHCH(D)COOH$, (S)-IDA, was prepared by selective deuteration of the complex $(-)_{563}$-mer(O)-cis(N_c)-[Co(IDA)(EDMA)] (EDMA = ethylenediaminemonoacetate). Using (S)-H_2IDA the complex μ-fac-{Co[(S)-IDA]Medien}$^+$ (Medien = N-methyl-diethylenetriamine) was prepared and optically resolved. On the basis of the ^1H n.m.r. spectrum of the (S)-IDA ligand the absolute configuration of the $(-)_{550}$ isomer was assigned ΔΛΔ. The complex cations trans(O)- and cis-β-mer(N)-cis(O)-{Co[(S)-Ala]AMDA}$^+$ [AMDA = (R,S)-8-amino-2-methyl-3,6-diazaoctanoate] have been synthesised and characterised.[71] Five optically active diastereoisomers of the trans(O) isomer were separated by cation exchange chromatography and their absolute configurations were assigned.



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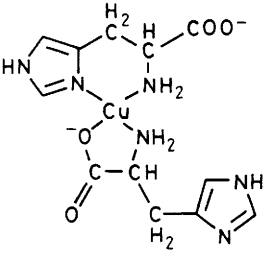

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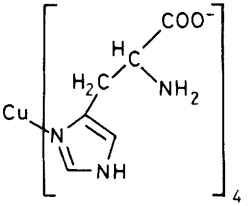

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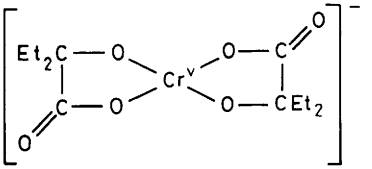

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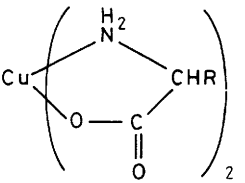

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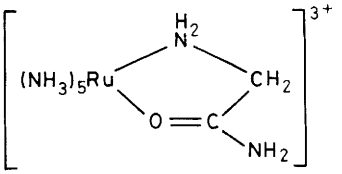

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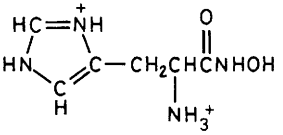


(27)

The enhanced N-H and C-H stretching vibrational circular dichroism spectra of some $Co(AA)_3$ complexes (AA = Gly, Ala ; mer and fac isomers, Λ and Δ enantiomers) in aqueous acid solution and of some $Cu(AA)_2$ complexes (AA = L-and D-Pro, L- and D-Thr) in d_6-DMSO have been analysed.[72] Evidence is obtained for the existence of hydrogen bonding between N-H groups and oxygen lone pairs or carbonyl group π electrons on an adjacent ligand which restricts ligand conformations in solution. Pulsed electron spin resonance spectroscopy utilising the electron spin echo envelope modulation technique was used to study the copper(II) binding sites in porcine kidney and bovine plasma amine oxidases.[73] For both proteins two magnetically distinct histidyl residues were identified as ligands. Using echo envelope data for a series of $[Cu(bipy)_n(H_2O)_{6-2n}]^{2+}$ (n = 0-3) complexes for comparison, water was identified as another ligand.

The Cu^{II}-$(His)_2$ complex in water at room temperature in the presence of excess ligand (to prevent aggregation on freezing) has been investigated by electron spin resonance spectroscopy.[74] Isotope substitution of ^{15}N for ^{14}N in the imidazole ring was used to distinguish between imidazole and amine coordination. Two species were found to be responsible for the spectra in the liquid phase. In the predominant one (80%) both ligands are coordinated 'histamine-like',(21), while in the second species, (22), one histidine is coordinated 'histamine-like' and the other 'glycine-like'. On freezing, imidazole groups which may have been present as weak axial ligands in the liquid phase replace amino and/or carboxylate ligands in the square plane to give complexes in which the metal ion is bound to four imidazole groups, (23).

A combination of potentiometric and spectrophotometric data has been used to detect bi- and tri- nuclear complexes between $[Co(NH_3)_5AA]^{2+}$ (AA = Gly, β-Ala), cis-$[CoN_4(Gly)]^{2+}$ (N_4 = two ethylenediamine ligands or triethylenetetramine) and Cu(II), Ni(II) and Co(II) in aqueous solution.[75] These techniques have also been used to determine the binding sites of some alcohol-containing ligands to copper(II).[76,77] These ligands include 3-amino-2-hydroxypropionic acid, 3-hydroxy-2-aminopropionic acid and 4-amino-3-hydroxybutyric acid. In monomeric complexes the OH group is deprotonated and coordinated at high pH.

The coordination of CH_3Hg^+ with amino acids has been investigated by Raman Spectroscopy.[78] The structures of complexes of UO_2^{2+} with iminodiacetate and nitrilotriacetate have been inferred from spectrophotometric data.[79] ^1H n.m.r. relaxation rate enhancements induced by Gd(III) have been measured in solutions containing Gly and Ala and metal-hydrogen distances have been calculated from the data.[80] From the results obtained it appears that the amino acids in these complexes are coordinated through the bidentate carboxylate groups. The interaction of the chiral shift reagent $Na[Eu(R- \text{ or } S- Pdta)(H_2O)_3]$ (Pdta = propylenediaminetetraacetate) with amino acids has been investigated.[81] This reagent was found to consistently induce larger upfield shifts in the H_α signals of the ^1H n.m.r. spectra of L-amino acids than in their D-enantiomers and hence shows promise for the determination of absolute configuration of underivatized α-amino acids. The induced shift is the result of equilibrium (1) and the separation of enantiomer signals is probably due to a difference in formation constants.

$$[Eu(R\text{-}Pdta)(H_2O)_3]^- + AA \rightleftharpoons [Eu(R\text{-}Pdta)AA(H_2O)_2]^{2-} \qquad (1)$$

Reactions in Solution.- The pyridoxal and metal ion catalysed oxidation of alanine and phenylalanine to pyruvic and phenylpyruvic acids respectively occur at easily measurable rates at 25°C in aqueous and methanol solutions.[82] The relative catalytic activities are Mn(II)>>Co(II)>>Cu(II)>>Ni(II). Two possible reaction mechanisms are proposed for the reaction of the Schiff base chelate, one involving coordination of dioxygen and the other involving oxygen attack on an α-deprotonated carbanionic intermediate. A previous communication claiming that an aluminium(III)-carbanion complex is a mandatory intermediate for the transamination process leading from ketimine to aldimine has been disputed and the complex was shown instead to be a metastable byproduct of a reversible side reaction.[83]

The rate and equilibrium constants for the anation of $[(H_2O)_5CrCH_2CN]^{2+}$ by amino acids $^+NR_3CH_2CO_2^-$ (R=H,CH_3) have been determined.[84] The rate constants are higher than normal for chromium(III) anations and this has been attributed to the trans labilising effect of the alkyl ligand. The anation of

$[Cr(H_2O)_5OH]^{2+}$ by methionine in aqueous methanol has also been investigated.[85] Species distribution curves for the 2:1 Cys-Cr(III) complex system in the pH range 4.5-7.0 have been constructed and the presence of the species $[Cr(Cys)_2]^-$, $Cr(Cys)H\text{-}Cys(H_2O)$ and $[Cr(H\text{-}Cys)_2(H_2O)_2]^+$ confirmed.[86] The kinetics of ring closure in $Cr(H\text{-}Cys)Cys(H_2O)$ have been investigated and a mechanism for this reaction has been proposed. The kinetics and mechanism of the acid hydrolysis of [N-(2-carboxyphenyl)iminodiacetato]glycinatochromium(III), $[CrL(Gly)]^-$, which results in the displacement of glycine and the formation of $CrL(H_2O)_2$, are reported.[6]

The oxidation of L-cysteine and thiolactic acid by the bis(2-ethyl-2-hydroxybutyrate)oxochromate(V) chelate, (24), has been studied in the pH range 2.68-4.09, equation (2).[87] The chromium(III) products are uncharged bischelates containing the

$$Cr^{V} + 2RSH \longrightarrow RSSR + Cr^{III} + 2H^{+} \qquad (2)$$

ligand monoanion and a unidentate ligand from the buffer or from the mercaptide reagent. The reaction is catalysed by chromium(IV) and involves thiyl radical intermediates. Photochemical and thermochemical redox reactions between chromium(VI) and amino acids (Gly, Ala, OH-Pro, Met) lead quantitatively to chromium(III) and do not involve a chromium(VI)-amino acid complex in the ground state.[88] The active participants in the thermal reaction are $HCrO_4^-$, the amino acid cation and H^+, and chromium(V) appears to be present as an intermediate.

The kinetics and mechanisms of the disproportionation of H_2O_2 in the presence of amino acid complexes of manganese(II) (Ser, Thr, Asp, Asn, His) [89,90] and iron(III) (Glu) [91] have been investigated. The reaction of iron(II)-aminopolycarboxylates with H_2O_2 has also been studied.[92] Reaction of $Fe(ClO_4)_2$ and $Fe(ClO_4)_3$ with propionic and butyric acids (HL) in aqueous solution leads to the formation of FeL^+, FeL_2, FeL^{2+}, FeL_2^+, $Fe_3L_6(OH)_2^+$, and the mixed-valence $Fe_3L_6(OH)_2$. The oxidation of cysteine by O_2 in the presence of these complexes occurs with maximum efficiency under conditions where the mixed-valence complexes dominate.[93]

The effect of added ligands (Pro, Gly, Ala) on the lifetime and ^{17}O hyperfine coupling constants of water bound to cobalt(II) was determined as a function of pH by ^{17}O n.m.r. spectroscopy.[94]

The C-formylation of glycine in the complex $[Co(en)_2Gly](O_2SCF_3)_2$ and the subsequent condensation of penicillamine with the C-formylglycinate ligand to give a penicilloato complex have been described in section 2.1. The homolysis and β-elimination reactions characteristic of alkyl-cobalt(III) species have been applied to the enantiospecific synthesis of substituted pyrrolidine structures which are found in kainoid natural products.[95]

The use of ultrasound to promote reactions has been applied to the complexes $Co(Gly)_3.2H_2O$ and $Cu(Gly)_2.2H_2O$.[96] These react with benzyl chloride at 45°C, pH 11.0, under ultrasound irradiation to give N-benzylglycine in high yield particularly with the latter complex. No such reaction was observed for $Ni(Gly)_2.2H_2O$. Arylchlorides having electron-withdrawing groups give higher yields of N-arylglycines, while those having electron-releasing groups produce the opposite effect. The kinetics and mechanism of anation of $cis[Co(en)_2(H_2O)_2]^{3+}$ by L-serine in aqueous ethanol have been studied.[97] The polarographic behaviour of the ternary nickel(II) complexes Ni(AA)AA' (AA = Gly, Ala, Ser; AA' = Gly, Ala, Ser, Thr, 2-ABA) has been studied in 0.1M $NaClO_4$.[98]

The interaction of a number of thiols including cysteine hydrochloride (1.43:1), penicillamine (1:1) and N-mercaptopropionylglycine (2:1) with copper(II) in frozen solutions has been the subject of an e.s.r. study, and from plots of $g_{||}$ vs. $|A_{||}|$ the donor sets SO_3, SO_3 and S_2O_2 respectively were assigned to these ligands in the complexes.[99] The thermal decomposition of the metastable complex $[Cu^{I}_{8}Cu^{II}_{6}(D\text{-pen})_{12}Cl]^{5-}$ (pen = penicillamine) in the absence of and in the presence of likely serum constituents has been investigated.[100] The results show that the complex is not stable in serum but slowly decomposes with the liberation of copper(II), which is immediately sequestered by serum albumin. The clear correlation between the antitumour activity of trans-bis(salicylaldoximato)copper(II) complexes and reactivity towards glutathione and cysteine has been established.[101] This adds further weight to the proposal that antitumour copper(II) complexes such as salicylaldoximates or thiosemicarbazones owe their activity to a reduction in intracellular thiol content and the production of cytotoxic copper(I) species. A number of amino acids (His, Thr, Glu, Gln, Tyr) were found to only weakly inhibit the copper(II)-catalysed

oxidation of ascorbate whereas the proteins catalase, superoxide dismutase and copper binding serum proteins cause strong inhibition.[102]

A number of reactions of bis(serinato)copper(II), (25, R = CH_2OH), in aqueous solution have been reported.[103] Reaction with 4-toluenesulphonyl chloride (TosCl) affords Cu(Tos-Ser)$_2$, (25, R = $CH_2OSO_2C_6H_4CH_3$), which can be oxidised to the C-formylglycinate (For-Gly) complex Cu(For-Gly)$_2$, (25, R = CHO). The decomposition of the copper(II) complexes Cu[$NH_2CH(R)COO$]$_2$ [R = $-(CH_2)_4NHOCOCH_2C_6H_5$, $-CH_2C_6H_4CH_2C_6H_5$] by tetrahydrothiazole-L-thione gives the derivatized amino acids Lys-$OCOCH_2C_6H_5$ and Tyr-$OCH_2C_6H_5$.[104] Thioureas have also been used as reagents for the enantiospecific decomposition of copper(II)-amino acid complexes.[105] The effect of amino acids (Glu and Asp) on the adsorption of copper(II) onto aluminium hydroxide from aqueous solution was studied by e.s.r. and reflectance spectroscopy.[106] Differential pulse anodic stripping voltammetry for trace copper in the presence of cysteine has been investigated.[107] Rate constants for the formation and dissociation of [Cu(bipy)AA]$^+$ and [Cu(phen)AA]$^+$ (AA = Pro, Val, Thr, Abu) at 25°C have been determined by the temperature jump method, and a Bronsted-type linear free-energy relationship was found to exist between the rate constants and the equilibrium constants.[108] Rate constants for the exchange of amino acids in ternary copper(II) complexes with diethylenetriamine were determined by n.m.r. spectroscopy.[109]

Alkylation of glycine as its Schiff base with (S)-2-[(N'-benzylpropyl)amino]benzophenone and complexed to nickel(II) was carried out in basic DMF or MeCN solutions.[110] These reactions were used for the synthesis of (S)-Ala, (S)-Val, (S)-Trp, (S)-Ahx and (S)-3,4-Phe-$(OMe)_2$. A stopped-flow spectrophotometric study shows that nickel(II) reacts with N-benzenesulphonyl-L-cysteine to give consecutively 1:1 and 2:1 complexes.[111] The nickel(II) complexes Ni(AA)H-AA (AA = Gly, Ala, Ser, Thr, Abu) undergo 2-electron irreversible electroreduction at the dropping mercury electrode.[112]

A new route for the synthesis of trans-β-lactams by the reaction of an amino acid ester zinc(II) enolate with an imine is described.[113] Glycolic acid and ammonia in the presence of

platinized CdS undergoes a photochemical reaction to produce glycine and methylamine.[114] While hole-generated $\cdot NH_2$ radicals are necessary for glycine formation, both photogenerated holes and electrons are necessary for the production of methylamine.

The oxidation of L-cysteine, penicillamine and thioglycolic acid by $[Mo(CN)_8]^{3-}$ in aqueous acid is second order in substrate and shows an inverse dependence on $[H^+]$.[115] This is attributed to deprotonation of the -SH and -COOH groups prior to electron transfer. No evidence for the formation of an intermediate complex has been found. The interactions of the dimeric molybdenum(IV) compounds $Mo_2O_4L_2(H_2O)_2$ (L = N-alkylphenothiazine) with L-cysteine and histidine have been investigated.[116]

Hydrolysis of the chelated glycinamide ($GlyNH_2$) complex $[Ru(NH_3)_5GlyNH_2]^{3+}$, (26), in aqueous acid solution (pH 1-3) gives a mixture of $[Ru(NH_3)_4Gly]^{2+}$ and cis-$[Ru(NH_3)_4(H_2O)_2]^{3+}$.[117] The reaction mechanism involves nucleophilic attack by water on the complexed carbonyl group and is the first demonstration of metal-promoted amide hydrolysis at room temperature in acid solution. The hydrolysis of $[Rh(Gly)(NH_3)_5]^{3+}$ in acid solution follows spontaneous and acid-catalysed pathways both of which have rate-determining steps involving fission of the metal-oxygen bond.[118] Base-catalysed hydrolysis of the N-bonded monodentate amino acid ester ligands in the complex $[Pd(dien)(AA\text{-}OR)]^{2+}$ (dien = diethylenetriamine; AA-OR = Gly-OEt, Ala-OMe, Ala-OEt, Ser-OMe, His-OMe, Amb-OEt, Leu-OEt, Val-OEt) at 25°C occurs 12-18 times faster than for the free ligand.[119]

Rate constants and equilibrium constants have been determined for the hydrolysis of cis(N,S)-Pt(AA)DMSO(Cl) complexes (AA = Gly, Sar, N,N-Me_2-Gly) under acid and base conditions at 35°C.[120] Hydrolysis of the Pt-Cl bond over a wide pH range involves attack by water. Rate constants for the silver(I)-catalysed hydrolysis reactions have also been reported. The reaction of excess $PdCl_2$ with methionine and S-methyl-L-cysteine in the pH range 1-3.5 involves rapid formation of a mononuclear chelate followed by a slower hydrolysis or polymerisation step.[121] It was concluded that the most reactive species in the chelation step was $[PdCl_3(OH)]^{2-}$. The reduction of palladium(II) in aqueous pyridine solution at the dropping mercury electrode was studied both in the absence of and in the presence of glycine.[122] Deprotection of

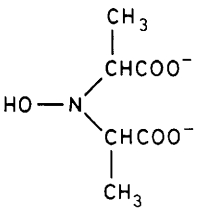
N-allyoxycarbonyl derivatives of amines and amino acids by palladium-catalysed hydrostannolysis with Bu_3SnH in the presence of a proton donor is described.[123]

^{15}N and ^{195}Pt n.m.r. spectroscopy has been used to study the reactions of the complexes $[Pt(^{15}NH_3)_2X(Y)]^{n+}$ (X=Y=H_2O, n=2 ; X=Y=OH, n=0 ; X=Y=Cl, n=0 ; X=$^{15}NH_3$, Y=H_2O, n=2 ; X=$^{15}NH_3$, Y=OH, n=1) with amino acids $^{+}NH_3(CH_2)_mCOO^{-}$ (m=1-3).[124] The reaction of glycine with the diaqua complex gives initially a monodentate O-bonded glycine complex (X=Gly, Y=H_2O, n=2) which undergoes facile ring closure to give $[Pt(^{15}NH_3)_2Gly]^{+}$. The other amino acids by contrast give mixtures of the kinetically stable, monodentate O-bonded complexes cis-$[Pt(^{15}NH_3)_2(O\text{-}AA)H_2O]^{2+}$ and cis-$[Pt(^{15}NH_3)_2(O\text{-}AA)_2]^{2+}$ as well as the bridged binuclear complex $[Pt_2(^{15}NH_3)_4\mu_{O,O}\text{-}AA(\mu\text{-}OH)]^{3+}$. At 37°C and pH=7 the dichloro complex reacts with glycine to give $[Pt(^{15}NH_3)_2Gly]^{+}$ but with the other amino acids to give mainly cis-$[Pt(^{15}NH_3)_2Cl(O\text{-}AA)]^{+}$. The complex $[Pt(NH_3)_3(H_2O)]^{2+}$ reacts with glycine to give initially $[Pt(NH_3)_3(O\text{-}Gly)]^{2+}$, which undergoes linkage isomerisation to $[Pt(NH_3)_3N\text{-}Gly]^{2+}$. In the case of the other amino acids the O-bonded isomers are stable indefinitely.

The effect of cis-$Pt(NH_3)_2Cl_2$ in combination with methionine was investigated on human NHIK 3025 cells cultured in vitro.[125] This amino acid reduces platinum uptake by the cells and reduces the cytotoxicity of cisplatin. An approach has been developed to predict the contact points between RNAs and proteins using trans-$Pt(NH_3)_2Cl_2$ as a crosslinking agent.[126]

The kinetics of formation of 1:1 lanthanide -picolinate complexes at 20°C in aqueous solutions containing t-butanol have been studied and a dissociative interchange mechanism with chelate ring closure as the rate-determining step has been proposed.[127]

Equilibrium Studies.- The principles governing the design of ligands for the treatment of iron overload conditions are the subject of a comprehensive report, and a total of forty new iron(III) chelators based on EDTA, phenolates, catecholates, aminophosphonates, hydroxamates and macrocyclic ligands are described.[128] The synthesis of the ligands, their affinity constants for iron(III) at pH 7.4 and their ability to mobilise iron in mice overloaded with this metal are described.



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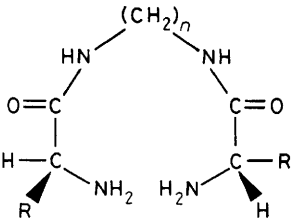
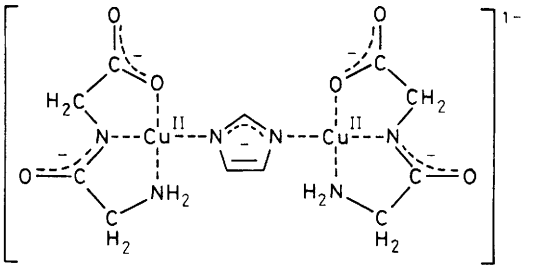


(29) n = 2 or 3

R = Me, Bzl, or Pr^i



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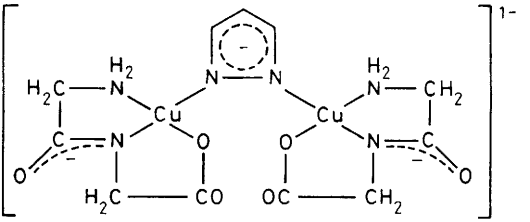

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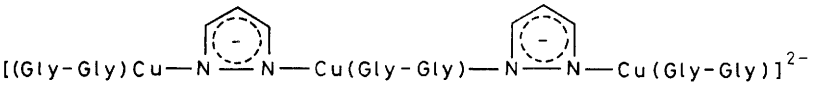

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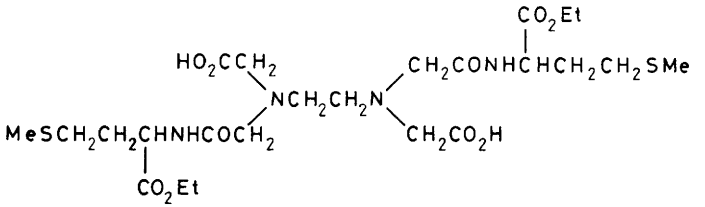

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Formation constants are reported for a number of complexes of hydroxamates derived from amino acids. The ligand α-amino-N-hydroxy-1H-imidazole-4-propanamide, (27), coordinates to Co(II), Ni(II) and Cu(II) through the N(3) of the imidazole ring, the 2-amino group and the oxygen of the hydroxamate group.[129] Ligand protonation constants and formation constants for protonated, deprotonated and hydrolysed complexes in these systems are reported. Formation constants are also reported for complexes of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) with glycinehydroxamic acid,[130] of Cu(II) with leucinehydroxamic acid,[131] and of Cu(II) and Ni(II) with tyrosinehydroxamic acid.[132] Whereas glycinehydroxamic acid is an N,N donor with respect to Cu(II) and Ni(II), it is an O,O donor with respect to the other ions.

In an effort to establish a data base of thermodynamic parameters for all metal ions and ligands in the environment, formation constants as well as enthalpies and entropies of formation have been compiled for a range of metal ions (alkali and alkaline-earth ions, d- and f-block ions, oxoions) and aminocarboxylate (EDTA, EDDA, IDA, CDTA, DTPA, MIDA, NTA) and carboxylate (oxalate and malonate) ligands.[133] The data base will provide much-needed information for the assessment of the toxicity and distribution of metal ions, ligands and their complexes. Formation constants are reported for complexes of Mg(II) with amino acids (Gly, Glu, Asp, pyroGlu) and other ligands (citrate, lactate, pyridoxine) under physiological conditions.[134] These along with other results are used to assess the bioavailability of Mg(II) from orally administered commercial preparations. Only Glu, Asp and citrate form neutral complexes, and their effects on magnesium uptake by enterocyte membranes are discussed. Calcium(II) interactions are also considered.

Formation constants are reported for complexes of N-hydroxy-α,α'-iminodipropionate (HIDP, 28) and the related ligands N-hydroxyiminodiacetate, iminodiacetate and imino-α,α'-dipropionate with alkaline-earth ions, 3d divalent ions, Cd(II) and Al(III).[135] The formation constants for 1:1 and 2:1 complexes follow the expected trends, except for the N-hydroxy derivatives, which show enormous selectivity for VO^{2+} forming 2:1 complexes with β_2 values in excess of 10^{20}. This selectivity is apparently due to coordination of VO^{2+} through the oxygen rather than the nitrogen atom. The ligand HIDP is responsible for the

accumulation of vanadium in amavadine, a naturally occurring vanadyl complex isolated from the poisonous mushroom Amanita Muscaria.

Formation constants have been measured for complexes of aminoethanephosphonic acids with Co(II), Ni(II), Cu(II) and Zn(II) at 25°C, I = 0.2M.[136] The ligands $RCH_2CH(NH_2)PO_3^{2-}$ (R = H, C_6H_5 C_6H_4OH) and $NH_2CH_2CH_2PO_3^{2-}$ coordinate similarly to their aminocarboxylate analogues, forming 1:1 and 2:1 complexes, although differences in the basicity, charge and size of the PO_3^{2-} group compared to the CO_2^- group are evident in the complexation properties.

^{13}C n.m.r. spectroscopy has been used to identify the major complexes present in solutions containing Zn(II) and 1,5-diamino-3-azapentane (dien) both in the absence of and in the presence of the other ligands, 1,2-diaminoethane, 1,3-diaminopropane, acetate, Gly and L-Ala.[137] Using a combination of potentiometric and calorimetric studies, stability constants and enthalpy changes are reported for complexation of UO_2^{2+} with β-alanine and γ-aminobutyric acid, and the results point to the non-involvement of the amino group in coordination.[138]

A number of (S,S)-N,N'-bis(aminoacyl)ethane- and propane-diamines,(29), have been synthesised and the equilibria with copper(II) in aqueous solution investigated.[139] These complex systems have been used for the chiral resolution of dansylamino acids in h.p.l.c. The distribution of complexes and their formation constants are reported.

References to further work on formation constants together with some comments are compiled in the Table.

3 Peptides

The chemistry of metal peptide complexes has attracted wide attention mainly because of the applications of such systems as models for the structures and reactions of metalloproteins. The vast majority of the work reported deals with equilibria in solutions containing copper(II)-peptide complexes.

Synthesis and Structures.- Imidazolate bridged, binuclear

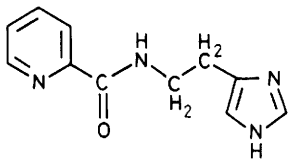
Table Formation constant measurements for metal-amino acid complexes

cation(s)	ligand, complexes	method, conditions, comments	Ref.
Alkali and alkaline-earth ions	Gly	spectrophotometry at 25°C, I = 1.0M	140
Mg^{2+}, Ca^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}	Cytidine and EDTA, NTA or IDA; 1:1:1 ternary complexes	potentiometry at 35°C, I = 0.1 M	141
Ca^{2+}, Pb^{2+}	N-alkyl and N,N-dialkyl amino acids	potentiometry at 25°C	142
VO_2^{2+}	5-sulfosalicylate and Gly, Ala or Phe; 1:1:1 ternary complexes	potentiometry	143
Fe^{3+}	N-(2-hydroxy)benzylglycine FeL^+, Fe(OH)L, $[Fe(OH)L]_2$	potentiometry at 30°C, I = 0.1M	144
Co^{2+}	IDA and Gly, Ala, Ape, Phe, Met, Ser or Asn; ternary complexes		145
Co^{2+}, Ni^{2+}, Cu^{2+}	N-(2-hydroxy-5-sulfo)-benzylglycine; MHL, ML^-, $ML(OH)^{2-}$ $MH_2L_2^{2-}$, MHL_2^{3-}, ML_2^{4-}	potentiometry at 25°C, I = 0.1M $NaClO_4$	146
Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}	Gly and substituted benzimidazoles; 1:1:1 complexes	potentiometry at 30°C in H_2O-dioxan, I = 0.2M $NaNO_3$	147
Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}	N-isobutyroyl-L-Lys, poly(N-methacryloyl-L-Lys)	potentiometry at 25°C, I = 0.1M	148

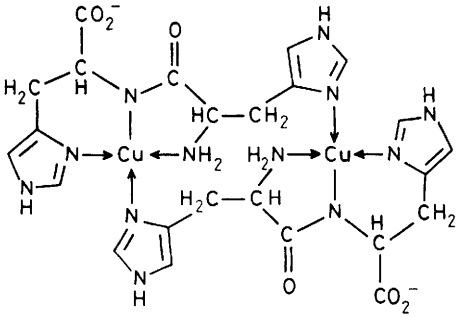
cation(s)	ligand, complexes	method, conditions, comments	Ref.
Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}	N-mesyl and N-tosyl Gly, Ala or Glu	potentiometry in 45% dioxan at 30°C	149
Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}	calmagite and IDA, Gly or en	potentiometry at 30°C, I = 0.1M	150
Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}	IDA, Gly or Ala and 3-hydroxy-2-methyl -4-pyrone; 1:1:1 ternary complexes	potentiometry at 30°C, I = 0.1M $NaClO_4$	151
Co^{2+}, Zn^{2+}	ATP and His, Asp or Glu; 1:1:1 ternary complexes	differential UV spectrophotometry	152
Ni^{2+}	S-carboxymethyl- and ethyl-L-Cys	thermometric titrimetry at 25°C, I = 2M	153
Ni^{2+}	1,4-diazacycloheptane-N,N'-dicarboxylates; 1:1 binary and 1:1:1 ternary complexes (with pyridine)	potentiometry, spectrophotometry	43
Cu^{2+}	pyridoxine and Asp, Ile, Gly, Glu and Val; binary and ternary complexes	303K, I = 0.1M	154
Cu^{2+}	Gly, Ala, β-Ala, Phe, Pro or His and 4- or 7-nitrobenzimidazoles; 1:1:1 ternary complexes	potentiometry at 30°C in 60% MeOH and I = 0.1M	155

cation(s)	ligand, complexes	method, conditions, comments	Ref.
Cu^{2+}	N-hexyl or octyl-IDA and Gly, Ala, β-Ala, 2- or 3-ABA; binary or ternary complexes	potentiometry at 25°C, I = 0.1M	156
Cu^{2+}	Hydrazides of Asp, Glu or isonicotinic acid; 1:1 and 2:1 complexes	potentiometry at 25°C, I = 0.1M KNO_3	157
Cu^{2+}	Gly and tartaric acid; 1:1:1 complex	spectrophotometry and potentiometry at 20°C, I = 0.5M	158
Cu^{2+}	Ala, Ser or Val and 5-nitrosalicylate; 1:1:1 ternary complexes	potentiometry at 25°C, I = 0.5M	159
Cu^{2+}	Gly, Ala or Val and en, $C_2O_4^{2-}$ or malonate; 1:1:1 ternary complexes	spectrophotometry	160
Cu^{2+}	N,N-dibenzyl-Gly, N-benzyl-Ala and $[CH_2N(Bz)CH_2COOH]_2$		161
Cu^{2+}	Asp or 2-Me-Asp; 1:1 and 2:1 complexes	potentiometry at 25°C, I = 0.5M	162
Cu^{2+}	Gly, Ala, Val, Leu, Phe, Trp or Met and bipy; ternary complexes	35°C, I = 0.2M KNO_3	163
Cu^{2+}	bipy or phen and Pro, Val, Thr or Abu	potentiometry at 25°C, I = 0.1M	164
Cu^{2+}	dopa or related ligands and Gly, Ala, Arg, Orn Lys, Asp, Glu; ternary complexes	potentiometry at 25°C, I = 0.1M	165

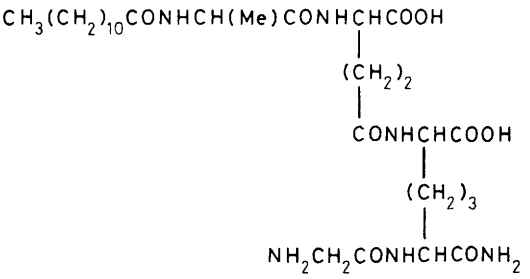
cation(s)	ligand, complexes	method, conditions, comments	Ref.
Zn^{2+}, Cd^{2+}	amino acids and IDA	30°C, I = 0.2M	166
Mo(Vl)	L-Asn, L-Asp	polarimetry, potentiometry	167
Pd^{2+}	dien and Gly, Ala 2-ABA, L-Val, DL-Ser, S-Me-Cys, 2-Me-Ala, L-Thr, Asp; binary and ternary complexes	potentiometry at 25°C, I = 0.1M	168
Pd^{2+}	dien and Gly-OEt, Ala-OEt, Ala-OMe, Ser-OMe, Ser-OEt or His-OMe; 1:1:1 complexes	potentiometry at 25°C	119
Hg^{2+}	dien and amino acids; 1:1:1 ternary complexes	potentiometry at 30 and 45°C, I = 0.1M	169
In^{3+}	L-Glu and L-Met, L-Val or L-Pro; 1:1:1 complexes	polarography	170
La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}	EDTA and Gly, Ala, β-Ala, Val, Leu or Asp	discussion based on published data	171
La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Gd^{3+}	Gly, Ala, Leu, Ile 1:1 and 2:1 complexes	potentiometry at 25°C, I = 0.2M	172
$SnMe_3(H_2O)_2^+$	a range of ligands including Gly, Asp, His, Cys, Pen and glutathione	potentiometry at 25°C, I = 0.3M	173



(34)



(35)



(36)

Glu - Ala - Lys - Ser - Gln - Gly - Gly - Ser - Asn

(37)

complexes containing copper(II) have been studied as models for the active Cu(II)-Zn(II) site of bovine superoxide dismutase. Hence the addition of imidazole (HIm) to a solution containing Cu(Gly-Gly)H_2O at pH 11 produced the dark-blue crystalline binuclear complex Na[Cu_2(Gly-Gly)$_2$Im].1.5 H_2O, (30).[174] At pH<9.8 this complex is decomposed by protonation, giving the mononuclear species Cu(Gly-Gly)H_2O and Cu(Gly-Gly)HIm, while at pH>11.5 it reacts with hydroxide ion to give [Cu(Gly-Gly)OH]$^-$ and [Cu(Gly-Gly)Im]$^-$. A number of complexes of copper(II) with Gly-Gly and pyrazole (Hpz) have been synthesised and their magnetic and spectroscopic properties compared with the imidazole complexes Cu(Gly-Gly)HIm.2H_2O and Na[Cu_2(Gly-Gly)$_2$Im].6H_2O.[175] These include the mononuclear Cu(Gly-Gly)Hpz, the binuclear Ca[Cu_2(Gly-Gly)$_2$pz]$_2$.8H_2O, (31), and the linear-chain trinuclear K_2[Cu_3(Gly-Gly)$_3$(pz)$_2$].5H_2O, (32). In the last two complexes there is weak antiferromagnetic coupling between the metal ions.

The ligand N,N'-1,2-ethanediylbis[N-(carboxymethyl)glycyl-L-methionine] ethyl ester (H_2L, 33) and its copper(II) complex, CuL.1.5 H_2O, have been synthesised and the structure of the latter has been determined by X-ray crystallography.[176] In this complex the metal ion lies in a tetragonally distorted N_2O_4 ligand environment containing the amine and carboxylate groups in the equatorial plane with the amide carbonyl groups axially coordinated. Neither the peptide nitrogen atom nor the thioether sulphur atom are involved in coordination under the conditions investigated.

Reaction of the ligand N-[2-(4-imidazolyl)ethyl]pyridine-2-carboxamide (Pypep, 34) with Et_4N[$FeCl_4$] affords [Fe(Pypep)$_2$]Cl.2H_2O, a synthetic analogue of Fe(III)-bleomycin.[177] The crystal and molecular structure of this complex has been determined. The metal ion lies in an octahedral N_6 ligand environment and the complex has a mer configuration. Mössbauer and magnetic susceptibility data confirm that the ferric ion is low spin. Chemical and electrochemical reduction of the complex has been carried out.

Solution Studies.- The interaction of the peptides L-Pro-L-His, D-Pro-L-His, L-His-L-His and D-His-L-His (HL) with copper(II) has been investigated by potentiometric and spectrophotometric methods.[178] The Pro-His isomers form the complexes CuL, Cu(H_{-1}L), Cu(H_{-2}L) and Cu(H_{-3}L), while the His-His isomers form Cu(H_2L)

Cu(HL), CuL, $Cu_2H_{-1}L_2$ and the dimer $[Cu(H_{-1}L]_2$, which contains each metal ion in a 4N ligand environment, (35). Stereoselectivity between the diastereoisomers towards the metal ion is explained in terms of conformational differences in the peptide chains. Thermodynamic selectivity was also found in the formation of amide deprotonated complexes of Cu(II) with L-Met-L-Met and D-Met-L-Met.[179] The differences of 0.3 in log β and 1.7 kcal mol^{-1} in ΔH° in favour of the L,L diastereoisomer has been attributed to hydrophobic interactions between the residues on the side chains, which is possible only for this isomer.

Complex formation between copper(II) and cyclo(L-histidyl-L-histidyl) has been investigated by calorimetric and e.s.r. methods and the results point to the formation of unusually large ring chelates in both the 1:1 and 2:1 complexes.[180] Stability constants have been determined for Cu(II) and Ni(II) complexes of the thioether containing amino acids S-Me-L-Cys and Met and the peptides Met-Gly, Gly-Met, Met-His and His-Met.[181] In the case of the Met-Gly and Met-His complexes evidence points to an axial interaction of the thioether group with the metal ion. Complex formation equilibria involving Cu(II), Ni(II) and the ligands Leu-NHMe, Met-NHMe, Phe-Met-NHMe and their thiocarbonyl analogues have been investigated.[182] While the presence of the thiocarbonyl group increases the stability of the 1:1 and 2:1 complexes, it does not prevent amide group deprotonation in basic solution. The pK_a values for these ionizations in the Cu(II)-Phe-Met-NHMe complex are 4.47 and 8.40 at 25°C, I = 0.2M. A potentiometric and spectrophotometric investigation shows that the SH group in cysteine-containing peptides is a very effective donor for Ni(II).[183]

Stability constants have been reported for complexes of Co(II), Ni(II), Cu(II) and Zn(II) with the dipeptides His-Phe and His-Tyr.[184] While no evidence for amide group deprotonation was observed, stability constants of the 1:1 and 2:1 Co(II) and Ni(II) complexes indicate a metal-ion aromatic-ring interaction. The phenolic group of His-Tyr does not participate in metal-ion coordination. Stability constants have also been obtained for ternary and quaternary complexes involving the ligands pyridoxamine, Gly-Gly and imidazole with the metal ions Co(II), Ni(II), Zn(II) and Cd(II).[185]

The interaction of copper(II) with the tyrosine-containing

tripeptides Gly-Leu-Tyr, Gly-Tyr-Gly and Tyr-Gly-Gly has been investigated and the results indicate that these ligands behave as simple tripeptides with the phenolic groups not involved in coordination to the metal ion.[186] The interaction of the tetrapeptide L-His-Gly-L-His-Gly with copper(II) under weakly alkaline conditions occurs through the amino group, two deprotonated peptide nitrogen atoms and an imidazole nitrogen from the third histidyl residue.[187] In the case of L-His-L-His-Gly-Gly the donor sites are the amino group, the adjacent deprotonated peptide nitrogen and an imidazole nitrogen from the second histidyl residue. The imidazole group from the terminal histidyl residues acts as a bridge between monomeric species. The synthetic immunostimulant tetrapeptide N^2-[N-(N-lauroyl-L-alanyl)--D-glutamyl]N^6-glycyl-2,6-diaminopimelamic acid, (36), interacts with copper(II) to form the complexes CuL, $Cu(H_{-1}L)$, $Cu(H_{-2}L)$ and at high pH $Cu(H_{-3}L)$.[188] Stability constants for these species are reported and only minor stereoselectivity between the D/D, L/L and meso isomers was observed.

The peptides $(Pro\text{-}Pro\text{-}Gly)_n$ (n = 1-3) which have been identified in the neuropeptide Nereidine have been synthesised and their interactions with copper(II) studied.[189] All the peptides form the species CuL and CuL_2 and at higher pH the species $Cu(H_{-1}L)$. The interaction of copper(II) with the tetrapeptides $Pro\text{-}Ala_3$, $Ala\text{-}Pro\text{-}Ala_2$, $Ala_2\text{-}Pro\text{-}Ala$ and $Ala_3\text{-}Pro$ has also been studied and 'break points' in coordination due to the lack of amide protons at Pro residues have been observed.

Thymulin is a recently identified metallo-nonapeptide, which is zinc dependent and which is involved in extrathymic T cell differentiation. Complex formation equilibria involving Zn(II) and the nonapeptide (L, 37) have been investigated under physiological conditions and the species ZnL_2H_2 and $Zn_2L(OH)_3$ have been identified.[190] The interaction of Cu(II) with the nonapeptide has been the subject of a detailed study by electronic absorption, c.d., e.s.r., ^1H and ^{13}C n.m.r. spectroscopy as a function of pH and ligand-to-metal ratio.[191] At low pH the metal-ion binding site involves the C-terminal Asn and two Ser residues, while at higher pH a binuclear species is formed. The N-terminal Glu residue is apparently not involved in binding either Cu(II) or Zn(II).

Stability constants are reported for complexes of hydroxamate

derivatives of Boc-Gly, Boc-Val and Boc-Ala-Gly with divalent (Mn, Co, Ni, Cu, Zn, Cd, Pb), trivalent (Fe, In), tetravalent (Th) and oxoions (UO_2^{2+}).[192] The kinetics of dissociation of $[Fe(Boc\text{-}Gly\text{-}NHO)]^{2+}$ have also been investigated.

The effect of the addition of Ala and the oligopeptides Ala_2, Ala-Val-Leu and Ala_4 to aqueous solutions of $Sc(NO_3)_3$ has been studied by ^{45}Sc n.m.r. spectroscopy.[193] Addition of Ala gives carboxylato-bonded complexes $[Sc(H_2O)_{6-n}(Ala)_n]^{3+}$ or $[Sc(H_2O)_{5-n}OH(Ala)_n]^{2+}$ which have ^{45}Sc signals lying at +70 ppm relative to $[Sc(H_2O)_6]^{3+}$ or $[Sc(H_2O)_3OH]^{2+}$. Exchanges between these species and the hexaquo or pentaaquohydroxo complexes have been investigated and the observed line widths suggest that the stabilities of the complexes follow the order $Ala<Ala_4<Ala_2<$ Ala-Val-Leu.

The kinetics of deprotonation of the amino acid amide and peptide ruthenium(III) chelates $[Ru(NH_3)_4NH_2CH_2CONRR']^{n+}$ (R=H, R'=H, Et, CH_2COO^- ; R=Me, R'=CH_2COO^- ; n=2 or 3) have been studied by stopped-flow spectrophotometry.[194] The observed rates of deprotonation suggest that the site of deprotonation is the methylene group of the chelate and not the dangling amide group as was previously proposed. In agreement with this it was found that the glycylsarcosine chelate which does not have an ionizable amide group has a similar pK_a value to the other complexes studied.

The reaction of iron(II)-tetraphenylporphyrin with Cu(III)-Aib_3 (Aib = α-aminoisobutyric acid) results in the formation of the binuclear complex Fe(III)TPPCu(II)Aib_3, for which spectroscopic, electrochemical and magnetic data are reported.[195] A weak antiferromagnetic interaction between the high-spin Fe(III) and Cu(II) is evident from e.s.r. studies.

The oxidation reduction potentials of Cu(II)-bleomycin, a cyclopeptide antibiotic, in the presence of dithionite and cysteine have been measured.[196] The values obtained lie within a range which would allow reduction of the complex to occur inside the cell. The reductions of cytochrome c and nitroblue tetrazolium chloride (NBT) occur in the presence of Cu(II)-bleomycin and cysteine.[197] Both reductions are inhibited by neocuproine, which is known to bind Cu(I), but only the reduction of NBT is inhibited by superoxide dismutase. It

therefore appears that Cu(I) is responsible for the reduction of cytochrome c while NBT is reduced by superoxide produced from Cu(I) and O_2. DNA chain breakage by bleomycin is enhanced in the presence of Cu(II)-bleomycin and cysteine.[198] These species do not enhance Fe(II)-bleomycin DNA breakage but activate inactive Fe(III)-bleomycin. This activation is due to the reduction of Fe(III) to Fe(II) by Cu(I)-bleomycin, which in turn is obtained by cysteine reduction of the Cu(II) complex.

The addition of alkylthiols to cytochrome c and its haem-octapeptide (H8PT) has been investigated by optical absorption and m.c.d. spectroscopy.[199] Similar association constants were obtained for both systems. The m.c.d. spectrum of the haem-octapeptide adduct was similar to that of low-spin cytochrome P-450, suggesting similar haem environments for both systems. The interaction of o-, m- and p-fluoroaniline with H8PT was studied by optical absorption and ^{19}F n.m.r. spectroscopy.[200] The line broadening and the chemical shifts in the ^{19}F signals have been interpreted in terms of fluoroaniline binding to the haem group. The kinetics of reduction of the haem undecapeptide and the haem nonapeptide from cytochrome c by dithionite have been studied and as with other haem proteins SO_2^- was found to be the dominant reducing agent.[201] The use of the reagents 3,4-toluenedithiol and o-xylene-α,α'-dithiol to investigate the chelating ability of peptides of the type Cys-AA-AA'-Cys (AA,AA' = amino acid residues) in 2Fe-2S model complexes is described.[202]

The interaction of ammonium metavanadate with glutathione and oxidised glutathione and its effect on the activity of glutathione reductase has been investigated.[203] The determination and characterisation of vanadium contaminant in bovine serum albumin are described.[204] Levels of this element determined by neutron activation analysis vary from 0.2-16.7 μg/g of protein, and this according to e.s.r. results is in the vanadate form.

The chromatographic behaviour of 49 peptides containing His, Asp and Lys residues on Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) Sephadex G25 iminodiacetic acid immobilised metal-ion affinity columns is reported.[205] Other papers on metal-ion–peptide interactions describe the interaction of the antiproliferative and antineoplasmic agent trans-bis(salicylaldoximato)copper(II) with glutathione and cysteine,[206] the interaction of thiomolybdate with albumin, thionein and their copper(II) complexes,[207] and c.d.

studies of Cd(II),[208] and Pd(II),[209] complexes with polymers derived from the amino-acids Ala, Asp and Glu.

References

1 P.A. Sutton and D.A. Buckingham, Acc.Chem.Res., 1987, 20, 357.
2 F. Jursik and B. Hajek, Chem.Listy, 1987, 81, 941.
3 U. Ozer, Chim.Acta Turc., 1985, 13, 253.
4 G. Roewer, S. Gahler and B. Berndt, J.Inf.Rec.Mater., 1986, 14, 433.
5 C.A. Green, Diss.Abstr.Int B, 1987, 47, 4156.
6 C. Chatterjee and S.V. Phadke, J.Chem.Soc.,Dalton Trans., 1987, 2095.
7 B.P. Murch, F. Bradley, P.D. Boyle, V. Papaefthymiou and L. Que Jr., J.Am.Chem.Soc., 1987, 109, 7993.
8 A.S. Borovik, B.P. Murch, L. Que Jr., V. Papaefthymion and E. Munck, J.Am.Chem.Soc., 1987, 109, 7190.
9 M.S. Sadykova, A.A. Shabilalov and M.K. Alyaviya, Uzb.Khim.Zh., 1986, 19.
10 J.M. Elizabathe and P.S. Zacharias, Polyhedron, 1987, 6, 969.
11 B.P. Straughan, O.M. Lam and A. Earnshaw, J.Chem.Soc.Dalton Trans., 1987, 97.
12 M.A. Cox, T.J. Goodwin, P. Jones, P.A. Williams, F.S. Stephens and R.S. Vagg, Inorg.Chim.Acta, 1987, 127, 49.
13 S. Tsuboyama, T. Sakurai and K. Tsuboyama, J.Chem.Soc., Dalton Trans., 1987, 721.
14 P. Jones, P.A. Williams, F.S. Stephens and R.S. Vagg, J.Coord.Chem., 1987, 16, 25.
15 D.J. Radanovic, M.I. Djuran, V.D. Miletic, C. Maricondi and B.E. Douglas, Inorg.Chem., 1988, 27, 1265.
16 M.J. Malinar, P.N. Radivojsa and M.B. Celap, Rev.Chim.Miner., 1986, 23, 753.
17 T. Kawamoto, H. Einaga and J. Hidaka, Bull.Chem.Soc.Jpn., 1987, 60, 919.
18 S.R. Niketic and W. Urland, Polyhedron, 1987, 6, 947.
19 K. Okamoto, H. Umehara, M. Nomoto, H. Einaga and J. Hidaka, Bull.Chem.Soc.Jpn., 1987, 60, 1709.
20 H. Umehara, T. Konno, K. Okamoto and J. Hidaka, Bull.Chem.Soc.Jpn., 1987, 60, 1367.
21 N.J. Curtis, A. Hammershoi, L.M. Nicolas, A.M. Sargeson and K.J. Watson, Acta Chem.Scand., 1987, 41A, 36.
22 M. Strasak and J. Laucokova, Proc.Conf.Coord.Chem., 1987, 391.
23 T. Ama, H. Kawaguchi and T. Yasui, Bull.Chem.Soc.Jpn., 1987, 60, 1183.
24 L.P. Battaglia, A.B. Corradi, L. Menabue, M. Saladini and M. Sola, J.Chem.Soc., Dalton Trans., 1987, 1333.
25 L. Menabue, M. Saladini, L.P. Battaglia and A. Corradi, Inorg.Chim.Acta, 1987, 138, 127.
26 L. Menabue and M. Saladini, Inorg.Chim.Acta, 1987, 135, 49.
27 K. Aoki and H. Yamazaki, J.Chem.Soc., Dalton Trans., 1987, 2017.
28 S. Itoh, Y. Yukawa, Y. Inomata and T. Takeuchi, Bull.Chem.Soc.Jpn., 1987, 60, 899.
29 C.O.B. De Miranda-Pinto, E.B. Paniago, S. Carvalho, M. Tabak and Y.P. Mascarenhas, Inorg.Chim.Acta, 1987, 137, 145.
30 G. Mendoza-Diaz, L.M.R. Martinez-Aguilera, R. Perez-Alonso, X. Solans and R. Moreno-Esparaza, Inorg.Chim.Acta, 1987, 138, 41.
31 N. Barba Behrens, G. Mendoza Diaz and D.M.L. Goodgame, Inorg.Chim.Acta, 1986, 125, 21.

32 M.A.S. Goher and T.C.W. Mak, Inorg.Chim.Acta, 1987, 127, L13.
33 H.D. Nguyen, B. Viossat, A. Busnot, J.M. Gonzalez Perez, S. Gonzalez Garcia and J. Gutierrez, Inorg.Chem., 1987, 26, 2365.
34 A. Fuertes, C. Miravitlles, E. Escriva, E. Coronado and D. Beltran, J.Chem.Soc., Dalton Trans., 1987, 1847.
35 M. Castillo, A. Criado, R. Guzman, J.J. Criado and B. Macias, Transition Met.Chem., 1987, 12, 225.
36 N.A. Emanuel and P.K. Bhattacharya, Polyhedron, 1987, 6, 845.
37 A.M. Abdel-Mawgoud and R. Abdel-Hamid, Monatsh.Chem., 1987, 118, 1219.
38 I. Grecu, R. Sandulescu and M. Neamtu, Rev.Chim., 1986, 37, 589.
39 M.S. Verma, P.K. Chitale and S.N. Mandloi, Egypt.J.Chem., 1985, 28, 121.
40 P.K. Chitale, S.N. Mandloi, N. Kothari and M.S. Verma, Pol.J.Chem., 1986, 59, 1001.
41 V.K. Rastogi, H.P. Mital, R.C. Saxena and N.P. Gupta, Proc.Natl.Acad.Sci.India, Sect.A, 1987, 57, 350.
42 V. Hondrellis, T. Kabanos, S.P. Perlopes and J.M. Tsangaris, Inorg.Chim.Acta, 1987, 136, 1.
43 Y. Fukuda, K. Yamagata and T.A. Kaden, Bull.Chem.Soc.Jpn., 1987, 60, 3569.
44 P. Bukovec, S. Milicev, N. Bukovec and M. Ceponi, Inorg.Chim.Acta, 1987, 137, 177.
45 D.T. Corwin, E.S. Gruff and S.A. Koch, J.Chem.Soc.,Chem. Commun., 1987, 966.
46 A. Colombo, R. Di Gioia, A. Pasini, T. Dasdia and F. Zunino, Inorg.Chim.Acta, 1986, 125, L1.
47 O. Otsuki, T. Nakatari, M. Nawata and Y. Arai, Chem.Abstr., 1987, 107, 191009V.
48 N. Jain, R. Mital, K.S. Ray, T.S. Srivastava and R.K. Bhattacharya, J.Inorg.Biochem., 1987, 31, 57.
49 S. Kasselouri, A. Garoufis and N. Hadjiliadis, Inorg. Chim.Acta, 1987, 135, L23.
50 S.K. McCully and M.P. Vezeridis, Cancer Invest., 1987, 5, 25.
51 R.M. Moriarty, Y-Y. Ku and U.S. Gill, J.Chem.Soc.,Chem. Commun., 1987, 1837.
52 M.A. Anderson, J.P.G. Richards, A.G. Stark, F.S. Stephens, R.S. Vagg and P.A. Williams, Inorg.Chem., 1986, 25, 4847.
53 B.L. Bhat and K.P. Dubey, Natl.Acad.Sci.Lett.(India), 1987, 10, 275.
54 T.M. Aminabhavi, N.S. Riradar, S.B. Patil, D.E. Hoffman and V.N. Biradar, Inorg.Chim.Acta, 1987, 135, 139.
55 V.S. Sergienko, A.E. Bukanova, T.P. Siderova, M.A. Porai-Koshits and L.K. Shubochkin, Zh.Neorg.Khim., 1987, 32, 1931.
56 I. Csoeregh, P. Kierkegaard, J. Legendziewicz and E. Huskowska, Acta Chem.Scand., 1987, 41A, 453.
57 Y. Zheng and K. Pan, Jiegon Huaxue, 1987, 6, 132.
58 R. Shanthi, K.S. Nagaraja and M.R. Udupa, Inorg.Chim.Acta, 1987, 133, 211.
59 L. Casella, M.Gullotti, A. Pintar, L. Messori, A. Rockenbauer and M. Gyor, Inorg.Chem., 1987, 26, 1031.
60 C. Plesch, G. Friebel, O. Svajlenova and J. Kratsmar Smogrovic, Inorg.Chim.Acta, 1987, 129, 81.
61 J. Jezierska, Polyhedron, 1987, 6, 1669.
62 M.R. Mahmoud, S.A. El-Gyar, A. Shaker and A.M. Abdel-Mawgoud, Monatsh.Chem., 1987, 118, 1289.
63 N. Thankarajan and K. Mohanan, J.Ind.Chem.Soc., 1986, 63, 861.
64 M.R. Mahmoud, S.E. El-Gyar, A.A. Moustafa and A. Shaker, Polyhedron, 1987, 6, 1017.
65 R. Roy, M.C. Saka and P.S. Roy, Inorg.Chim.Acta, 1987, 129, 265.

66 U.M. Shodiev, Z.M. Musaev, O.F. Khodzhaev, Ya.S. Usmankhodzhaeva and N.A. Parpiev, Uzb.Khim.Zh., 1986, 23.
67 U.M. Shodiev, Z.M. Musaev, O.F. Khodzhaev, Y.S. Usmankhodzhaeva and N.A. Parpiev, Koord.Khim., 1987, 13, 179.
68 E.D. Gotsis and D. Fiat, Polyhedron, 1987, 6, 2053.
69 E.D. Gotsis and D. Fiat, Polyhedron 1987, 6, 2037.
70 T. Ama, S. Kaizaki, H. Kawaguchi and T. Yasui, Chem.Letts., 1987, 441.
71 C. Fukuhara, T. Kinjo, K. Katsura and E. Kyuno, Inorg.Chim.Acta, 1987, 130, 33.
72 T.B. Freedman, D.A. Young, M. Reza Oboodi and L.A. Nafie, J.Am.Chem.Soc., 1987, 109, 1551.
73 J. McCracken, J. Peisach and D.M. Dooley, J.Am.Chem.Soc., 1987, 109, 4064.
74 M. Pasenkiewicz - Gierula, W. Froncisz, R. Basosi, W.E. Antholine and J.S. Hyde, Inorg.Chem., 1987, 26, 801.
75 P. Mohanty, P.C. Tripathy, A.C. Dash and R.K. Nanda, Ind.J.Chem., 1987, 26A, 392.
76 T. Kiss, C. Simon and Z. Vachter. J.Coord.Chem., 1987, 16, 225.
77 T. Kiss, C. Simon and Z. Vachter, Magy.Kem.Foly. 1987, 93, 1.
78 S. Alex and R. Savoie, Can.J.Chem., 1987, 65, 491.
79 V.A. Perfiler, V.T. Mishchenko and N.S. Poluektov, Dokl.Akad.Nauk.SSSR, 1986, 291, 898.
80 R.S. Reid and B. Podanyi, Can.J.Chem., 1987, 65, 1508.
81 K. Kabuto and Y. Sasaki, J.Chem.Soc.,Chem.Commun., 1987, 670.
82 K. Tatsumoto, M. Haruta and A.E. Martell, Inorg.Chim.Acta, 1987, 138, 231.
83 R. Martin, Inorg.Chem., 1987, 26, 2197.
84 M.J. Sisley and R.B. Jordan, Inorg.Chem., 1987, 26, 2833.
85 H.G. Mustofy Mitra and G.S. De, J.Ind.Chem.Soc., 1986, 63, 1040.
86 P.O. Brien, J.D. Pedrosa De Jesus and T.M. Santos, Inorg.Chim.Acta, 1987, 131, 5.
87 S.K. Ghosh, R.N. Bose and E.S. Gould, Inorg.Chem., 1987, 26, 3722.
88 M. Bolte, B. Robert and J. Lemaire, Can.J.Chem., 1986, 64, 1964.
89 D.G. Batyr, S.V. Kilmininov and Ch.T.T. Phuong, Izv.Akad.Nauk.Mold.SSR, Ser Biol.Khim.Nauk, 1986, 46.
90 D.G. Batyr, V.G. Isak and S.V. Kilmininov, Koord.Khim., 1987, 13, 1376.
91 V.G. Isak, N.T. Nyung and A. Ya. Sychev, Zh.Fiz.Khim., 1987, 61, 2212.
92 J.D. Rush and W.H. Koppenol, J.Inorg.Biochem., 1987, 29, 199.
93 Kh.M. Yakubov, V.E. Bessarabenko and E. Ya. Offengenden, Zh.Obshch.Khim., 1987, 57, 1681.
94 E.D. Gorsis and D. Fiat, Magn.Reson.Chem., 1987, 25, 407.
95 J.E. Baldwin and C.-S. Li, J.Chem.Soc., Chem.Commun., 1987, 166.
96 G.S. Reddy and G.G. Smith, Inorg.Chim.Acta, 1987, 133, 1.
97 D. Chatterjee and G.S. De, Transition Met.Chem., 1987, 12, 193.
98 P.K. Chitale, V.S. Verma and M.S. Verma, Proc.Natl.Acad.Sci. India,Sect.A, 1987, 57, 115.
99 E.W. Ainscough, A.G. Bingham and A.M. Brodie, Inorg.Chim.Acta, 1987, 138, 175.
100 U. Deuschle and U. Weser, Inorg.Chim.Acta, 1987, 135, 5.
101 H. Elo, Inorg.Chim.Acta, 1987, 136, L33.
102 R.A. Loevstad, Int.J.Biochem., 1987, 19, 309.
103 M. Castillo, A. Griado, E. Ramirez, J. Criado and B. Macias, Transition Met.Chem., 1987, 12, 184.
104 K.H. Hoenig, L. Kaul, M. Kuge and M. Schuster, Liebigs Ann.Chem., 1987, 1115.

105 Y. Ye, G. Gu, N. Yu, C. Li, Y. Lin and Q. Xing, Kexue Tongbao, 1986, 31, 1320.
106 G. Micera, L.S. Erre and R. Dallocchio, Colloids Surf. 1987, 28, 147.
107 C. Sun, W. Huang and X. Zhang, Fenxi Huaxue, 1987, 15, 435.
108 Y.T. Chen, H.K. Lin and H.Q. Liu, Inorg.Chim.Acta., 1987, 126, 107.
109 E.L. Gogolashvili, V.G. Shtyrlin and A.V. Zakharov, React.Kinet.Catal.Lett., 1987, 33, 167.
110 Y.N. Belokon, N.I. Chernoglazova, N.S. Garbalinskaya, M.B. Saporovskaya, K.A. Kochetkov and V.M. Belikov, Izv.Akad.Nauk SSSR, Ser.Khim., 1986, 2340.
111 G.N. Mukherjee, P. Dhar and B. Chakraborty, J.Ind.Chem.Soc., 1986, 63, 801.
112 P.K. Chitale, V.S. Verma and M.S. Verma, Proc.Natl.Acad.Sci., India, Sect.A., 1987, 57, 115.
113 J.T.B.H. Jastrazebski, F.H. Van der Steen and G. Van Koten, Recl.Trav.Chim.Pays-Bas, 1987, 106, 516.
114 J. Onue and T. Kawai, J.Chem.Soc.,Chem.Commun., 1987, 1480.
115 M. Olatunji and R.C. Okechukwu, Inorg.Chim.Acta, 1987, 131, 89.
116 B. Keshavan and J. Seetharamappa, Polyhedron, 1987, 6, 465.
117 Y. Ilan and A. Kfir, Inorg.Chem., 1987, 26, 2872.
118 C. Chatterjee and A.S. Bali, Bull.Chem.Soc.Jpn., 1987, 60, 1210.
119 M.M. Shoukry, E.M. Khairy and A. Saeed, Transition Met.Chem., 1987, 12, 315.
120 L.E. Erikson, M. Godfrey and R.G. Larsen, Inorg.Chem., 1987, 26, 992.
121 H.M. Marafie, N. Shuaib and M.S. El-Ezaby, Polyhedron, 1987, 6, 1391.
122 R. Das, P.S. Verma and K. Zutshi, Acta Cienc.Indica Chem., 1986, 12, 109.
123 O. Dangles, F. Guibe, G. Balavoine, S. Lavielle and A. Marquet, J. Org.Chem., 1987, 52, 4984.
124 T.G. Appleton, J.R. Hall and S.F. Ralph, Aust.J.Chem., 1986, 39, 1347.
125 J.E. Melvik and E.O. Pettersen, Inorg.Chim.Acta, 1987, 137, 115.
126 M.A. Tukalo, M.D. Kubler, D. Kern, M. Mougel, C. Ehresmann, J.P. Ebel, B. Ehresmann and R. Giege, Biochem., 1987, 26, 5200.
127 T.E. Eriksen, I. Grenthe and I. Puigdomenech, Inorg.Chim.Acta, 1987, 126, 131.
128 A.E. Martell, R.J. Motekaitis, I. Murase, L.F. Sala, R. Stoldt, C.Y. Ng, H. Rosenkrantz and J.J. Metterville, Inorg.Chim.Acta, 1987, 138, 215.
129 E. Leporti, J.Chem.Soc.,Dalton Trans., 1987, 435.
130 E.B. Paniago and S. Carvalho, Inorg.Chim.Acta, 1987, 136, 159.
131 B. Kurzak, K. Kurzak and J. Jezierska, Inorg.Chim.Acta, 1987, 130, 189.
132 E. Leporati, J.Chem.Soc.,Dalton Trans., 1987, 1409.
133 R.M. Smith and A.E. Martell, Sci.Total Environ., 1987, 64, 125.
134 C. Blaquire and G. Berthon, Inorg.Chim.Acta, 1987, 135, 179.
135 G. Anderegg, E. Koch and E. Bayer, Inorg.Chim.Acta, 1987, 127, 183.
136 T. Kiss, J. Balla, G. Nagy, H. Kozlowski and J. Kowalik, Inorg.Chim.Acta, 1987, 138, 25.
137 D.N. Hague and A.D. Moreton, J.Chem.Soc.,Dalton Trans., 1987, 2889.
138 A. Bismondo, L. Rizzo, P. Di Bernardo and P.L. Zanonato, J.Chem.Soc.,Dalton Trans., 1987, 695.

139 E. Armani, R. Marchelli, A. Dossena, G. Casnati and F. Dallavalle, Helv.Chim.Acta, 1986, 69, 1916.
140 L. Harju, Talanta, 1987, 34, 817.
141 P.R. Reddy and K. Sudhakar, Proc.Ind.Acad.Sci.Chem.Sci., 1987, 98, 289.
142 F. Arnaud-Neu, M.J. Schwing-Neill, F. M'Henni and Z. Mighri, Analusis, 1987, 15, 193.
143 N.P. Gupta, R.C. Saxena, S.K. Sangal, A.K. Manglik and V. Gupta, Chim.Acta Turc., 1986, 14, 323.
144 J. Xu, J. He and H. Zhang, Ziran Kexueban, 1986, 25, 405. (Chem.Abstr., 1987, 107, 13565r).
145 C. Fan and J. Zhang, Chem.Abstr., 1987, 107, 13594z.
146 M. Chandra and A.K. Dey, Acta Chim.Hung., 1987, 124, 259.
147 G.N. Mukherjee and A. Sen, J.Ind.Chem.Soc., 1987, 64, 325.
148 A. Lekchiri, M. Morcellet and M. Wozniak, Polyhedron, 1987, 6, 633.
149 Y. Moroguchi, T. Ikematsu and S. Kojima, Chem.Abstr., 1987, 107, 122142w.
150 B. Satyanarcyana, K.L. Omprakash, K.J. Charyulu, A.V.C. Pal and M.L.N. Reddy, Acta Cienc.Indica,Chem., 1986, 12, 23.
151 M.N. Rao, K.L. Omprakash, K.J. Charyulu, A.V. Chandrapal and M.L.N. Reddy, Acta Cienc.Indica,Chem., 1986, 12, 31.
152 N.K. Daridenko and V.A. Raspopina, Zh.Neorg.Khim., 1987, 32, 1140.
153 R. Aruga, Transition Met.Chem., 1987, 12, 235.
154 R.K. Saxena, R. Kumari, C.P.S. Chandel and C.M. Gupta, J.Electrochem.Soc.India, 1987, 36, 135.
155 N. Vishnumurthy and P. Lingaiah, Indian J.Chem.,Sect.A, 1986, 25, 876.
156 T. Yang, C.Chen and X. Zhang, Zhongnam Kuangye Xueyuan Xuebao, 1987, 18, 331.
157 A. Tsvetanova, M. Miteva and H. Boneva, J.Prakt.Chem., 1987, 329, 534.
158 A.S. Simeu, G.E. Ermolina and A.K. Molodkin, Zh.Neorg.Khim., 1987, 32, 2295.
159 P.K. Migal, Chem.Abstr., 1987, 107, 122145z.
160 V.G. Shtyrlin, A.V. Zakharov, N.N. Kireeva and Z.A. Saprykova, Koord.Khim., 1987, 13, 875.
161 E. Uhlig and K. Fischer, Z.Anorg.Allg.Chem., 1986, 539, 195.
162 P.O. Lehtonen, Finn.Chem.Lett., 1987, 14, 21.
163 K. Prasad and M.S. Mohan, J.Coord.Chem., 1987, 16, 1.
164 Y.T. Chen, H.K. Lin and H.Q. Liu, Inorg.Chim.Acta, 1987, 126, 107.
165 P.G. Daniele, P. Amico, O. Zerbinati and G. Ostacoli, Ann.Chim., 1986, 76, 393.
166 J.D. Joshi, J.Ind.Chem.Soc., 1986, 63, 929.
167 Z. Shen, H. Xu and Z. Chen, Wuli Huaxue Xuebao, 1987, 3, 265.
168 M.M. Shoukry, A.E. Mahgoub and W.M. Hosney, Transition Met.Chem., 1987, 12, 77.
169 R.R. Sharma, B.L. Bhat and K.P. Dubey, J.Ind.Chem.Soc., 1987, 64, 437.
170 S.L. Jain, J.Ind.Chem.Soc., 1986, 63, 805.
171 S.N. Limaye and M.C. Saxena, Chem.Abstr., 1987, 107, 243948x.
172 M. Philip, M.P. Peerzada and J.D. Joshi, J.Ind.Chem.Soc., 1987, 64, 436.
173 M.J. Hynes and M.O'Dowd, J.Chem.Soc.,Dalton Trans., 1987, 563.
174 M. Sato, M. Ikeda, M. Fukuda, T. Ikeda and J. Nakaya, Inorg.Chim.Acta, 1987, 136, 47.
175 W. Mori, T. Sakurai, A. Nakahara and Y. Nakao, Inorg.Chim. Acta, 1987, 132, 247.
176 R.A. Bulman, N. Jobanputra, R. Kuroda, A. McKinnon and P.J. Sadler, Inorg.Chem., 1987, 26, 2483.

177 X. Tao, D.W. Stephan and P.K. Mascharak, Inorg.Chem., 1987, 26, 754.
178 C.E. Livera, L.D. Petit, M. Bataille, B. Perly, H. Kozlowski and B. Radomska, J.Chem.Soc.,Dalton Trans., 1987, 661.
179 R.P. Bonomo, G. Maccarrone, E. Rizzarelli and M. Vidali, Inorg.Chem., 1987, 26, 2893.
180 G. Arena, R.P. Bonomo, G. Impellizzeri, R.M. Izatt, J.D. Lamb and E. Rizzarelli, Inorg.Chem., 1987, 26, 795.
181 I. Sovago and G. Petocz, J.Chem.Soc.,Dalton Trans., 1987,1717.
182 T. Kowalik, H. Kozlowski, I. Sovago, K. Varnagy, G. Kupryszewski and K. Rolka, J.Chem.Soc.,Dalton Trans., 1987, 1.
183 H. Kozlowski, B. Decock - Le Reverend, D. Ficheux, C. Loucheux and I. Sovago, J.Inorg.Biochem., 1987, 29, 187.
184 B. Radomska, T. Kiss and I. Sovago, J.Chem.Res.Synop., 1987, 156.
185 N.M. Moussa, H.M. Marafie, M.S. El-Ezaby and M. Rashad, Polyhedron, 1987, 6, 1477.
186 T. Kiss, J.Chem.Soc.,Dalton Trans., 1987, 1263.
187 J. Ueda, N. Ikota, A. Hanaki and K. Koga, Inorg.Chim.Acta, 1987, 135, 43.
188 B. Decock - Le Reverend, C. Loucheux, C. Livera, L.D. Petit, D. Migliore-Samour and P. Jolles, Inorg.Chim.Acta, 1987, 136, 173.
189 L.D. Petit, C. Livera, I. Steel, M. Battaille, C. Cardon and G. Formicka-Kozlowska, Polyhedron, 1987, 6, 45.
190 B. Hacht and G. Berthon, Inorg.Chim.Acta, 1987, 136, 165.
191 J.P. Laussac, M. Pasdeloup, P. Lefrancier, M. Dardenne and J.F. Bach, New J.Chem., 1987, 11, 67.
192 C.A. Chang, V.C. Sekhar, B.S. Garg, F.S. Guziec and T.C. Russo, Inorg.Chim.Acta, 1987, 135, 11.
193 D. Rehder and M. Speh, Inorg.Chim.Acta, 1987, 135, 73.
194 Y. Ilan, Inorg.Chem., 1987, 26, 2454.
195 M.C. Elliott, N.C. Jain, B.K. Cranmer and A.W. Hamburg, Inorg.Chem., 1987, 26, 3655.
196 D.L. Melnyk, S.B. Horwitz and J. Peisach, Inorg.Chim.Acta, 1987, 138, 75.
197 K. Takahashi and T. Takita, Jpn.J.Antibiot., 1987, 40, 735.
198 K. Takahashi and T. Takita, Jpn.J.Antibiot., 1987, 40, 542.
199 A.J.M. Sadeque, T. Shimizu and M. Hatano, Inorg.Chim.Acta, 1987, 135, 109.
200 A.J.M. Sadeque, T. Shimizu and M. Hatano, Inorg.Chim.Acta, 1987, 135, 203.
201 J. Peterson and M.T. Wilson, Inorg.Chim.Acta, 1987, 135, 101.
202 N. Ueyama, S. Ueno and A. Nakamura, Bull.Chem.Soc.Jpn., 1987, 60 283.
203 M.D. Cohen, A.C. Sen and C-I. Wei, Inorg.Chim.Acta, 1987, 138, 91.
204 H. Sakurai, M. Nishida, K. Kida, M. Koyama and J. Takada, Inorg.Chim.Acta, 1987, 138, 149.
205 M.C. Smith, T.C. Furman and C. Pidgeon, Inorg.Chem., 1987, 26, 1965.
206 H. Elo, Inorg.Chim.Acta, 1987, 136, L33.
207 N.J. Clarke, S.H. Laurie and D.E. Pratt, Inorg.Chim.Acta, 1987, 138, 103.
208 T. Kuroto and M. Kasagi, Polym.J.(Tokyo), 1987, 19, 363.
209 C. Methenitis, J. Morcellet and M. Morcellet, Eur.Polym.J., 1987, 23, 403

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