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Amino Acids and Peptides

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Amino Acids and Peptides

Volume 19

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Amino Acids and Peptides

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A Review of the Literature Published during 1986

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Preface

We have not been made aware of any major general works on the chemistry of amino acids and peptides, although there has of course been the usual crop of specialist monographs[1] and symposium proceedings. On the other hand, there have been two noteworthy texts covering the pharmacological background which provides the principal justification and motivation for a large part of the chemical effort surveyed in these Specialist Periodical Reports. In their 'Principles of Endocrine Pharmacology', Thomas and Keenan[2] give clear and up-to-date (at mid-1985) accounts of the functions and pharmacology of hypothalamic hormones, the anterior pituitary hormones, the posterior pituitary hormones, parathyroid hormone, calcitonin, and insulin. The fifth edition[3] of a well-known introduction to neuropharmacology is also commended: it has a most stimulating discussion of the neuroactive peptides. This is an area of great complexity, which increases relentlessly with the discovery of new peptide factors and activities, and it will be a long time before all the pieces of the puzzle fall into place. It is already clear that the interpretation of the roles of the neuropeptides will lead towards an understanding of the workings of the brain and provide a basis for the rational design of drugs for use in neurological and psychiatric disorders. Quite a lot can be said about what the neuropeptides can do in experimental systems, but what are they for in Nature? At first it was thought that they were like the neurotransmitters of classical pharmacology. Now they are generally regarded as modulators of neural activity - a high sounding but imprecise phrase, like a politician's answer to a tricky question. But there is room for speculation and hypothesis at a fundamental level here, and it has been argued[4] (in a new journal which looks like one which should be watched) that 'peptides are an overelaborated form of messenger for engaging in the relatively simple informational events associated with neurotransmission', and suggested that there may be other functions, to do with nerve growth and development.

The advice with which Cooper, Bloom, and Roth[3] conclude their book is sound: 'Stay tuned, the data flow fast'.

Balliol College, Oxford *John Jones*
July 1987

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4. J. S. Morley, Drug Design and Delivery, 1986, 1, 47. This new journal is published by Harwood Academic Publishers GmbH.

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Abbreviations

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

1
Amino Acids

BY G. C. BARRETT

1 Introduction

The coverage is predominantly derived from the chemical literature, though much of the interest in the amino acids lies in their biological context. The list of references at the end of this Chapter (p.40) reveals many citations from biological journals and secondary sources, however. The 'cut-off point' as far as this Chapter is concerned is to exclude coverage of the distribution of amino acids and metabolic and biosynthetic aspects and biological roles.

2 Textbooks and Reviews

Reviews of a specialist nature are cited in the appropriate Sections of this Chapter. This Section lists more general references: a supplementary list of nomenclature recommendations (IUPAC-IUB) covers selenium-containing amino acids;[1] N-hydroxyamino acids;[2] L-proline and L-hydroxyproline as chiral auxiliary agents in asymmetric synthesis;[3] historical account of the discovery of γ-aminobutyric acid;[4] and arginine with special emphasis on evolutionary and metabolic aspects.[5] Monographs and compendia include a volume entitled 'Glutamate, Glutamine, and Related Compounds' that contains authoritative coverage of many other amino acids of similar functionality;[6] Proceedings volumes;[7] comprehensive analytical coverage;[8] and more broadly based texts.[9]

3 Naturally Occurring Amino Acids

3.1 Occurrence of Known Amino Acids.- This Section includes examples of unusual occurrence of simple, familiar amino acids, either in the free form or in a non-peptide coupling.

D-Leucine is found, not merely in trace amounts, in aerial parts of Coronilla varia and in seeds of Coronilla scorpioides.[10] S-(β-Carboxyethyl)cysteine is the major free amino acid (up to 2.9% dry weight) in seeds of several Calliandra species, and survives in leaves of these plants at early stages of germination.[11] Since this derivative is moderately insecticidal, young plants have chemical defence against at least some of their natural adversaries.

Culture media of Streptomyces cattleya contain (2S)-amino-(3R)-hydroxypent-4-ynoic acid ("β-ethynyl serine").[12] The detection of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid in beer and wine has been reported;[13] it is accompanied by its 1-methyl homologue.

Argiopine, a fortuitously named ion-channel blocking agent from the spider Argiope iobata,

contains arginine and asparagine linked through their carboxy groups by the polyamine moiety $-NH(CH_2)_3NH(CH_2)_3NH(CH_2)_5NH-$, the side-chain amide being substituted by a 2,4-dihydroxyphenylacetic acid grouping.[14]

3.2 Uncommon Amino Acids in Peptides and Proteins.- This would be a much larger section if it covered the title comprehensively; it is restricted to representative citations.

The aquatic fern Azolla caroliniana contains(N-γ-L-glutamyl-D-amino)phenylpropanoic acid.[15] The modified nucleoside N-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]-L-threonine occurs in the urine of patients with certain types of breast cancer and may be of diagnostic value in this context.[16]

Hydrolysis of the glycopeptide antibiotic aricidin A gives (2R,2'S)-actinoidinic acid (as a mixture of two atropisomers) and the phenylglycine derivative (1).[17] More familiar but still uncommon amino acids reported as substituents of proteins are D-aspartic acid in myelin and myelin basic protein;[18] γ-N-methyl asparagine in allophycocyanin;[19] and histidinoalanine, a crosslinking residue in a Macrocallista nimbosa protein.[20] This crosslink is surmised to derive from non-enzymic condensation of phosphoserine and histidine residues,[20] though since this protein also contains phosphothreonine this conclusion would be more plausible if analogous "histidinobutyrine" crosslinks could also be hunted for.

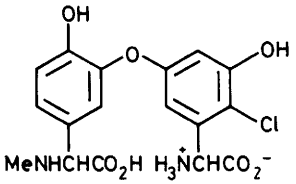
3.2 New Natural Amino Acids.- Xylem sap of Pisum sativum contains an amino-chlorobutanoic acid $C_4H_8NO_2Cl$;[21] while further structural studies can be expected for this compound, more complete assignments have been reported for N^{δ}-(1-carboxyethyl)-L-ornithine from Streptococcus lactis grown in ornithine-supplemented media.[22] Synthesis of this compound from poly(L-ornithine) or N^{α}-benzyloxycarbonyl-L-ornithine gave a 1:1 mixture of diastereoisomers, one of which was identical with the natural material.

Seven new amino acids have been found in the red alga Chondria armata,[23] but the information from Chemical Abstracts is limited to domoilactone B (2) and two palitoxin analogues. The strongly insecticidal properties of these amino acids towards cockroach will ensure the availability of more complete information on this research. Ectothiorhodispira halochloris yields ectoine (3), shown by X-ray analysis to exist in the zwitterionic form.[24]

An unusual type of derivative, D-β-lysylmethanediamine, occurs in Streptomyces nashvillensis.[25]

The earlier finding[26] that α-amino-γ,δ-dihydroxyadipic acid is a constituent of normal human urine is now corrected;[27] it is an artefact from boiling urea and D-glucuronolactone with 6M hydrochloric acid.

3.3 New Amino Acids from Hydrolysates.- This Section covers new amino acids found in peptides and proteins and related condensation products. 2,2'-Bityrosine has been detected



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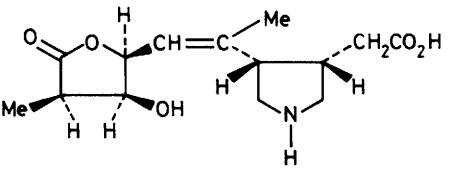

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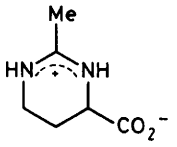

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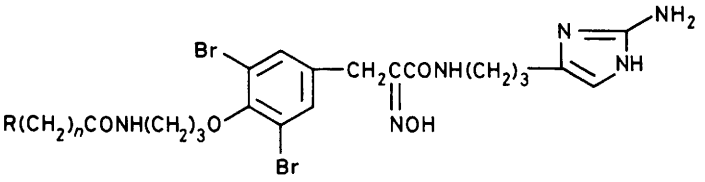

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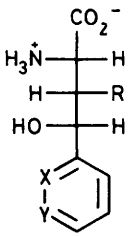
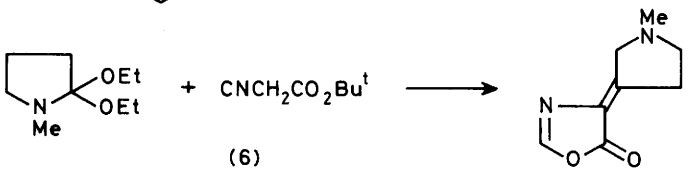


(5) X = N , Y = CH , R = Me
X = CH , Y = N , R = Me
X = N , Y = CH , R = H



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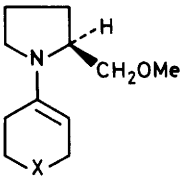

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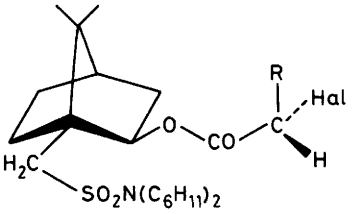


(8)

in yeast acospore wall protein in previously unknown racemic and meso forms.[28] Hydrolysis of proteins that have been chemically modified through azo-coupling of lysine residues releases the modifed residues unaltered when $MeSO_3H$ is used, but when aqueous HCl is used for the hydrolysis α-amino-ε-hydroxycaproic acid and α-amino-ε-chlorocaproic acid are formed.[29]

2-Aminoethylphosphonic acid, claimed to have been found in hydrolysates of ruminate stomach contents, is thought to be a mis-interpretation.[30]

Lipopurealins A (4; R = Me, n = 12) and homologues B (4; R = ^{i}Pr, n = 11) and C (4; R = Me, n = 14) are novel bromotyrosine derivatives from the marine sponge Psammaplysilla purea.[31] Nikkomycin from Streptomyces tendae releases three novel amino acids (5) on hydrolysis, whose structures have been confirmed by synthesis.[32]

4 Chemical Synthesis and Resolution

4.1 General Methods of Synthesis of α-Amino Acids.- This Section collects together those papers that illustrate the use of standard methods (the objectives of these papers are mentioned elsewhere in this Chapter), and also the development of alternative methods. Several papers in the following Section on Asymmetric Synthesis describe the use of standard general methods.

Acylaminomalonates, Ac- or Z-$NHCH(CO_2Et)_2$,[33-39] and other glycine derivatives, e.g. $Ph_2C{=}NCH_2CO_2Me$,[40,41] are alkylated by alkenes,[33,41] alkyl halides,[34-38,40] or αβ-unsaturated aldehydes (Michael addition leading to Z/E-3-ethylproline[39]). Analogous alkylation of 'azlactones' continues in use;[80,145] a new azlactone synthesis[42] uses the glycine derivative t-butyl isocyanoacetate (6) in a condensation that is closely analogous to the standard use of (6) for the synthesis of αβ-dehydro amino acids through reaction with aldehydes or ketones.[43]

Several methods exist for the amination of carboxylic acid derivatives, either employing ammonia with an α-halo-acid[44] or amines with triflates of α-hydroxyacids.[45] In the latter study based on (S)-lactic acid derivatives, decreasing reactivity of various leaving groups ($MeCHRCO_2Et$: R = $CF_3SO_3 \gg Br > MeSO_3 > TolSO_3 > Cl$) is accompanied by increasing tendency towards racemization and elimination.[45] Reductive amination of α-keto-acids using NADH and NADPH with NH_3 has been given a novel aspect in the use of photoinduced regeneration of the reducing agent.[46]

The use of nitrosobenzene for the introduction of a nitrogen functional group into a silyl enol ether, PhNO + $(Me_3SiO)_2C{=}CR^1R^2 \rightarrow PhNHCR^1R^2CO_2H$, involves $LiAlH_4$ reduction of the intermediate adduct.[47] Nitro-alkanoate esters are reduced by catalyzed hydrogen transfer (ammonium formate and Pd-C).[48]

The hydrolysis of α-aminonitriles to corresponding amides is markedly catalyzed by thiols; for example 2-mercaptoethanol leads to 90% conversion in 17 hours at room temperature in aqueous solution at pH 6.5.[49]

Further study of the amidocarbonylation of allylic alcohols has led to improvements in

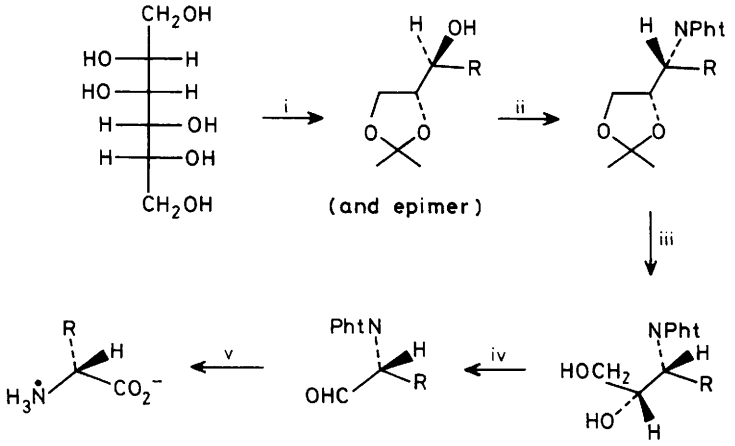
details: $R^1R^2C{=}CHCH_2OH + AcNH_2 + CO + H_2 \rightarrow R^1R^2CHCH_2CH(NHAc)CO_2H$ under mild conditions through the use of the catalyst system $HRh(CO)(PPh_3)_2 + Co_2(CO)_8$.[50]

4.2 Asymmetric Synthesis of α-Amino Acids. Electrophilic amination by BocN=NBoc of chiral silylketene acetals[51] and of camphane esters[52] leads to α-hydrazino acids. These are readily reduced (H_2/Pt) to α-amino acids and provide valuable new routes as alternatives to well established methodology. In the latter category, the 'asymmetric Strecker synthesis' in which (S)-1-phenylethylamine is condensed with NaCN and $PhCH_2COMe$ to give (R)-α-methyl phenylalanine,[53] numerous examples of alkylation of glycine derivatives ($Ph_2C{=}NCH_2CO_2Me$) and allyl acetate catalyzed by a chiral Pd catalyst,[54] $(MeS)_2C{=}NCH_2CONR^1R^2$ where $-NR^1R^2$ is a chiral 2,5-bis(methoxymethyl)pyrrolidine,[55] and the D-camphor imine of t-butyl glycinate [56]), and analogous Schiff bases ($RCH{=}NCHMePh + BrCN \rightarrow RCH(CN)NBrCHMePh$,[57] and $PhCON{=}CHCO_2R$ + enamines (7)[58]) provide a range of optical efficiency. While modest enantioselectivity (up to 57% [54]) is frequently obtained, some of these methods are exceedingly enantio- and diastereoselective (better than 97%,[55] 100%[58]), alkylation by enamines being postulated to proceed via a Diels-Alder-like transition state.[58]

Amination processes of a conventional type are involved in the reaction of α-halogeno-10-sulphonamido-isobornyl esters(8) with NaN_3[59] and of α-keto-acids mediated by polymer-bound NADH and leucine dehydrogenase.[60] Both lead to nearly 100% enantioselective syntheses of a variety of simple aliphatic L-α-amino acids including L-alloisoleucine[59] and L-t-leucine.[60] Other examples of chiral auxiliaries[3] are D-mannitol (conversion into diaziridines, thence to N-toluene-p-sulphonyl-L-α-aminobutyric acid,[61a] or conversion into (R)-phthalimido-aldehydes and D-amino acids: Scheme 1[61b]). (R,R)-Tartaric acid has been used for the preparation of N-Boc-L-erythro-β-benzyloxyaspartate through partial debenzylation, then conventional stages.[62] Enantioselective protonation of lithium enolates by chiral acids alters the optical purity of an amino acid, the extent determined by the lithium counter-ion.[63]

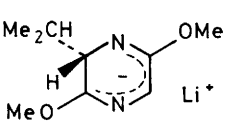
Chiral heterocyclic compounds are being worked hard for the present purpose, with the bislactim ethers (e.g. 9) derived from L-valylglycine di-oxopiperazine having been in use by Schollkopf's group for several years. Chlorination by Cl_3CCCl_3 followed by reaction with a malonic ester gives β-carboxy-D-aspartic acid diesters,[64] while more conventional alkylation methods lead to γ-diethoxyphosphinyl-L-butyrine.[65] The oxazinone (10) from erythro-αβ-diphenyl-β-hydroxyethylamine enantiomers is a useful electrophilic glycine synthon when R = Br (prepared from 10; R = H by reaction with N-bromosuccinimide) that reacts with carbon nucleophiles.[66,67] The (-) isomer after alkylation in this way gives L-α-amino acids through hydrolysis and hydrogenolysis;[66] one example[67] in which displacement of the bromine substituent is brought about by $^2H_2$ has been described, leading to (S)-chiral glycine.

Seebach's exploitation of the enantioselectivity accompanying alkylation of lithium



Reagents: i, Established route; ii, Mitsunobu reaction (DEAD, Ph_3P), phthalimide; iii, H_3O^+; iv, $Pb(OAc)_4$; v, oxidation, deprotection

Scheme 1



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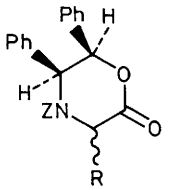

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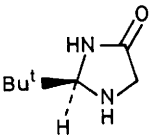

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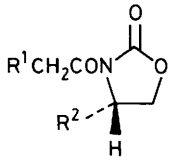

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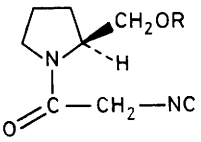


(13)

enolates of imidazolidines (11; see also Vol.18, p. 5) has been extended to other examples,[68-71] including analogous oxazolidinones.[70,71] Condensation of pivalaldehyde with glycinamide gives (11), which can be resolved in the conventional way using (S)-PhCH(OH)CO_2H,[69] while use of an L-amino acid in the condensation gives the oxazolidinone corresponding to (11) with a cis relationship between the 2-t-butyl group and the 4-substituent.[71] Use of an N-alkanoyl oxazolidinone (12) as a chiral glycine synthon for the synthesis of N-methyl-β-hydroxy amino acids through syn-diastereoselective aldol addition of the stannous enolate of (12; $R^2 = {}^nBu$) has been illustrated for N-methyl-3-hydroxy-4-methyloct-6-enoic acid, an unusual α-amino acid in cyclosporin.[72] The novel aminating agents BocN=NBoc[73;cf. 51,52] and $RO_2CN{=}NCO_2R$[74] react with near-100% stereoselectivity with (12) in the form of its Li enolate, the resulting (S)-hydrazino acids being hydrolysed (LiOH), deblocked, and hydrogenolyzed (H_2/Ni) to give the amino acids. N-Isocyanoacetyl-L-prolinol derivatives (13) have served the corresponding purpose in syntheses of enantiomers of α-disubstituted amino acids.[75]

Aldol reactions ($CNCH_2CO_2Me$ + RCHO)[76] and hydrogenations of 2-acylaminocrotonates[77] show a wide range of enantio- and diastereoselectivities with the influence of chiral catalysts. Bis(cyclohexylisocyanide)gold(I) tetrafluoroborate and an (R)-ferrocenylphosphine are very effective in this respect for the aldol reaction,[76] while a range of chiral Rh(I) phosphines of familiar types has shown mixed ability (less than 26%,[77a] 100%[77b]). 'Asymmetric hydrogenation' (H_2 can be replaced by 80% aqueous HCO_2H[77c]) has been reviewed in relation to the commercial synthesis of L-dopa.[77d] Closely related studies have been described for the hydrogenation of alkylidene derivatives of glycyl-L-alanine dioxopiperazine,[78] leading to L-amino acids in better than 94% e.e., and α-nitrocaprolactam catalyzed by $PdCl_2$-(S)-phenylethylamine (giving L-lysine in only 11% e.e.);[79] aminolysis of 2-methyl-4-(4-acetylaminobutyl)oxazolin-5-one with (S)-phenylethylamine gives mainly the L-lysine-containing diastereoisomer.[80]

A review has appeared[81] concerning applications of enzymes in asymmetric synthesis.

4.3 Synthesis of β- and Higher Homologous Amino Acids.- These systems can be made available through standard methods of introduction of amine and carboxy functional groups, and there are few characteristic routes.

Addition of ammonia to αβ-unsaturated acids at 15-30 Kbar yields β-amino acids.[82] An alternative conventional approach to these compounds, exemplified in the synthesis of 3-amino-3-(2-nitrophenyl)propionic acid from o-nitrobenzaldehyde, malonic acid, and NH_4OAc in AcOH, offers a 'one-pot' procedure.[83] Asymmetric synthesis is illustrated in the threo-selective condensation of Z-(O-vinyloxy)boranes with imines (14)→(15).[84] The addition of a chiral primary amine to an αβ-unsaturated ester at 5-15 Kbar is generally highly enantioselective, especially so in the case of 8-(2-naphthyl)menthylamine (better than 99%).[85]

Seebach has taken up the procedure for decarboxylative electrochemical methoxylation of amino acids (see Vol. 17, p. 26) to provide a conversion of (2S,4R)-hydroxyproline into (R)-3-amino-3-hydroxybutanoic acid ("GABOB"), as shown in Scheme 2.[86,87]

Proline isomers (16) can be prepared by cyclization of azomethine ylides formed between alkenes, amines, and formaldehyde.[88]

4.4 Prebiotic Synthesis Models for Amino Acids.- A number of enterprising experiments have been described under this heading in recent Volumes of this Report. These are joined by an account of the formation of the polymer "Titan tholin" by continuous d.c. discharge through N_2 and CH_4 (9:1) at 0.2 mbar pressure.[89] This mixture and energy source simulates the turbulent cloud-top atmosphere of Jupiter's moon; hydrolysis of the polymer with 6M-hydrochloric acid leads to glycine, aspartic acid, alanine, and β-alanine, with 12 other amino acids in lesser proportions. A review has appeared[90] covering HCN polymers as a potential prebiotic source of amino acids.

An efficient system for the synthesis of amino acids that may be relevant to the primordial scene is the ammonolysis of keto-acids in aqueous ammonia, mediated by visible light and dyes.[91]

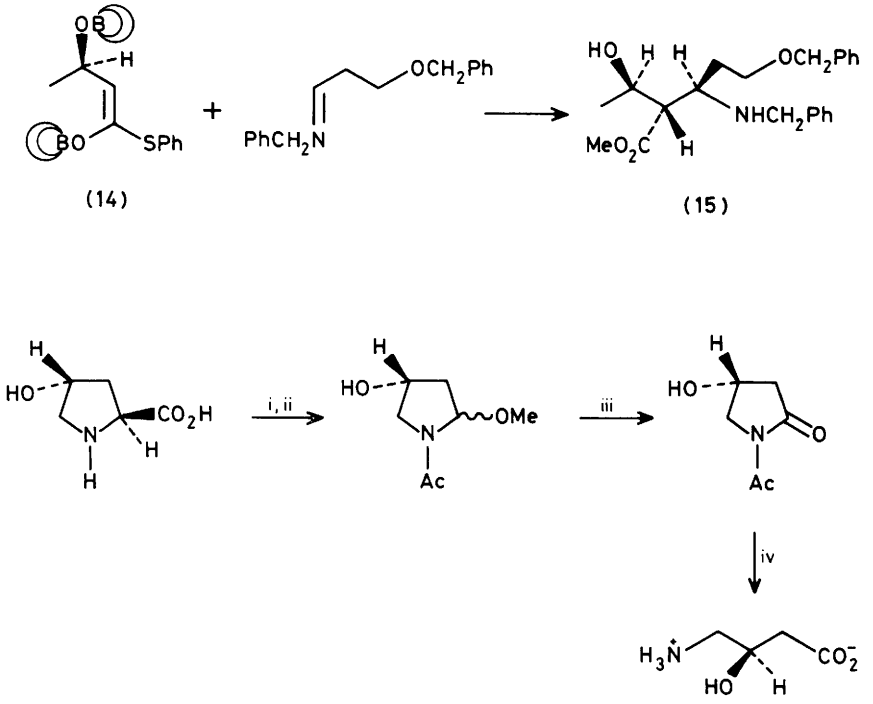
Conventional experiments, repeating the earliest laboratory demonstrations, have been described for photolysis of CH_4 with HCN, CO_2, and other simple compounds;[92] of HCHO with aqueous $K_4Fe(CN)_6$;[93] and electric discharge studies with CH_4, N_2, H_2O, NH_4^+ and metal salts;[94] and similar mixtures also including PH_3.[95] Amino acids are formed in all these cases.

4.5 Synthesis of Protein Amino Acids and Other Naturally Occurring α-Amino Acids.- As in previous Volumes, there is insufficient space here for the ever more voluminous literature concerning enzymic synthesis of protein amino acids. This important area can only be acknowledged through representative citations here, but it is well served with reviews[96] and is accessible through Section 16 (Fermentation and Bio-industrial Chemistry) of Chemical Abstracts.

Selected papers[97,98] and a compendium[99] describe the use of immobilized cells of Alcaligenes metalcaligenes for the synthesis of L-aspartic acid from ammonium fumarate;[97] mixed enzymes (serine hydroxymethyltransferase with β-tyrosinase) for the synthesis of L-tyrosine from glycine and phenol;[98] and individual treatment of the microbiological production of each of the protein amino acids.[99]

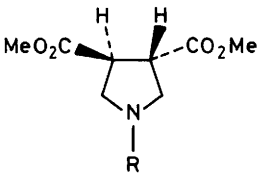
Several of the papers discussed in other sections (synthesis and reactions of amino acids) lead incidentally to the synthesis of natural amino acids, and a full appraisal of syntheses achieved should take in these other Sections.

Simple aliphatic α-amino acids that have received attention are (S)-2-cyclopropylalanine, a constituent of the mushroom Amanita virgineoides (synthesis from L-allylglycine);[100]



Reagents: i, e, MeOH, carrier electrolyte; ii, Ac_2O; iii, AcO_2H; iv, 4M–HCl

Scheme 2



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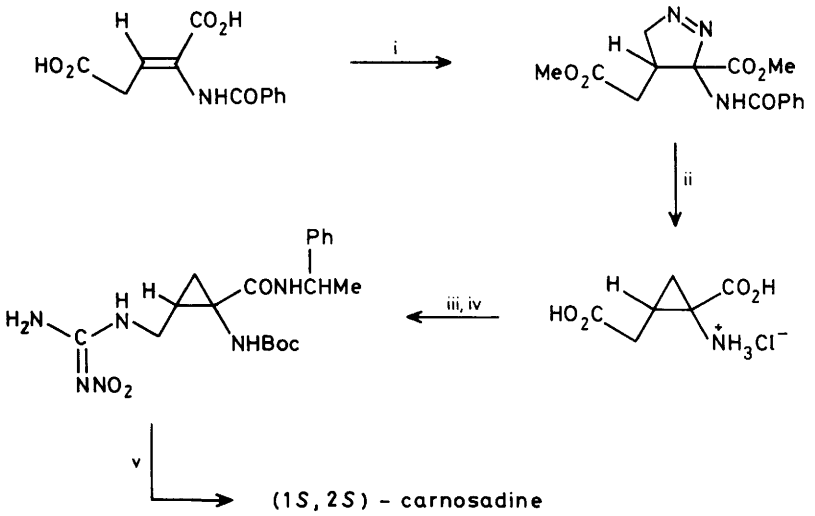
1-aminocyclopropanecarboxylic acid (through the use of methyl 4-bromo-2-phthalimidobutyrate, a compound more generally useful in synthesis as shown by syntheses of DL-phosphinothricin and DL-2-amino-4-phosphonobutyric acid);[101] and carnosadine (1-amino-2-guanidinomethylcyclopropane-1-carboxylic acid) from Z-(N-benzoyl)-αβ-dehydroglutamic acid (Scheme 3).[102] Numerous studies, mainly biosynthetic as far as chemical interest is concerned, have continued to appear for 1-aminocyclopropanecarboxylic acid, including an interesting proof that synthesis from S-adenosyl-[4-$^2H_2$]-L-methionine through the use of 1-aminocyclopropanecarboxylic acid synthase involves inversion of configuration at C-2.[103] In an extension of this project, in which ^{2}H-n.m.r. played a key role, a 1:1 mixture of (3S,4R)-[3,4-$^2H_2$]-(2S)-adenosylmethionine and its (3R,4R) isomer was converted into a 1:1 mixture of the two meso isomers of 1-aminocyclopropanecarboxylic acid labelled by ^{2}H. This is consistent with the inversion of configuration at C-4 that implies direct nucleophilic displacement of the sulphonium grouping.[104]

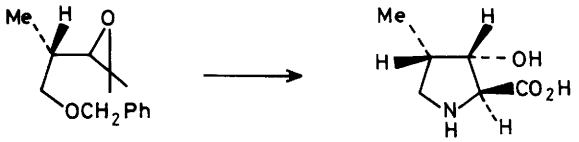
Proline and its analogues feature prominently in the recent literature, with syntheses[105,143] of some amino acids (17)-(19) of the echinocandins employing largely conventional routes from starting materials shown; synthesis of (2S,4S)-4-phenylproline, notable for the retention of configuration observed in displacement of the corresponding 4-tosyloxyproline with lithium diphenylcuprate;[106] and a general synthesis employing 1,3-dipolar cycloaddition of an N-alkyl thiazolium salt (20) to an αβ-unsaturated ester leading to 4-ethoxycarbonylprolines.[107] Acromelic acid A (21), the toxic principle of the poisonous mushroom Clitocybe acromelalga, has been synthesised from L-α-kainic acid (Scheme 4).[108] The allo isomer accompanying kainic acid (opposite configuration at the isopropenyl-substituted carbon atom) as neuroexcitatory amino acids in the alga Diginea simplex Ag., has been synthesised through the dipolar cycloaddition to an azomethine ylide that has become a favoured strategy in this area of stereoselective synthesis (Scheme 5).[109] Hydroxylated prolines (22) and analogous pipecolic acids have been synthesized enantiospecifically from D-ribonolactone[110] and from D-glucuronolactone,[111] respectively. In the former case, introduction of the azide grouping at C-2 of D-ribonolactone occurs with retention of configuration, surprisingly, and routine elaboration of the resulting compound gives the D-proline derivative (2R,3S,4R)-dihydroxyproline. A similar strategy leads to (2S,3R,4R,5S)-trihydroxypipecolic acid and its (2R) epimer and bulgecinine [alias (2S,4S,5R)-4-hydroxy-5-hydroxymethylproline].[111]

Highly stereoselective syntheses have been described for L-saccharopine, (2S,5'S)-N^{ϵ}-(1,3-dicarboxypropyl)lysine, and related N-carboxyalkylamino acids ('opines'; see Vol. 18, p. 1) through aminolysis of triflates of chiral hydroxy-acids.[112,45] Other simple aliphatic amino acids synthesised recently include L-canaline (O-amino-L-homoserine)[113] and polyoxamic acid (22; from the threose derivative (23) through Overman - Claisen rearrangement of the derived



Reagents : i, CH_2N_2, MeOH; ii, hν; 6M – HCl; iii, MeOH, H^+; Boc_2O; NH_3; Br_2/NaOH; iv, ZCl; (*R*)-(+)-$PhCHMeNH_2$; DCCI; H_2 – Pt; 3,5-dimethyl-1-nitroguanyl-pyrazole; v, H_2 – Pd; 6M-HCl

Scheme 3



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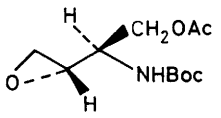

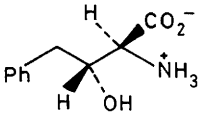

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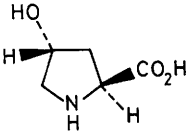

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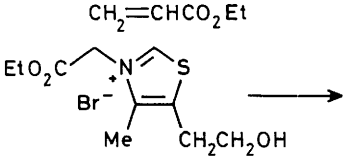

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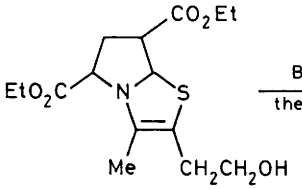
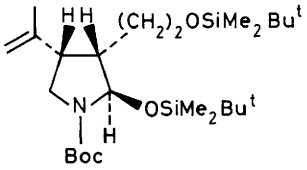
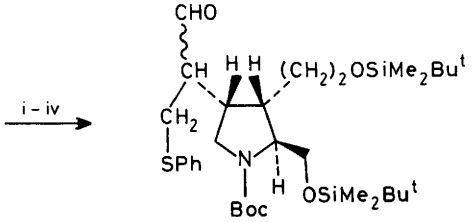
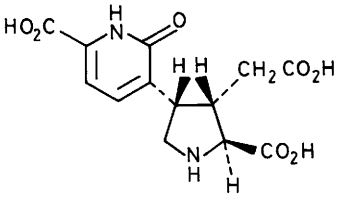


prolines



from L-α-kainic acid by $LiAlH_4$ reduction of *N*-Boc dimethyl ester and silylation





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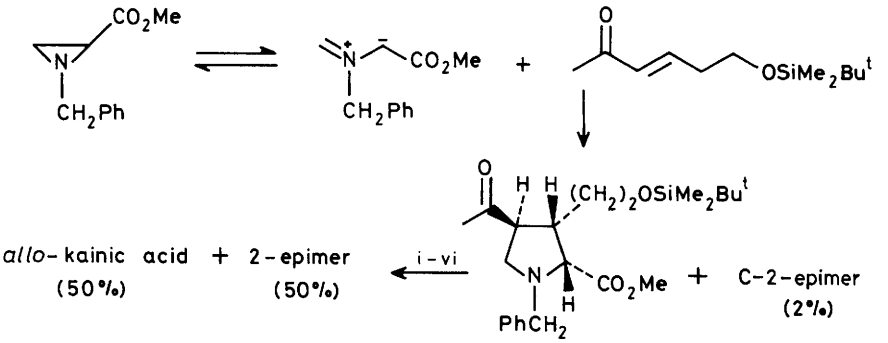
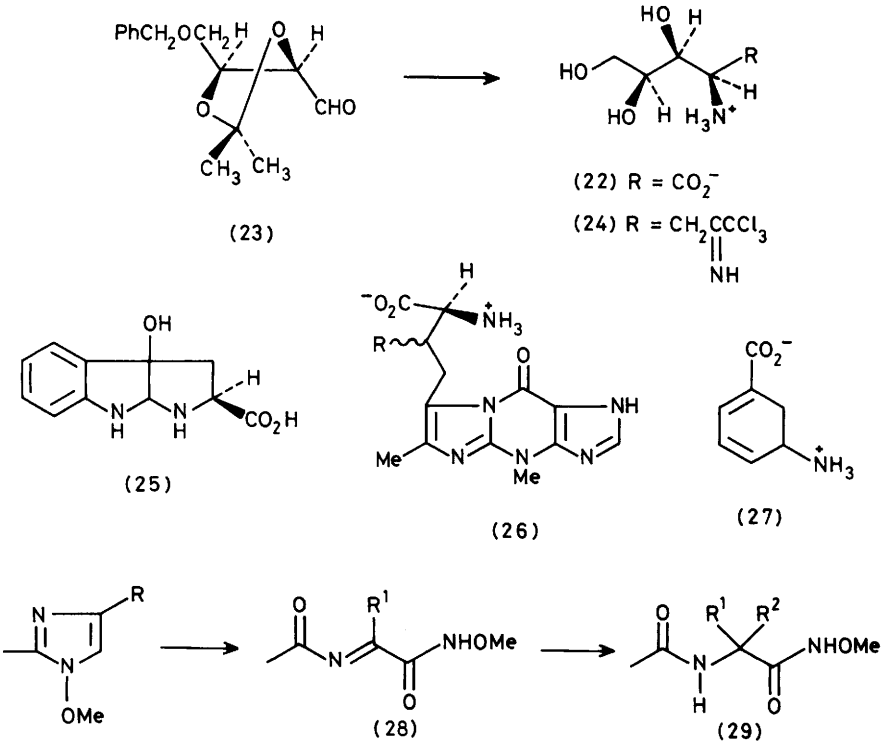
Reagents: i, *m*-chloroperbenzoic acid; ii, Li tetramethylpiperidide; iii, MnO_2; iv, PhSH; v, construction of 3-(*o*-picolyl) ring; vi, oxidation of desilylated intermediate to the tricarboxylic acid; vii, rearrangement of the pyridine *N*-oxide; viii, Boc removal

Scheme 4



Reagents: i, PPh_3CH_2; ii, F^-; iii, CrO_3-acetone; iv, CH_2N_2; v, $ClCOOCHClCH_3$; vi, NaOH, for C-2-epimerization

Scheme 5



imine (24)).[114]

Aromatic and heterocyclic amino acids featured in the recent literature include tryptathionine, formed between cysteine and the pyrrolo[2,3-b]indole (25);[115] quisqualic acid, synthesized from β-chloroalanine[116] (see also Vol. 18, p.16); and the extraordinary wybutine (26; R = H) a fluorescent minor base from yeast tRNA, whose oxygenated derivative (R = OH or R = COOH) has been located in animal and plant sources. Disagreement has arisen over structural details obtained with minute amounts of the materials, and synthesis of (2S,3S)-β-hydroxywybutine and its 2S,3R isomer has identified one or other of these as 'most likely' structure.[117] The natural GABA-T inhibitor gabaculine (Streptomyces toyocaensis) is now available through a fifth synthesis that is conceptually different from its predecessors, all of which have been based on the functionalization of a cyclohexenecarboxylic acid. 5-Ethoxypyrrolid-2-one was N-silylated and its 3-phenylsulphenyl derivative was alkylated with 5-iodo-1-trimethylsilylpent-2-yne. Desilylation in HCOOH was accompanied by ring closure to a 7-azabicyclo[3.2.1]oct-2-ene from which gabaculine was secured through straightforward elaboration,[118] as the racemate (27).

4.6 Synthesis of α-Alkyl Analogues of Protein Amino Acids.- Reference is made elsewhere in this Chapter to the title compounds (e.g. refs 47, 53). Acylimines are a novel source,[119] adding organometallic compounds to give αα-disubstituted N-acylglycinamides in good yields when the amide function is methoxylated (28)→(29). The starting materials are formed by singlet oxygenation of corresponding imidazoles.[119]

4.7 Synthesis of Other Aliphatic Amino Acids.- Later Sections deal with side-chain functionalized amino acids, and this Section discusses close relatives of protein amino acids.

Side-chain extension of protected γ-iodobutyrine (prepared from homoserine) through nucleophilic displacement by a lithium dialkyl cuprate offers a general entry to long-chain homologues.[120] A different carbon-carbon bond-forming strategy, used for the synthesis of (1R, 2S)-2-methyl-1-aminocyclopropane-1-carboxylic acid,[121] was the outcome of consideration of other established methods for this type of amino acid. It was concluded that condensation of 1,2-dibromopropane with ethyl isocyanoacetate was the method of choice in this case.

Friedel-Crafts acylation of benzene by ethyl N-methoxycarbonyl-L-aspartate through the side-chain carboxy group leads to (S)-phenacylglycine derivatives.[122]

Several proline analogues have been synthesized through demonstrations in a crop of papers of the potential of new methods for this category. 5,5-Dichloro-L-pyrrolidinecarboxylic esters formed from corresponding L-pyrrolines as N-chlorocarbonyl derivatives by reaction with $COCl_2$ can be converted into proline esters in 78% overall yield by dehydrochlorination followed by hydrogenation.[123] cis-5-Alkyl-[124] and trans-4-cyclohexyl-prolines have been

synthesized from N-benzyloxycarbonyl-L-glutamic acid and from L-pyroglutamic acid, respectively, deriving their absolute configurations from that of the starting materials. Ring closures, in the former case to the oxazolidinone (30), which on ammonolysis undergoes ring opening and reclosure to the prolinamide, and in the latter case to the bicyclic derivative (31), which is formed from benzaldehyde and the hydroxymethylpyrrolidone derived from L-pyroglutamic acid, are crucial to each route.[125] The chiral lithium enolate from (31) is alkylated by cyclohexyl bromide and elaborated through conventional methods into the L-proline analogue. Photocyclization of secondary amines $PhCOCHR^1CH_2NRCHR^2CO_2R^3$ yields mixtures of cyclopropanones and 3-hydroxyprolines.[126]

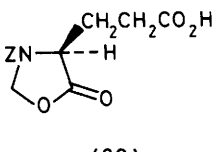
Uses of readily available L-amino acids for the synthesis of elusive analogues continue to be well represented, a further illustration of the versatility of L-glutamic acid being its use in the synthesis of (S)-$H_3\overset{+}{N}CH_2CH(OH)CH_2CH_2CO_2H$ via (S)-5-carboxybutyrolactone (elaboration of the carboxy group into CH_2NH_2).[127] Other aliphatic amino acids with well separated functional groups include 2,3-diaminopropanoic acid, prepared as its N^2-Boc-N^3-benzyl ester through addition of benzylamine to Boc-dehydroalanine methyl ester,[128] and unsaturated $\alpha\varepsilon$-diaminopimelic acids carrying γ-methyl, methylene, or chloro substituent.[129] At the other end of the scale, the aminoglycines, e.g. $BocNHCH(NHZ)CO_2H$, can be prepared from Z-hydroxyglycines (i.e. ZNH_2+ glyoxylic acid) through conversion into the sulphide with Pr^iSH and reaction with $BocNH_2$ and $HgCl_2$.[130]

4.8 Synthesis of Halogenoalkyl Amino Acids.- An enzyme-catalyzed route is to be seen as unusual in this area, and conversion of halogenofumaric acids into β-halogenoaspartic acids through β-methylaspartase-mediated addition of NH_3 is a valuable entry to (2S,3R) isomers.[131]

The usual method for introducing a halogen substituent into an amino acid, remembering the relatively easily modified incumbent functional groups, is through side-chain unsaturation via hydrogen halide addition to a derived oxirane. This approach has been used in a synthesis of (2S,3R,4R)-4-chloro-3-hydroxyproline from the protected 3,4-dehydroproline,[132] and for a synthesis of 4-fluoro-L-threonine.[133] In this latter case, stereospecific introduction of the halogen substituent into a chiral oxirane derived from benzyl 4-hydroxybut-2-enyl ether was followed by construction of the amino acid through standard general methods.[133]

Separate routes to threo and allo isomers of $\gamma\gamma\gamma$-trifluorothreonines have been reported, reduction of $CF_3COC(=NOMe)CO_2Et$ and hydrolysis giving the allo isomer, and hydrolysis of 4-trifluoromethyloxazolin-2-one-5-carboxylic acid giving the threo isomer.[133] $\beta\beta\beta$-Trifluoroalanine has been prepared by azidolysis of $FCOCH(CF_3)CO_2Me$ and hydrolysis of the resulting isocyanate.[134]

4.9 Synthesis of Amino Acids with Unsaturated Side-Chains.- A general amino acid



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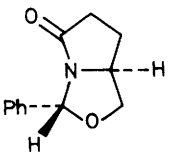

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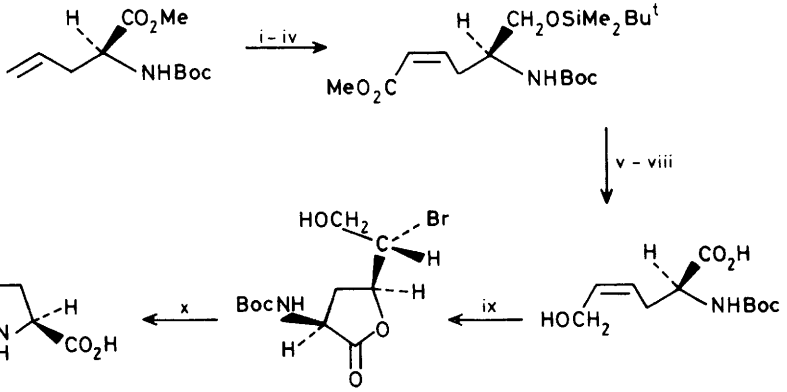
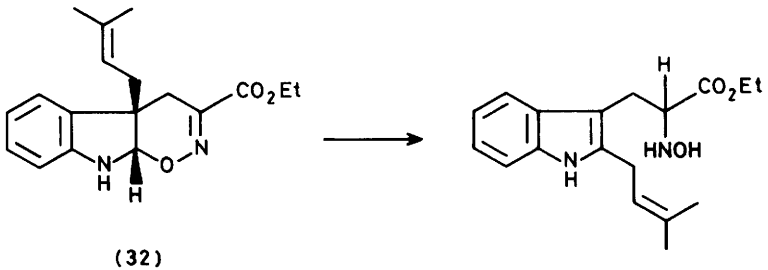


Reagents: i, $LiAlH_4$;ii, TBDMSCl ; iii, O_3 ; iv, Wittig synthesis; v, DIBAL reduction ; vi, Ac_2O ; vii, oxidation; viii, 0.5 M-NaOH; ix, NBS ; x, deprotection

Scheme 6



(32)

(33)

synthesis employing a cationic glycine synthon, $ZNHCHClCO_2Me$, has been applied to a synthesis of vinylglycine and other $\beta\gamma$-unsaturated amino acids,[135] through condensation with organomagnesium reagents. Differently based routes include alkylation of diethyl acetamidomalonate with $Me_3SiC{\equiv}CSO_2Ph$ followed by sulphone cleavage, a method that permits stereospecific labelling at each vinyl proton,[136] and an alternative (seeVol.17,p.13) route to optically pure L-vinylglycine from L-glutamic acid.[137] The latter method involves successive Hunsdiecker reaction ($CO_2H \rightarrow Br$) and conversion into the 2-pyridyl selenide, followed by oxidative elimination (O_3).[137] Another acetamidomalonate route leads to β-methyleneglutamic acid through alkylation by the allene $H_2C{=}C{=}CHCO_2Et$;[33] yet another leads to 2-amino-4-hexynoic acid.[38]

Allylglycines $R^2SO_2NHCH(CO_2Bu)CHR^2CR^3{=}CHR^4$ are formed from an N-sulphinylsulphonamide RSO_2NSO and glyoxylate ester through an ene reaction with an alkene.[138]

2-Aminocrotonates ('dehydro-amino acids') have received attention over many years, and the novel eliminative route from $CF_3CON(SiMe_3)CH(CO_2Me)CHRSiMe_3$ should offer entry to new examples of this class carrying sensitive side-chains.[139] The synthesis of 'dehydroprolines' has been reviewed.[140]

Higher homologous unsaturated amino acids, like their saturated analogues, are generally prepared through standard methods for the introduction of either amine or carboxy group into the otherwise complete structure. Gabriel synthesis of $PhtCH_2C{\equiv}CCO_2H$ and partial reduction and deblocking ($EtNH_2$) gives (Z)-$H_3\overset{+}{N}CH_2CH{=}CHCO_2H$;[141] Schmidt rearrangement of (E)-$(HO_2CCH_2CH{=})_2$ yields (E)-5-aminopent-3-enoic acid;[142] the double bond can be moved into conjugation with E/Z isomerization through the use of a strong base. Corresponding alkynes have been formed through the same amination approach.[142]

4.10 Synthesis of Hydroxyalkyl Amino Acids.- Ohfune's group continues to provide elegant syntheses of uncommon amino acids (see also ref.105), especially hydroxylated derivatives, and the 'halolactonization' approach by which (S)-allylglycine is converted into (19) has been used with other alkenyl amino acids.[143] (-)-Bulgecinine has been provided with another synthesis using this approach (Scheme 6), and the general nature of this route was illustrated for several β-hydroxy-α-amino acids from 2-amino-4-pentenoic acid derivatives.[143] The same systems can be prepared through condensation of aldehydes or ketones with [N,N-bis(trimethylsilyl)-amino]ketene bis(trimethylsilyl)acetal, a method that avoids the use of a strong base.[144] The key intermediate $(Me_3Si)_2NCH{=}C(OSiMe_3)_2$ is readily available by reaction of glycine with Me_3SiNEt_2 followed by conversion into the lithium enolate and silylation (Me_3SiCl).

4.11 Synthesis of Amino Acids with Aromatic or Heteroaromatic Side-Chains.- Syntheses from familiar (azlactone)[145] and novel [$CH_2{=}C(N{=}O)CO_2Et$][146] alanine derivatives have been

described for 2-fluoro-L-histidine and 2-substituted tryptophans, respectively. Rearrangement is observed in the ring-opening of the 2-(dimethylallyl)indole - nitrosoalkene adduct (32) to give the N-hydroxy tryptophan homologue (33), after reduction of the precursor oxime using trimethylaminoborane and HCl in ethanol.[146]

Modification of the side-chains of readily available amino acids (O-phenylation of N-acetyl-L-tyrosine methyl ester [NaH and C_6H_6-$Mn(CO)_3$],[147] iodination of p-trimethylsilylphenylalanine with I_2/Ag^+ to give p-iodophenylalanine,[148] and radical halogenation of protected tryptophans to give 2-halogenation products[149]) continues a long series of papers over the years in the same vein.

4.12 Synthesis of N-Substituted Amino Acids.- The problems of synthesis of simple N-alkyl amino acids from the amino acids themselves have been largely overcome in recent years, and a synthesis of $N^{\epsilon}N^{\epsilon}N^{\epsilon}$-trimethyllysine from a suitably protected starting material is illustrative of the general approach.[150]

4.13 Synthesis of Amino Acids containing Sulphur or Selenium.- General methods have been applied to the synthesis of ββ-dialkyl cysteines through reaction of P_4S_{10} with an N-formyl dehydroamino acid ester[43] and preparation of selenocysteines through alkylation of a Schiff base of methyl glycinate with bromomethyl selenides.[40]

Other papers under this heading describe modifications of amino acids in straightforward ways. 5-Thioxoproline can be prepared from pyroglutamic acid through reaction with Lawesson's reagent.[151] Cysteine is the starting material for the synthesis of bis(S-cysteinyl)selenide using selinite anion as reagent,[152] and homocysteine for the preparation of S-adenosylhomocysteine through reaction of the derived N-TFA disulphide methyl ester with adenosine and Bu_3P;[153] and of S-(N^6N^6-dimethyladenosyl)-L-methionine from 6-chloro-9-(β-D-ribofuranosyl)purine by reaction with Me_2NH to give 5'-chloro-5'deoxy N^6N^6-dimethyladenosine, the synthesis being completed by methylation of the resulting homocysteine analogue.[154] DL-5,5-Dimethyl-4-thiazolidinecarboxylic acid has been prepared from DL-penicillamine and formaldehyde.[155]

4.14 Amino Acids Synthesized for the First Time.- The following, additional to other new amino acids named elsewhere in this Chapter, have been prepared through routine methods: 2-amino-4-alkenoic acids;[41] cyclopropylglycines;[41] (2S,9S)-2-amino-8-oxo-9,10-epoxydecanoic acid;[55] 1-adamantylglycines and 2-adamantylalanines;[44] 1,3-bis(2-glycinyl)adamantanes;[44] (2R,5R)-5-hydroxymethylproline;[37] DL-homolysine;[35] 1-amidinylpyro-L-glutamic acid;[156] p-benzoyl-L-phenylalanine;[157] 3'-carboxy-D-phenylalanine, and its 4'-methyl- and 4'-hydroxy analogues;[158] 4'-methoxy-3'-formylphenylalanine and its oxime;[158] and 2'-, 5'- or 6'-fluorodopas.[36]

4.15 Synthesis of Labelled Amino Acids.- Many of the methods described here are standard general methods, but others illustrate novel solutions to problems of synthesis of selectively labelled amino acids. As in previous Volumes, the coverage is in a sequence of increasing atomic number of the labelled atom.

Addition of $^2H_2$ to 2-cyanoethyl acetamidomalonic acid diethyl ester gives [5,5-$^2H_2$]-DL-ornithine after conventional elaboration.[159] Catalyzed halogen exchange between $^2H_2$ and N-acetyl-4-chloro- and -iodo-L-phenylalaninamides competes unfavourably with exchange with ^1H (from H_2O) and scrambling is also observed.[160] Catalyzed β-deuteriation of GABA and homoserine requiring pyridoxal and its 5'-phosphate[161] and direct substitution of the halogen atom in N-benzoyl-3-chloro-L-valine methyl ester by ^2H[162] are processes that do not suffer side-reactions. NaB^2H_4 is the source of the label in Boc-[4,4-$^2H_2$]-L-proline, prepared from hydroxy-L-proline via the oxo analogue (RuO_4 - $NaIO_4$) through[4-^2H]-hydroxy-L-proline and the iodo analogue (Ph_3P/diethyl azodicarboxylate/MeI).[163] Chiral ^2H-glycine features in an exchange study involving a chiral cobalt(II) N-2-picolinylglycine complex and ^2H_2O[164] and also results from L-glutamic acid subjected to enzymic and chemical degradation in the presence of ^2H_2O.[165]

Various ^2H, ^{13}C, ^{15}N, and ^{18}O isotopomers of L-tyrosine have been prepared from the correspondingly labelled phenol and L-serine through the use of β-tyrosinase,[166] and the L-phenylalanines derived from them by chemical degradation have also been described.

Processes analogous to some of those described above have led to ^3H-labelled amino acids, namely addition of $^3H_2$ to L-2-amino-4-hexynoic acid,[38] to a protected dehydro-2-fluoro-histidine,[145] and a protected dehydro-3-(2-naphthyl)alanine.[167] ^3H - Halogen exchange has been employed for the preparation of [4'-^3H]-DL-phenylalanine from p-chlorophenylalanine,[168] [2,5-$^3H_2$]-L-histidine from 2,5-di-iodo-L-histidine,[169] and [4-^3H]-L-glutamic acid.[170] Pd-Catalyzed exchange of $^3H_2$ was used in these studies,[168,169] and NaB^3H_4 was employed in the case of labelled glutamic acid, where curiously only the (2S,4S)-4-halogenoglutamic acid (as its dimethyl ester) underwent satisfactory ^3H - halogen exchange.[170] The diastereoisomer was obtained, however, by the alternative enzymatic exchange method.[170]

^{13}C-Formaldehyde has been extended to $EtO_2CCH_2{}^{13}CHO$ via 1,3-dithian and used in the Strecker synthesis giving the labelled aspartic acid.[171] The [1,4-$^{13}C_2$]-L-aspartic acid isotopomer is obtainable through aspartase-catalyzed addition of ammonia to [1,4-$^{13}C_2$]-fumaric acid[172] and the [4-^{13}C]-L-aspartic acid from an acetamidomalonate synthesis.[172] Acylase mediates the conversion of the intermediate cyanomethyl derivative into β-[^{13}C-cyano]-L-alanine in this route.[172] The amidocarbonylation synthesis of amino acids has been used only rarely in syntheses of labelled compounds, a recent example being [1-^{13}C]-L-isoleucine;[173] cobalt(II) acetate-catalyzed condensation of acetamide, (RS)-EtCHMeCHO, and ^{13}CO in the presence of hydrogen is followed by resolution of the resulting N-acetylamino acid using

hog renal acylase.[173] o-[Carboxy-^{13}C]-phenylalanine has been prepared from o-bromotoluene and $^{13}CO_2$, followed by conversion of the resulting labelled o-toluic acid into the o-[carboxy-^{13}C]benzyl bromide and use in the acetamidomalonate synthesis.[174]

^{11}C-Labelled amino acids are worthwhile targets only if rapid synthesis and use in metabolic and transport studies is ensured, because of the short half-life of this isotope. A 30 minute synthesis of isocyanoacetic acid ($CNCH_2Li$ + $^{11}CO_2$) has been reported,[175] opening up some options for use in synthesis of [1-^{11}C]amino acids. S-Adenosyl-[^{11}C]-L-methionine has been prepared from the labelled methionine and rat liver extract.[176] The general field of [^{11}C]-amino acid synthesis has been reviewed.[177]

Purification of [^{15}N]-amino acids, prepared through standard procedures from $^{15}NH_3$, by anion-exchange preparative chromatography has been achieved.[178]

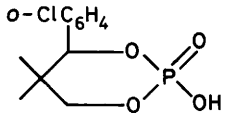
A number of papers on 6-[^{18}F]-dopa synthesis (and its use for tracing dopamine metabolism in the brain by positron tomography[179]) has appeared, employing $^{18}F_2$ with 6-trimethylsilyl-3,4-dimethyldopa ethyl ester as its N-salicylidene derivative[180] or direct reaction of the amino acid with $AcO^{18}F$ (less than 8% yield)[181] or with $HB^{18}F_4$ (high yield).[182] Introduction of ^{18}F into 5-amino-3,4-dimethoxybenzaldehyde through the Schiemann reaction, followed by the elaboration of the aldehyde group into the alanyl moiety through standard methods, provides a satisfactory route to 5-[^{18}F]-dopa.[183] Direct fluorination of phenylalanine with $^{18}F_2$ gives a mixture of o-, m- and p-^{18}F derivatives.[184]

Incorporation of ^{73}Se from $^{73}SeO_2$ into selenomethionine can be accomplished by *Saccharomyces cerevisiae* and *E.coli*.[185]

4.16 Resolution of DL-Amino Acids.- While this Section is at least as well endowed with useful recent literature as in previous Volumes of this Report, and therefore surprisingly lengthy for such a well researched topic, there are also papers discussed elsewhere in this Chapter on the analytical resolution of amino acids.

Chemical and physical techniques to achieve the separation of enantiomers continue to be based on familiar principles. Adduct formation of DL-amino acids with L-phenylalanine is strongly enantioselective in a number of cases, permitting D-amino acids to be crystallized out in 75 - 100% optical purity.[186] More reliable general methods have been used for the resolution of DL-*threo*-β-hydroxyvaline and its DL-*erythro* isomer via L-tyrosinehydrazide salt formation,[187] of *cis*-3-ethyl-DL-proline and its DL-*trans* isomer by (+)-dibenzoyltartaric acid salt formation,[39] of tert-leucine by (+)-camphor-10-sulphonic acid,[188] and of homomethionine through salt formation with the chiral phosphoric acid (34).[189] Derivative formation between DL-amino acids and (35) is followed by separation of the resulting diastereoisomers and removal of the chiral 'handle' with $NaBH_4$, in an efficient resolution method.[190]

Preferential crystallization resolution of N-acetyl-DL-phenylglycine (as its NH_4^+ salt)[191]



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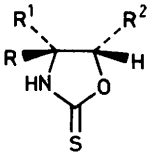

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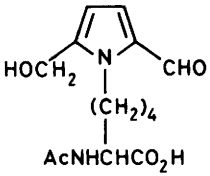

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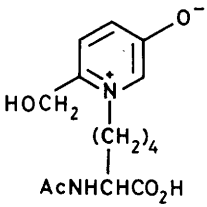

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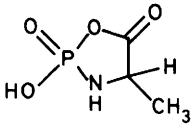

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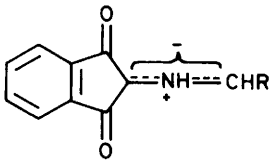

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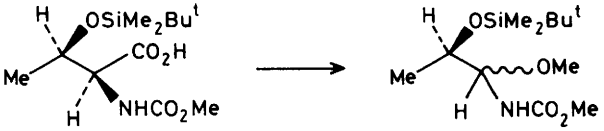

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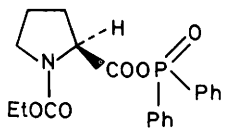

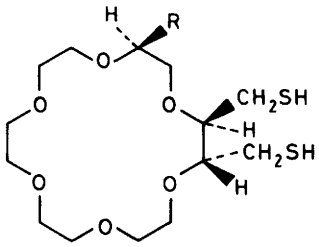

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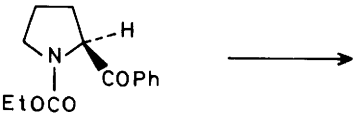


(42)

and N-acetyl-DL-phenylalanine (as its pentylammonium salt)[192] has been achieved. Again, any rational link between structure and tendency towards preferential crystallization still seems elusive.

Chromatographic and related methods are often useful since they are direct, and economical of time. Many of the analytical methods (Sections 7.1, 7.4) for determining enantiomer ratios can be scaled up for preparative separations, in principle. Another example of the use of the simplest chiral stationary phase, cellulose, has been reported: the preparative separation of enantiomers of N-(2,4-dinitrophenyl)amino acids.[193] Pirkle has developed an application of long-chain esters of N-(2-naphthyl)-L-valine attached to silica as stationary phase for the resolution of DL-amino acid 3,5-dinitrobenzamides[194] and the reverse approach, resolution of N-acyl-DL-amino acid esters on a chiral stationary phase prepared from N-(3,5-dinitrobenzoyl)-L-leucine or D-phenylglycine.[195] The thorough study is being rewarded with very substantial separation factors in some cases, and has more recently been extended to N-(2-naphthyl)alanine alanine esters.[196] A chiral stationary phase formed by immobilizing bovine serum albumin has been used in exploratory studies of resolution of N-acetyl-DL-amino acid derivatives.[197]

Other physical methods of resolution include a promising observation that macroporous acrylic polymers, when prepared so that the architecture of the polymer becomes chiral through what is described as a 'coulombic' influence by D-phenylalanine ethyl ester (or its L-isomer), show a modest resolution capability towards DL-phenylalanine ethyl ester when used in column chromatography.[198] L-Tryptophan is adsorbed more strongly than its D-isomer on silica coated with membranes formed by plasmolysis of (+)-camphor,[199] and a distantly related basis applies to the resolution of racemates over polystyrene-supported liquid membrane carrying a chiral crown ether,[200] and through electrophoresis in a chiral electrolyte.[201]

Uses of enzymes in resolution of DL-amino acids are: α-chymotrypsin (aromatic amino acid esters);[202] aminopeptidase from Pseudomonas putida (homomethioninamide);[189] and hog renal acylase (trifluoroalanine;[203] see also refs. 172,173). A review has appeared[204] dealing with uses of L-aminoacylases and N-acyl-L-lysine amidohydrolase in resolution of DL-amino acids. Microbial sources of enzymes such as these may themselves be used for the same purpose, an example being preferential degradation of L-isomers of N-acetyl-DL-tryptophan and N-benzoyl-DL-alanine (Nocardia restrictus) and of the D-isomer of N-acetyl-DL-tryptophan ethyl ester (Arthrobacter oxydans; the L-isomer is preferentially hydrolyzed by Nocardia corallina).[197]

Some enzymic methods involve the 'destruction' of one enantiomer to achieve resolution of a DL-amino acid (though none of the examples in the preceding paragraph are in this category). The differential degradation of enantiomers of an amino acid by radiation is a topic that has featured in the literature for many years, because of its relevance to the ascendancy of L-amino

acids in biological systems. Polarized electrons arising from ^{90}Sr - ^{90}Y β-decay cause some 10% greater degradation of solid D-alanine than of its L-analogue, as shown by e.s.r. spectral analysis of radical yield.[205] Results such as these have been claimed, and disputed, over the years; but persuasive attempts continue to be made to the effect that each member of a pair of enantiomers is of slightly different energy due to parity-violating weak neutral current perturbation of ground-state electronic energy.[206] The result of this, in energy content, is that the existence of L-amino acids is favoured to the extent of approximately 10^{-14} J mol^{-1},[206] too small an amount to have led to the present situation.[207] A new type of chiral interaction, expressed in the relative affinities for enantiomeric amino acids towards water[208] or the coupling of the magnetic moment induced by the chiral interaction with the Earth's magnetic field,[209] has been proposed.

5 Physical and Stereochemical Studies of Amino Acids

5.1 Crystal Structures of Amino Acids and Their Derivatives.- While there is a predominant 'fact-gathering' motive to many of the reported crystal structures of these compounds, there are examples of experimental verification of calculated molecular parameters, and of comparison of information with that obtained by solid-state n.m.r. spectrometry.

Amino acids for which more accurate data than those in the literature have been determined are L-leucine[210] and D-enantiomers of methionine and tyrosine.[211] L-Citrulline dihydrate,[212] salts formed between L-arginine and D-aspartic acid or D-glutamic acid,[213] DL-homocysteine thiolactone perchlorate,[214] and p-fluoro-DL-[2,3,5,6-$^2H_4$]phenylalanine[215] have also been studied, the last-mentioned example assisting the interpretation of the solid-state n.m.r. spectrum.

Amino acid derivatives subjected to X-ray analysis are N-monochloroacetylglycine and the αα-di-ethyl and -di-propyl homologues,[216] and various αα-dimethylglycine (alias α-aminoisobutyric acid) derivatives.[217] The parameters found for the proline and hydroxyproline moieties in their simple derivatives correlate well with those deduced from conformational energy calculations.[218] NN'-Bis(L-phenylalanyl) hydrazide[219] and dimethyl N-phthaloyl-4-bromo-DL-glutamate[220] and unsaturated derivatives (Z)-N-benzoyl-αβ-dehydroleucine[221] and (Z)-4-benzylidene-2-methyloxazolin-5-one[222] have been studied.

5.2 Nuclear Magnetic Resonance Spectrometry.- Conformational information has been inferred from n.m.r. studies of amino acid adenylate anhydrides of amino acids with hydrophobic side-chains.[223] At low pH in dilute solutions, the side-chain is shown to participate in the same type of intercalative interaction with the adenine ring that has already been established for aromatic side-chains. N-Acetyl t-butyl-DL-valinate has provided a surprising example of

discrimination of one enantiomer in favour of another, in studies of this racemate to which small additions of one enantiomer were made (i.e. $[D] \neq [L]$). It was found that CCl_4 solutions showed n.m.r. non-equivalence due to the formation of N-H ····· O=C hydrogen-bonded diastereoisomeric dimers.[224]

^1H-, ^{19}F-, and ^{31}P-N.m.r. has been used in estimations of enantiomeric purity for (S)-[2-^2H]-glycine as its (-)-N-camphanyl derivative;[67] of amino acids derivatized with Mosher's acid (2-trifluoromethyl-2-methoxyphenylacetic acid);[225] and methylthiophosphoric di-amides formed between an amino acid ester and $MeP(S)Cl_2$.[226] In the last-mentioned example the derivatives consist of a mixture of racemate with two meso isomers due to the chirality at both carbon and phosphorus centres, that show well resolved ^{31}P singlets which, on integration, give a direct measure of optical purity.[226]

^2H-N.m.r. nuclear relaxation rates for aqueous solutions over a wide pH range reveal details of molecular dynamics of [γ-2,2-$^2H_2$]aminobutyric acid, [2-^2H]-DL-glutamic acid, and [2-^2H]-DL-lysine.[227] A similarly targetted study[228] has been made of polycrystalline [4,4-$^2H_2$]-DL-proline and of [2,3,5,6-$^2H_4$]-p-fluoro-DL-phenylalanine,[215] assisted in the latter case with a detailed X-ray crystal structure determination.

^{13}C-N.m.r. study of L-histidine prepared in differently protonated forms from solutions of various pH illustrates further the increasing interest in the finer details of the solid-state behaviour of amino acids.[229] Homonuclear coupling is revealed by the magic-angle technique for solid-state glycine zwitterion labelled at both carbon atoms by ^{13}C that can be interpreted in terms of rotamer composition. Different crystalline forms of DL-, D-, and L-methionine are conformationally pure, but each contains a different conformation,[230] as yet undefined.

^{17}O-N.m.r. of solutions of L-alanine and L-proline in ^2H_2O - dimethyl sulphoxide provide unique information on the hydration states of the carboxy group.[232] Two water molecules are hydrogen-bonded in neutral and basic solutions, and a third is involved in acidic media. In dimethyl sulphoxide solutions the solute appears to exist in the dimeric form. ^{17}O-N.m.r. data have been reported for the labelled L-tyrosine isotopomers.[233]

5.3 Optical Rotatory Dispersion and Circular Dichroism.- While routine applications for assignments of absolute configuration continue to be excluded, even the two papers selected are based on well established principles. The c.d. of N-2,4-dinitrophenyl-L-amino acids carrying a polycyclic aromatic side-chain shows a negative Cotton effect centred at 400 nm,[234] in accordance with the rule linking chirality with sign of longest-wavelength Cotton effect. On this basis, the laevorotatory isomer of 3-(9-anthryl)alanine is assigned the D-configuration. An interesting example of the power of the technique is the discovery of induced Cotton effects by tryptophan, tyrosine and phenylalanine into gossypol.[235] Solutions at pH 7.6 show negative c.d. at 424-426 and 300 nm, and positive c.d. centred at 355 nm, indicating weak complex

formation between the aromatic moieties in the amino acids and the pigment.

5.4 Mass Spectrometry.- In contrast with the preceding Section, this is well supplied with innovative papers associated with the rapid progress in instrumentation. Again, routine mass spectrometric analysis is excluded, and mention of a study of the N-trifluoroacetyl derivative of n-butyl ^{2}H-leucinate to establish positions of the label[236] is made to give the reader an indication of the sort of material which, although worthy and useful, is based on established principles and supported by standard textbook coverage and not covered further here.

A curious observation has been published[237] to the effect that c.i.m.s. of enantiomers of an amino acid derivative can show characteristic differences when (+)-amyl alcohol is a constituent of the reagent gas. The implication that this might be exploited to permit mass spectrometric assignment of absolute configuration to an amino acid is sure to be followed up. The c.i.m.s. technique has been applied to N-2,4-dinitrophenylamino acid esters, leading to very simple negative-ion spectra in which the molecular ion is the base peak.[238]

Various fast-atom bombardment studies, mostly in the tandem mode, have been reported for amino acids. These provide improved spectra for problem cases (argininosuccinic acid and citrulline as their n-butyl esters,[239] L-carnitine [240]) but also give more insight into fragmentation processes more generally. Two isomeric immonium ions formed from N-isobutylglycine and N-methylvaline by f.a.b. can be distinguished by their collision-induced fragmentation characteristics and therefore mixtures containing them can be analyzed.[241] In terms of structure determination of an 'unknown' amino acid, the characteristic fragmentation patterns permit an unequivocal distinction to be made between C- and N-alkylated isomers.

Unimolecular reactions of metastable cluster ions formed by f.a.b. of amino acids have been interpreted to provide an order of relative proton affinities: Arg > His > Lys > Trp.[242]

5.5 Other Spectroscopic Studies.- The pattern for preceding Sections (exclusion of routine papers) continues here. Observations from readily accessible methods that appear to provide novel insights include reduction of the molar absorptivity of bilirubin by all common amino acids except arginine[243] and indications from calorimetric and infrared spectrometric studies that proline aggregation in aqueous solutions may account for a role for this amino acid in stabilizing biological macromolecules under reduced water stress (Vol.18, p.26).[244] An extraordinary technique in which molecules are cooled to near absolute zero in supersonic beams has been applied to tryptophan, so that it could be shown that two conformers exist under these conditions.[245] The conclusion was drawn from electronic spectra with the implication that there must be two gas-phase conformations of this amino acid of essentially identical energy.

Considerable progress is being made with Raman spectroscopic techniques, and the literature

on amino acids is duly reflecting this. Surface-enhanced Raman spectra of amino acids[246] adsorbed on silver have been interpreted to show that both amino and carboxy functional groups are points of adsorption. Ultraviolet excitation resonance Raman spectroscopy can provide finer details of structure - spectra relationships. An application to aromatic[247,248] and heteroaromatic[247,249] amino acids includes observations that nitration of tyrosine shifts the spectral features and abolishes its 200nm excited Raman spectrum, and that a similar effect is observed for the 218nm excitation feature of tryptophan as a result of (2-hydroxy-5-nitro)-benzylation.[247]

More convential Raman spectroscopic study has been incorporated with infrared and n.m.r. analysis of conformational equilibria for thiazolidine-4-carboxylic acid and its 2- and/or 5-methyl derivatives.[250]

5.6 Other Physical Studies.- Papers collected for this Section range from simple observations on purely physical phenomena to applications of physical methods other than spectroscopic for determination of data. In the former category, the observations usually relate to certain biological models, and include adsorption studies (intercalation of L-histidine, L-lysine, and L-arginine in γ-zirconium phosphate,[251] phenylalanine - cyclodextrin[252] and cyclomaltohexose[253] inclusion complexes) and radiation protection (N-acetylcysteine[254] and zinc aspartate[255]). The effect of tyrosine on reducing critical micellar concentrations of bile salts[256] and an effect of proline (see also ref. 244) in increasing the area occupied by membrane phospholipid molecules[257] have been reported.

A fascinating study[258] of transport of L-amino acids employs an oil layer between two aqueous layers, one of which contains a chiral detergent and the other contains the amino acids. As followed by changes in the electric potential across the aqueous layers, which varied with time in a pattern that is characteristic of the detergent chirality, the transport behaviour could be interpreted as a novel method to determine chirality. A related transport study through an ion-exchange membrane to which a potential gradient is applied has been modelled on the biological cell membrane system.[259]

Fundamental physical parameters have emerged from studies of N-acetyl-L-prolinamide and N-acetyl-N-methyl-L-alaninamide in binary mixtures, in terms of interpreting the excess free energy of the mixtures and their enthalpic virial coefficients to show interactions between solute molecules.[260] A related objective, the estimation of transfer free energies of amino acid side-chains from water to N-methylacetamide, similarly aims to extend knowledge on aspects of interactions that occur within proteins.[261] Simpler methods can be used to determine the hydrophobicity of amino acid side-chains, but vapour pressure measurements[262] may have only limited validity.[263] Dissociation constants of amino acids can be determined[264] by the ionophoretic technique; a simple interpretation of the variation of electrophoretic

mobility with pH of the electrolyte shows results in "fair agreement" with those of other methods. The related isotachophoretic indexes vary with pH and this has been interpreted to give absolute mobility values and pK_a values for 26 amino acids.[265] An equally simple method based on polarimetric measurements yields protonation microconstants for L-amino acids.[266]

The long-running interest in interactions between amino acids and DNA continues with interpretations of simple physical data on mixtures.[267]

The polarographic behaviour of L-pyrrolidine-2-carboxylic acid and its lead(II) and cadmium(II) complexes[268] and of DL-norleucine[269] has been described.

5.7 Molecular Orbital Calculations.- Continuing an interest covered at the end of the preceding Section, calculations have been presented[270] for the binding energies of amino acid side-chains to O-6 and N-7 of guanine and adenine . Allowed molecular conformations for Pirkle's chiral stationary phases (see refs. 194-196) have been computed for one specific case, viz. 3,5-dinitrobenzoyl-L-alaninamide linked to silica through the amide nitrogen atom with a $-(CH_2)_3-$ group.[271] Because force field parameters for aromatic nitro groups do not yet exist, these authors have based their computations on the 3,5-di-formylbenzoyl analogue, and have concluded that the chiral stationary phase presents chiral recognition through an anti conformation of the methine hydrogen at the chiral centre relative to the benzoyl carbonyl group.[271]

Support for spectroscopic studies is provided by calculations of lower electronic energy levels for the glycine zwitterion, which are in good agreement with the spacing implied by absorption spectra.[272]

6 Chemical Studies of Amino Acids

6.1 Racemization.- Kinetic studies for racemization of representative L-amino acids at 142°C in aqueous solutions of differing pH yield the 6 absolute rate constants that correspond with each ionic species for catalysis by H_3O^+ and OH^-.[273] The heterogeneous-catalyzed racemization of L-alanine (a strong base anion exchanger substituted with 5-sulphosalicylaldehyde as the Cu^{++} salt) has also been studied kinetically.[274]

The use of a racemase is still the province of very few groups, but the catalyzed 2-^{1}H - 2-^{2}H exchange by α-amino-ε-caprolactam racemase in ^2H_2O will be an attractive labelling method in certain cases.[275]

The exploitation of amino acid racemization for dating of relatively young fossil samples has given cause for concern due mainly to the uncertainties of effects, particularly catalytic, of the medium in which the fossil was located. A further problem, of course, is the chance that the amino acids being studied may have accumulated in the sample over time, from the

environment, and it has been pointed out[276] that there might be evidence on the absolute indigeneity of the amino acids arrived at by considering the proportions of the stable isotopes for carbon and nitrogen in the amino acids from the fossil, in relation to those of other amino acids collected at the fossil site. Major discordances have been reported between dates determined by aspartic acid racemization and those from accelerator mass spectrometric ^{14}C analysis, for bone samples,[277] and the sources of error in the amino acid dating method are concluded to be the uncertainties outlined above. Another factor that is perhaps unlikely to have arisen is the acceleration of the racemization of aspartic acid residues in eye lens protein that accompanies accelerated aging by X- or γ-irradiation.[278] Isoleucine epimerization has been used in dating of nine outcrop raised marine deposits through analysis of epimer ratios for sediment shells (Glycymeris violascens and Glycymeris glycymeris), leading to dates for three marine terraces of 120 000, 200 000, and 350 000 y that agree with estimates derived from ^{230}Th - ^{234}U decay.[279] Another way of using isoleucine racemization takes account of changes at both chiral centres.[280] The application referred to in the preceding sentence is based on epimer formation at the α-carbon, for which the half-life (the time taken to reach a D-alloisoleucine to L-isoleucine ratio of about 0.4) is between 10^5 - 10^7 y. The other stereoisomers result from β-epimerization, and rates for this process have been measured in H_2O at 250°C.[280] All four stereoisomers were found in ancient biogenic carbonates, so β-epimerization might be an index of value in the study of such samples.

6.2 General Reactions of Amino Acids.- This title is used to collect recent advances in reactions involving the amino and carboxy functional groups of amino acids.

A regular feature of this section is heterocyclic synthesis through the Maillard reaction between amino acids and carbohydrates and other reactions leading to heterocyclic ring formation. In the simplest combination in the Maillard reaction, between glycine and D-glucose, N-butylacetamide and N-butylformamide are formed as well as pyrroles (twelve such products as well as 5,6,7,8-tetrahydroindolizin-6-one and its 8-hydroxymethyl derivative are formed in the reaction of hydroxyproline and arabinose or erythrose[281]), suggesting that an early stage in the reaction creates dicarbonyl compounds.[282] There is some conflict between two reports, in one of which lysine is stated to react with glucose to yield the pyrrole (36),[34] while in the other the product is claimed to be the 1-alkyl-3-oxidopyridinium betaine (37).[283] Synthesis of (36) appears to have settled the point but further consideration may be called for, in view of the numerous products commonly found in such reactions. Fluorescent 1,4-dihydropyridines are formed in the Michael addition of malondialdehyde and a simple aldehyde with an amino acid,[284] and α-chymotrypsin catalyzes the formation of fluorescent products between amino acid amides and 1-methyl-3,4-dihydro-β-carboline-3-carboxylic acid methyl ester.[285] 2-Methoxy-2,4-diphenyl-3(2H)-furanone has been proposed[286] as a useful alternative reagent

for fluorescent derivative formation with amino acids (the reagent is closely similar to fluorescamine).

Well established heterocyclic syntheses are based on the reaction of both amino and carboxy groups, and a new example involves the condensation of alanine with sodium cyclotriphosphate at pH 10 -12, to give (38) as well as the phosphonamide $(HO)_2P(O)NHCHMeCO_2H$.[287] Curiously, β-alanine gives $(HO)_2P(O)OP(O)(OH)OP(O)(OH)NHCH_2CH_2CO_2H$ under these conditions. Oxazolin-5-ones form easily from N-acylamino acids and can be converted into α-acylaminoalkyl β-difluoroalkyl ketones[288] and benzamidoalkyl mono-, di-, and trifluoromethyl ketones[289] by a modified Dakin-West reaction with appropriate acyl chlorides or acid anhydrides. Oxazolinones may be intermediates in oxidative decarboxylation of N-acylglycine using KO_2[290] or lead tetra-acetate.[291] Yields of amides are modest in these reactions, the side-products in the latter case being the N-formyl and N-acetoxymethyl amides.[291]

2-Cyclohexen-1-one is a better catalyst than di-t-butyl peroxide for the decarboylation of amino acids;[292] 'peroxidizing methyl linoleate' brings about the standard Strecker degradation of amino acids,[293] a classic example of which (reaction with ninhydrin) is now shown to involve the 1,3-dipole (39).[294] The methaneiminium methylide $CH_2\overset{+}{=}NHCH_2^-$ (more correctly represented in a delocalized structure; cf. (39)) is formed by decarboxylative condensation of glycine with formaldehyde.[295] In both these studies, evidence for the assertions comes from trapping experiments using simple dipolarophiles.

Studies of the kinetics of oxidative degradation of amino acids by familiar oxidants continue to fascinate a number of research groups.[296] Metabolic oxidation, exemplified by a study using Raja erinacea liver mitochondria,[297] is a broader topic outside the scope of this Chapter.

Oxidation of amino acids with Fremy's salt converts amino acids successively into hydroxy- and keto-acids without oxidative decarboxylation[298] (a number of the other oxidants described in ref. 296 bring about the same conversion). α-Hydroxy acids are formed from amino acids by reaction with HNO_2 with high, but not complete, stereospecificity.[299,300] However, the useful chiral synthon (S)-3-hydroxybutyrolactone is available from L-aspartic acid through straightforward elaboration of (S)-malic acid that forms from the amino acid with HNO_2.[300]

Photolytic decarboxylation of N-acetyl-N-nitroso-amino acids is accompanied by N-N cleavage.[301] These facts needed unravelling since the eventual products are 1,2,4-oxadiazoles formed between the initially formed N-acetylimines and NO^-.

The final example of decarboxylation in this year's review is accomplished by electrolytic methoxylation, a useful entry to chiral amines since the resulting N,O-acetal (e.g. (40) from a protected L-threonine) undergoes $TiCl_4$-catalyzed substitution with high diastereoselectivity.[87] Scheme 2 uses the same decarboxylation process in an alternative synthesis.[86]

A distantly related asymmetric synthesis, the Rh(I)-chiral phosphine-catalyzed hydrogenation

of N-(α-ketoacyl)amino acid esters, is a useful route to chiral α-hydroxy acids.[302]

N-Substitution reactions of amino acids providing synthetically valuable derivatives have been reported: N-maleyl and N-dichloromaleylamino acids;[303] Boc- and Z-amino acids prepared using benzotriazol-1-yl carbonates,[304] and through the usual reagent for preparation of Boc derivatives (Boc_2O: reactions of ^{17}O-labelled amino acids with this reagent in MeOH or DMF occur without loss of the label);[305] and use of Fmoc N-succinimidoyl ester for the preparation of Fmoc-amino acids.[306] N-Benzyloxycarbonylamino acids and allyloxycarbonyl analogues can be converted into triethylallyloxycarbonyl analogues, through reaction with triethylsilane (not the N-triethylsilyl analogues as reported earlier), and, using t-butyldimethylsilane and 0.05 equiv. $Pd(OAc)_2$, into the t-butyldimethylsilyloxycarbonyl analogues.[307] Palladium-catalyzed hydrostannolysis by Bu_3SnH of allyl- and allyloxycarbonylamino acid derivatives offers a novel, very mild, deprotection method.[308]

A novel variation of the Bucherer reaction of amino acids enables a preparation of L-N-(2-naphthyl)amino acids to be conducted conveniently and on a sufficiently large scale for the development of chiral stationary phases from them.[309] The method is essentially a condensation reaction of the L-amino acid, 2-naphthol, and $NaHSO_3$ in aqueous media at pH 8.

Kinetic studies have been reported for the reaction of trinitrobenzenesulphonic acid with amino acids[310] and for the nitrosation of proline, cysteine, and sarcosine.[311] A corresponding study of the formation of N-acetyl-N^{in}-nitrosotryptophan also covers the kinetics of the de-nitrosation process.[312]

Direct esterification procedures based on the enhanced ambident reactivity of the amino acid zwitterion with an alkyl halide rely on the much higher proportion of zwitterion in DMSO in comparison with H_2O.[313] Isolated examples of simple esterification in this way in aqueous solutions[314] have appeared in the literature. The thoughtful article by Hughes, Bergam and Grabowski, though mainly concerned with spectroscopic determination of pK_a values of amino acids in DMSO, will stimulate further attempts to simplify direct esterification. Rates of esterification of 5'-adenosine monophosphate by N-acetylamino acids differ greatly for amino acids with A as the middle letter of their anticodons (Phe > Leu > Val > Ile) but not for others.[315]

Ester hydrolysis is represented in a comparison of uncatalyzed and Cu^{++}-promoted reactions of p-nitrophenyl glycinate.[316] The major crop of papers concerns enantioselective hydrolysis, catalyzed by chirally substituted polyethylene imines (benzoyloxycarbonyl-DL-phenylalanine p-nitrophenyl ester),[317] imidazole in chiral reversed micelles (a series of amino acid p-nitrophenyl esters),[318] and N-benzoyloxycarbonyl-L-Phe-L-His-L-Leu-OH (N-dodecanoyl-DL-phenylalanine p-nitrophenyl ester).[319] A comparison is made in the last-mentioned study with the diastereoselectivity of cleavage of N-benzyloxycarbonyl-DL-Pro-L-Pro-$OC_6H_4NO_2$ by a functional iodosobenzoate surfactant showing that both processes respond similarly to dimensions of the coaggregate micellar systems.[319] Chiral crown ethers (**41**) show modest chiral recognition

in thiolysis of DL-amino acid p-nitrophenyl esters.[320]

Simple exchange of anion has been described for amino acid benzyl ester toluene-p-sulphonates, leading to the corresponding hydrochlorides.[321]

Other manipulations of the carboxy function of amino acids have been reported for formation of Fmoc-amino acid chlorides from the acids using $SOCl_2$ in CH_2Cl_2 (and the use of 4-piperidylmethylamine for Fmoc cleavage),[322] formation of N-alkylhydroxamate esters by reaction with $ClCH{=}\overset{+}{N}Me_2\ Cl^-$ and N-methylmorpholine, followed by reaction of a hydroxylamine to displace the leaving group: $RCO_2H \rightarrow RCO_2CH{=}\overset{+}{N}Me_2\ Cl^- \rightarrow RCONR'OH$,[323] and the reduction of esters to aldehydes[324] and N-ethoxycarbonyl-L-proline to the phenylmethanol (42) by H^- - selectride reduction of the phenylketone derived (93% e.e.) by Grignard addition to the mixed anhydride.[325]

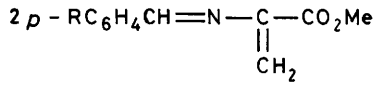
6.3 Specific Reactions of Amino Acids. Side-chain manipulations described here are also, incidentally, often useful methods for the conversion of one amino acid into another.

Aliphatic side-chains in N-benzoylamino acid methyl ester undergo slower H-abstraction with N-bromosuccinimide (a source of $Br^\cdot$ under irradiation in CCl_4) than glycine.[326] Ethylene formation from 1-aminocyclopropanecarboxylic acid can be accomplished *in vitro* through Mn^{2+} catalyzed degradation by H_2O_2[327] and through lipoxygenase-catalyzed oxidation.[328] In the latter study the atoms of the cyanide ion that results are C-1 and N-1.[328]

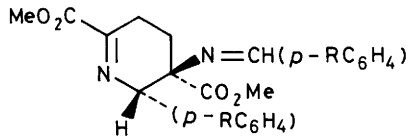
$\alpha\omega$-Aminodicarboxylic acids can be formed from cyclic α-amino acids through an improved RuO_4-oxidation, followed by hydrolysis of the resulting lactam.[329] The related formal dehydrogenation of proline under physiological conditions, giving pyrrol-1-ine-5-carboxylic acid, involves catalytic transfer of H^- via NADP(H).[330] Other unsaturated side-chain studies include unusual cyclodimerization of Schiff bases (43)→(44)[331] and selective reduction by DIBAL hydride + $BF_3.Et_2O$ of $\alpha\beta$-unsaturated γ-amino acid esters (readily available from α-amino acids) to give corresponding allylic alcohols.[332]

Pyrolysis of cysteine at 130 - 220° during 30 - 120 min in a sealed glass tube gives NH_3 and cystine as major identified products, while at 180° for longer periods thiophen, Et_2S_2, 4-ethyl-2-methylpyridine, di- and tetramethylthiolans, 1,2-dithiane, and N-ethylacetamide are formed.[333] The sulphur-containing amino acids yield SO_4^{--} through heating in water or HCl (the highest conversion occurring in 1M HCl), particularly readily for cysteine.[334] This surprising result, so long undiscovered, implies that analyses of sulphated proteins or glycoproteins may need correction of the sulphate figure to take account of contributions from these amino acids.

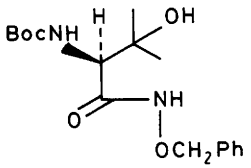
L-Lysine and L-ornithine bis (N-nitrososulphonamide)s undergo regioselective N-nitrososulphonamide - sulphonate rearrangement to give N-toluene-p-sulphonyl-L-proline and -pipecolic acids, respectively.[335] The near-relative amino acid, asparagine, undergoes adduct formation with $H^{13}CHO$ that enables ^{13}C-n.m.r. detection of free asparagine in potato.[336]

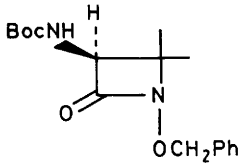

2 p - RC6H4CH=N—C—CO2Me
CH2

(43)


MeO2C
N=CH(p-RC6H4)
N
CO2Me
H
(p-RC6H4)

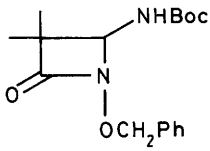
(44)


BocNH
H
OH
NH
O
OCH2Ph

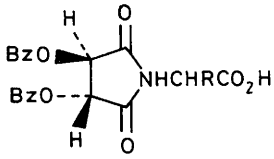

BocNH
H
N
O
OCH2Ph

(45)

+


NHBoc
N
O
OCH2Ph

(46)


H
O
BzO
NHCHRCO2H
BzO
H
O

(47)

A $^{15}N/^{14}N$ kinetic isotope effect has been established in the transamination of aspartic acid by α-ketoglutaric acid.[337] L-Serine is represented in preparation of its O-trimethylsilyl-N^{α}-trifluoroacetyl diethylamide,[338] in conversion into an oxetanone under Mitsunobo conditions[339] (see also Vol.18, p.32; these conditions also convert N^{α}-Boc β-hydroxy-L-valine O-benzyl hydroxamate into azetidinones (45) and (46)[340]). L-Serine has been used as a substrate for attack by HO• in H_2O at pH 3 - 7, leading to $\cdot CH(OH)CH(CO_2^-)NH_3^+$ and thence to NH_4^+ and $OCH\dot{C}HCO_2^-$.[341] In the last-mentioned study, corresponding products were found in reactions of HO• with threonine.

Migration of a 4-substituent in the aromatic moiety of [ring-$^2H_5$]-L-phenylalanine to position 3 or 5 (the NIH shift) occurs in vivo (species: man) in hydroxylation to L-tyrosine.[342] The redox condensation system consisting of an alkanol with Ph_3P - diethyl azodicarboxylate (the Mitsunobo reagent) brings about O-alkylation of tyrosine.[343] Iron(III) complexes catalyze the H_2O_2 oxidation of DOPA at pH 7.[344]

Substitution reactions of the indole moiety of tryptophan include a surprising N^{in}-acylation by aldehydes in aqueous media at pH 6.8, which can also lead to N^{in}-(1-hydroxyalkyl) adducts, but no reaction occurs with ketones.[345] The N^{in}-proton can also be substituted by the 3,4-dihydroxyphenyl group through reaction in acidic media with o-benzoquinone.[346] Indole C-hydroxylation can be accomplished in aqueous media with $FeSO_4$ and ascorbic acid;[347] the more extensive result usually observed with such 'Udenfriend reagents' (Fe^{++}/EDTA/ascorbic acid/O_2) is melanin formation, both from tryptophan and 'melanin-like' material from tyrosine.[348]

6.4 Non-Enzymic Models of Biochemical Processes Involving Amino Acids.- Spectroscopic evidence has been interpreted to derive binding constants for L-tryptophan to the Trp repressor of E.coli.[349] Disulphide bonds of proteins of lysosomes are more likely to undergo thiolysis by cysteine in vivo, rather than alternative thiols.[350]

These papers indicate a general rationale for this Section, but this Chapter contains several overlapping Sections so that certain topics that might have been located here have, instead, found an alternative relevant destination.

6.5 Effects of Electromagnetic Radiation on Amino Acids.- A group of papers dealing with relatively drastic treatment of aliphatic amino acids is a regular feature of this Section. This year this topic is represented by e.s.r. and ENDOR studies of X-irradiated single crystals of DL-proline hydrochloride (yielding radicals through α-deprotonation and ring cleavage),[351] X-ray-or γ-ray-accelerated racemization of aspartyl residues in proteins,[278] and autoradiolysis in solid D- and L-^{14}C-leucine resulting in β-radiation-induced decarboxylation.[352] E.s.r. analysis of the autodecomposition products reveals a 10% larger concentration of radicals in the D-leucine samples, a figure identical with that for D- and L-alanine irradiated with

polarized electrons produced during ^{90}Sr - ^{90}Y β-decay.[205]

Ultraviolet photolysis of N-acetylamino acids liberates free radicals through amide cleavage, and causes decarboxylation;[353] these substrates do not provide satisfactory models for the u.v. photolysis of peptides and proteins.[353] Arginine is degraded into aspartic acid, serine, norvaline, ornithine, urea, and NH_3 through u.v.-stimulated H_2O_2 oxidation;[354] in this system u.v. irradiation alone or H_2O_2 alone causes no degradation.

Extension of studies of one-electron oxidation of DOPA and cysteinylDOPAs through pulse radiolysis of aqueous solutions has been described.[355] Azide radicals formed from NaN_3 in the conditions yield unstable quinones and dopachromes. Electron-donating groups in substituted phenylalanines and tyrosine accelerate lumiflavin-sensitized photo-oxidation;[356] the formation of 'dityrosine' through ^{60}Co-γ-irradiation of aqueous tyrosine solutions can be detected by characteristic fluorescence at 410 nm.[357] The fluorescence of tyrosine itself in acidic solutions has been thoroughly investigated.[358]

An interesting variation of what has become a routine area of study is the observation that tryptophan radicals cause oxidation of tyrosine in aqueous solutions of any pH, while tyrosine radicals achieve the corresponding effect on tryptophan only in strongly acidic or strongly alkaline solutions.[359] Tryptophan free radicals involving the indole moiety can be 'repaired' by anti-oxidants.[360] Photo-oxidation of tryptophan in aqueous solutions gives cis- and trans-3α-hydroperoxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carboxylic acid as primary products through singlet-O_2 formed by red-light irradiation in the presence of sulphonated phthalocyanine - metal chelates.[361] Another pathway may be involved in the case of the Mn complex, but the tricyclic photoproduct is common to many other such studies, e.g. dye-sensitized photo-oxygenation at pH 3.6 - 7.1.[362] However, the indole-opened product, kynurenine, o-$HCONHC_6H_4COCH_2CH(CO_2^-)NH_3^+$, is formed in these conditions at pH 7.7 - 8.4.[362] 3-Indolecarboxaldehydes are formed by u.v. degradation of the side-chain of protected tryptophans in acid media through oxidation by pyrimido[5,4-g]pteridine N-oxide, with the corresponding protected glycine as accompanying product.[363] This new cleavage mode may account for the accumulation of 3-indolecarboxaldehyde in animals and plants. Another intriguing role for tryptophan is presumed in interpretation of visible light activation of retinal in bacteriorhodopsin followed by energy transfer to tryptophan revealed by fluorescence emission at 350 nm.[364] Five photolysis products, two unidentified accompanying kynurenine, N-formylkynurenine, and tryptamine, form in laser-irradiated (337 nm) aqueous tryptophan.[365] Radiolysis gives kynurenine and 5-hydroxtryptophan.[366] An e.s.r. study of photoionization products of tryptophan formed in media containing spin-traps to intercept hydrated electrons has been published.[367]

7 Analytical Methods

7.1 Gas-Liquid Chromatography.- Despite the continuing surge in h.p.l.c. analytical methods, there is no diminution in the volume of papers describing g.l.c. analysis.

Derivativization is an essential first step in the sample preparation stage of g.l.c. analysis of amino acids, and the novelty of procedures detailed here can be inferred from the extent of the discussion. Silylation is chosen only by a minority of workers, but the fact that t-butyldimethylsilyl derivatives are some 10000 times more stable than their trimethylsilyl analogues[368] will attract some to follow leaders using N-methyl-N-t-butyldimethylsilyltrifluoroacetamide as derivatization reagent for amino acids. Already[369] their use in analyses of glutamine and asparagine avoids problematical side-reactions that generate pyroglutamic acid and deaminated artefacts. Standard silylation practice has been employed in g.l.c. - m.s. analysis of phenylalanine and tyrosine that have been variously ^{2}H-labelled,[370] though N-trifluoroacetylated pentafluorobenzyl esters were also used in this study. N-Trifluoroacetylation[371-373,22] and N-heptafluorobutyroylation[374-379,21] remain the most widely used procedures that follow esterification with pentafluorobenzyl alcohol,[370] isobutanol,[371,374-379,21] n-butanol,[372,22] n-propanol.[373] N-Pentafluorobenzoylation is preferred by some workers.[379] Arenesulphonyl derivatives are rarely used, but a novel example is of 1-dibutylamino-5-naphthalenesulphonyl derivatization, followed by trimethylsilylation for an attack on the analytical problem posed by the existence of more than 90 ninhydrin-positive compounds in the urine of healthy subjects, only about 46 of which have been identified previously.[380]

The amino acids that have benefitted from these and other g.l.c. analytical studies[381-385] include various aminobutyric acids,[373] including a new chlorine-substituted homologue found in xylem sap of Pisum sativum,[21] pipecolic acid,[382] 3-methylhistidine,[371,381] N^5-(1-carboxyethyl)ornithine,[22] cysteic acid,[383] (employing the N-isobutoxycarbonyl or dibutylsulphamoyl methyl ester or dibutylamide derivatives), and cysteinesulphinic acid.[384]

Some of the papers cited here include some appraisal of practical innovations (stability of the stationary phase "Supelcoport",[376] applicability of an N-selective detector,[377] and fused-silica capillary columns[385]). Creatinine has been established as a major problem of interference in g.l.c. of amino acids in urine.[374]

Estimation of enantiomer ratios by g.l.c. of derivatized amino acids over chiral stationary phases (polysiloxanes treated with chiral derivatives[386-388] including acylated L-phenylalanine t-butyl ester as a novel enantiomer discriminant) has continued, with applications for aspartic acid in aged human eye lens protein hydrolysates[386] and asparagine in ancient samples.[387]

7.2 Ion-Exchange Chromatography.- Automated ion-exchange amino acid analysis has been reviewed,[389] and precautions in sample preparation[389,390] including de-salting[391] have

been discussed. Brief reference can be made to representative applications of the amino acid analyzer: for analysis of crosslinking amino acids and 5-hydroxylysine from hydrolyzed elastin and collagen;[392] an independent study of pyridinoline and its 2'-deoxy analogue;[393] lysine content of fermentation products;[394] 3-methylhistidine;[395] and tryptophan.[396]

7.3 Thin-Layer Chromatography.- Quantitative analysis by t.l.c. is a more reproducible technique than it once was, due to the availability of more homogeneous materials and to improvements in practical aspects.[397]

The use of tin(IV) arsenosilicate as a cation-exchange t.l.c. medium for analysis of amino acids may have some advantages, since aromatic and acidic amino acids show greater mobility than on silica gel while basic amino acids stay at the origin.[398] Other non-routine papers give detailed data on t.l.c. of methylated lysines and arginines,[399] interpretation of t.l.c. mobility to yield an order of lipophilicity of amino acids,[400] identification of amino acids as their 4,4-dimethylamino-azobenzene-4'-naphthalenesulphonyl derivatives,[401] and estimation of optical purity through t.l.c separation of enantiomers on chirally modified silica gel.[402,403] Commercially available 'Chiralplates'® consist of reverse-phase silica gel impregnated with Cu^{++} and a chiral proline derivative, operating on the ligand-exchange principle.[403]

7.4. High-Performance Liquid Chromatography.- A number of reviews have appeared on this vigorously expanding topic, two of a general nature[404,405] and two dealing with pre-column derivatization with o-phthaldialdehyde.[406,407] One covers also electrochemical detectors as they are used in amino acid analysis.[407]

Aspects of experimental technique are covered in a study of the use of alkanesulphonate salts as mobile-phase additives[408] and laser-induced fluorescence detection for o-phthaldialdehyde - mercaptoethanol derivatives and their naphthalenedialdehyde analogues.[409] This sensitive method allows the detection of between 4 and 15 femtomoles of the o-phthaldialdehyde derivatives or 200 - 500 amol amounts of the naphthalenedialdehyde analogues. Several orders of lower magnitude are therefore becoming possible, in relation to current levels around 10 pmol for the o-phthaldialdehyde derivatives using commercial instrumentation.

Deamination of L-amino acids by an L-amino acid oxidase creates a change in ionic strength, and amino acids in h.p.l.c. eluent can be estimated by the conductimetric approach with an immobilized enzyme detector.[410] Crown ether-containing mobile-phase separation parameters for amino acids[411] and improved separations using ammonium tungstophosphate-coated silica gel[412] have been explored.

Familiar subdivisions of the topic appear in the current literature, with some waxing and *waning of emphasis*. o-Phthaldialdehyde - mercaptoethanol occupies the pre-eminent position now, for pre-column derivatization reagent.[413-424] In one of these examples, the

necessity to cleave imino acids by HOCl to generate the fluorophore leads to post-column reaction for detection of proline and hydroxyproline.[420] Two other applications use wholly post-column derivatization following ion-exchange h.p.l.c. for assays of $N^{\epsilon}N^{\epsilon}N^{\epsilon}$-trimethyllysine[425] and S-methylmethionine.[426] Few papers describe the use of ninhydrin, but those that do adopt the usual post-column mode.[427,428] Harduf advocates colorimetry at 405nm and considers it advantageous to avoid switching detector wavelengths, as is common practice.[428]

The other burgeoning application is the direct phenylthiocarbamoylation by phenylisothiocyanate that appears to have been fully developed and side-reactions well understood.[429-439] An excellent short account of the method[436] will inform new and hesitant users. 1 pmol levels of 1-aminocyclopropanecarboxylic acid in apple tissue have been located by the method,[430] so it certainly compares favourably from this point of view with the o-phthaldialdehyde method. A novel combination of the two methods has been used for the assay of proline and hydroxyproline after pre-column derivatization with o-phthaldialdehyde and separation of the derivatives, followed by phenylthiocarbamoylation of the unreacted imino acids.[439]

Electrochemical detection is featured in an increasing proportion of h.p.l.c. studies of amino acids,[440-445] these papers covering homocysteine,[440] cystine,[441] methionine,[442] tyrosine,[443] and tryptophan,[444,445] and detection limits can be as low as 1 ng.[442]

Phenylthiohydantoins, whether they arise from sequencing or are prepared directly from amino acids (e.g. 3-nitrotyrosine + 0.5M HCl + PhNCS, room temp. during 24 h[446]), continue to offer satisfactory h.p.l.c. characterisation of amino acids.[447-453] 4'-(NN-Dimethylamino)azobenzenethiohydantoins form the basis of one of these papers, accompanied by their 4'-sulphonate analogues;[453] all the other papers involve the phenylthiohydantoins themselves.

Dansylamino acids[454-457] and their close analogues (dabsylamino acids[458]) are extending their long service in the context of analysis and characterization at about 20 pmol levels,[454] although laser fluorimetric detection permits 10^{-16} mol levels to be reached.[456]

Alternative derivatives are those formed with 4-chloro-7-nitrobenzofurazan[459,460] and with its 4-fluoro analogue,[461] p-bromophenacyl esters from amino acid betaines,[462] Fmoc amino acids,[463] and xanthydrol derivatization of N-acetyl-L-glutamine.[464] Fluorescent 1,N^6-etheno derivatives are formed from the reaction between decarboxylated S-adenosylmethionine and $ClCH_2CHO$,[465] and the innate fluorescence of o- and m-tyrosines at 305 nm ($\lambda_{excit.}$ 275 nm) has also been used in detection of these amino acids during h.p.l.c.[466]

The amino acids on which these and other[467-470] h.p.l.c. papers have focussed, if not stated as part of the preceding narrative, are: tryptophan[444,445,467] and its radiolytic decomposition products,[366] methylated lysines, arginines, histidines, and methionine;[381,415,426,457] O-phosphoserine, threonine and tyrosine,[417] γ-carboxyglutamic acid,[417] glutamic acid,[417] ^{32}P-phosphorylated amino acids,[468] histidine and its 1- and 3-methyl derivatives,[419] phenylalanine and

tyrosine,[424] S-adenosylmethionine,[469,470] arginine,[428] asparagine and glutamine,[431] tyrosine O-sulphate,[433] 4-fluorophenylalanine,[434] hydroxyproline and proline.[437,439]

Losses during protein hydrolysis have been accepted for many years as the price to pay for convenient and reliable methods for most of the amino acids. Cysteine, labelled with 4-(amino sulphonyl)-7-fluoro-[2.1.3]-benzoxadiazole while bound within the protein, is released using 6M-hydrochloric acid and then derivatized for h.p.l.c. analysis. Although methionine is largely destroyed by this milder acid hydrolysis, lysine is quantitatively recovered.[461]

Determination of enantiomer ratios by h.p.l.c. has been based on three main variations: the most common approach is the use of a chiral stationary phase,[471-476] with the use of chiral mobile phases becoming more widely used.[477-479] The classical approach in which the enantiomer mixture is converted into a pair of diastereoisomers through reaction with a chiral reagent is also valid, and dibenzoyl-L-tartaric anhydride has been used for this purpose to give the imides (47).[480] A further example of the formation of diastereoisomeric mixtures has been provided in the context of the well established o-phthaldialdehyde derivatization method. If N-acetyl-L-cysteine is used as thiol co-reactant, instead of the usual mercaptoethanol, then optically impure amino acids will yield corresponding proportions of diastereoisomeric iso-indoles that can be estimated by h.p.l.c. over achiral stationary phases.[478]

Pirkle's group is studying potential chiral stationary phases in a most thorough manner (see also refs. 194-196) and has described their use with enantiomeric purity determinations for α- and β-substituted β-alanines.[471] Silica gel made chiral through bonding to a D-phenylglycine derivative[472] also continues existing studies, and has been used with N-(3,5-dinitrobenzoyl)amino acid isopropyl esters. A chiral naphthylamine stationary-phase approach[473] and bovine serum albumin bonded to silica[474] also exploit the same general principle, the latter being a detailed study showing the particular suitable N-substituent for each amino acid (e.g. o-carboxybenzoyl for DL-alanine, phthaloyl for threonine).[474] The ligand-exchange principle (as used in t.l.c. resolution, ref. 392), in which an L-amino acid is bonded to the stationary phase and chelated to Cu^{++}, continues to develop satisfactorily, e.g. for the resolution of perfluoro-α,α-dialkyl glycines.[475,476] The same principle with mobile phases containing L-arginine - Cu^{++}[477] and either L-proline or L-histidine with Cu^{++}[478] has been used for the resolution of N-dansyl amino acids. N-Acetyl-L-valine t-butylamide has been used as a chiral hydrogen-bonding additive to the nonaqueous phase in silica-gel chromatography for the resolution of N-acetyl-amino acid t-butyl esters.[479]

A further category of h.p.l.c. enantiomer analysis employs a micropolarimeter-cum-refractive index detector at the outlet of a normal (achiral) h.p.l.c. column.[481] The sensitivity claimed (50ng - 2 μg) can only refer to compounds whose pure enantiomers have large specific rotations, and the method operates best only for samples that are close to maximum optical purity.

7.5 Fluorescence Methods.- The o-phthaldialdehyde method is covered in the context of its coupling with h.p.l.c., in the preceding section. Its reliability is assurable only under standardized procedures since degradation of the 1-alkythio-2-alkyliso-indoles occurs during and after derivatization. An important observation,[482] that degradation is strongly accelerated by excess o-phthaldialdehyde, implies the use of minimum reagent (or gradual addition). The low picomole detection limit for these derivatives has been underlined,[483] compared with about 3 nmol limits for the ninhydrin colorimetric method.

2,3-Naphthalenedicarboxaldehyde[409] reacts to give 2-substituted 1-cyanobenzo[f]iso-indoles by condensation with amino acids and CN^-;[484] the highly fluorescent derivatives will no doubt be taken up in competition with the o-phthaldialdehyde method for amino acid analysis.

The inherent fluorescence of certain amino acid side-chains has been mentioned in the preceding Section, and fluorescence energy acceptance by 4'-aminophenylalanine[485] might be useful in analysis of this amino acid.

Fluorimetric analysis of amino acids has been reviewed.[486]

7.6 Other Analytical Methods.- Dansylamino acids have been separated by paper electrophoresis[487] and by isoelectric focussing in immobilized pH gradient phases.[488]

7.7 Determination of Specific Amino Acids.- Most of the methods described here are specific because enzymatic assays are involved or because of colorimetry (in its broadest sense) derived from characteristic functional-group reactions for particular amino acids.

Three L-glutamic acid assays based on either L-glutamine synthetase[489] or L-glutamic acid dehydrogenase[490,491] include novel coupling with a second enzyme system - a reductase and luciferase that generate luminescence proportional to the amino acid,[490] and a transaminase that generates NADH in an enzyme electrode system.[491] One of these permits assay down to 100 pmol.[491] Arginase cleavage of L-arginine into ornithine and urea and assay of the urea by standard methods[492] illustrate conventional methodology, and immobilized bacteria - pO_2 sensor assay of L-tryptophan,[493] L-lysine α-oxidase electrode,[494] and other immobilized enzyme methods for L-amino acids[495] illustrate another strand of established methodology. An improved radioenzymic carnitine assay has been reported.[496]

Cysteine colorimetry using Ellman's thiol reagent can be inaccurate when surfactants are also present.[497] Another sort of interference applies to HOCl cleavage of 1-aminocyclopropane-carboxylic acid into ethylene, thought to be a specific reaction - but ethanol can also undergo cleavage to the same product.[498] Other presumed inocuous organic compounds, such as aliphatic amines, result in low ethylene yields from the amino acid.[498] Standard colorimetry is represented by Chloramine-T degradation of hydroxyproline (see also Vol.17, p.39)[499] and N-bromosuccinimide substitution of tryptophan (using a stopped-flow technique).[500] Analysis

of S-methylmethionine and S-adenosylmethionine in plant tissue down to 100 pmol levels has been accomplished through a dual-isotope dansylation procedure, depending on separation of the amino acids on phosphocellulose followed by thermal degradation to homoserine and estimation of the derived [^{3}H]dansyl[^{14}C]homoserine.[501]

Authoritative recent reviews of assay of specific amino acids are contained in refs 6 and 8.

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2
Peptide Synthesis

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

1. Introduction

The last year has seen a continued increase in the number of peptides prepared by direct chemical synthesis. Solid phase peptide synthesis has now become the dominant method, certainly for exploratory purposes, and many large peptides have been prepared using both the polystyrene and polyamide based methods.

There appears to have been somewhat of a decline in the amount of work devoted to new protecting groups and coupling methods, and it might be assumed that the methods that we now have available are adequate to cope with most synthetic problems. The protection of some side-chains is still causing difficulties and in a few cases there is still room for improvement. The problems of racemisation have also in the main been reduced to an acceptable level and it is now rare to see any proof of optical homogeneity, particularly in solid phase prepared material.

The use of enzymes in peptide synthesis and the application of semi-synthesis to the preparation of large peptides and proteins has become considerably more important and these techniques have facilitated the preparation of a number of modified proteins. The design of non-natural peptides and proteins is frequently discussed and some general considerations on this topic have been made.[1]

The Proceedings of the 19th European Peptide Symposium in Halkidi, Greece,[2] and the Proceedings of the 24th Japanese Peptide Symposium[3] have been published, and in this review no attempt has been made to examine these proceedings in detail. The Proceedings of a Peptide Seminar in Shanghai has also been published as a Supplement to Volume 25 of Biopolymers.[4] In this Seminar both review lectures and new research material were presented.

The toxicity of reagents used in peptide synthesis for the introduction of protecting groups and the mediation of coupling reactions is not frequently considered. However, in a recent paper[5] a note of caution is sounded as many

reagents have been tested in the Ames Salmonella mammalian microsome assay and although most reagents (11 synthetic reagents were tested) were found to be safe bis-(oxo-oxazolidinyl)phosphinic chloride (Bop-Cl) and fluorenylmethyl-chloroformate (Fmoc-chloride) gave rise to some degree of concern regarding their mutagenic activity and the paper recommends caution in the use and disposal of these reagents. Boc_2O, Z-Cl, Bop, HONSu, and HOBt were all given a clean bill of health in this assay.

As in previous years the current chapter covers the majority of all published literature in this area and only those papers of particular interest are discussed in the general text. The remainder of the papers are cited in the Appendices and information is presented in this section which covers new peptides, synthetic intermediates and methods of purification.

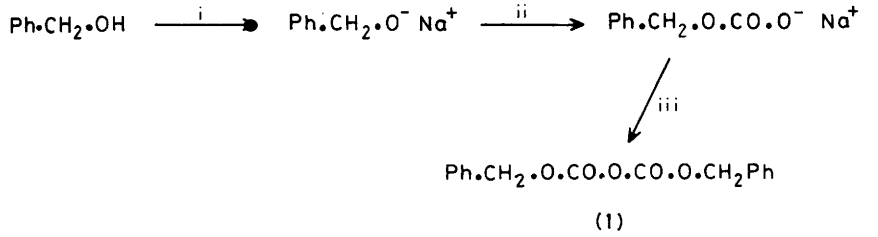
2. Methods

2.1 Protective Groups

2.1.1 Established Methods of Amino Group Protection

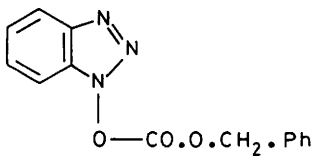
Dibenzylpyrocarbonate (1) has been prepared by two groups of workers.[6,7] The pyrocarbonate (1) is synthesised by the route outlined in Scheme 1, the oily product of the reaction crystallises and is stable to prolonged storage. Reaction of dibenzylpyrocarbonate with amino acids is best carried out under pH stat control, and generally gives yields in the region of 70 - 90%, the reaction usually being complete in one hour. An alternative reagent (2) has been prepared[6] by reaction of benzylchloroformate with hydroxybenzotriazole in the presence of triethylamine using THF as solvent. With this compound, which is a known anti-inflammatory agent, up to twenty-four hours may be required for acylation due to it's lower acylating power.

The formation of dipeptides during introduction of the benzyloxycarbonyl group was studied,[7] and in order to minimise this side-reaction the pH needs to be carefully regulated. Straightforward acylation using benzylchloro-formate in the presence of aqueous sodium hydroxide at pH 8.5 gives a 93% yield, the product being Z-Gly-OH(96.5%) and $Z\text{-}Gly_2\text{-}OH$(0.7%). With dibenzyl-pyrocarbonate however, using dioxan/water as the solvent, relatively high levels of dipeptide formation (1.8%) were noted when triethylamine was used as the base; however when sodium hydroxide was used at pH 9 an overall yield of (91%) was achieved and the product contained much less than 0.1% of the corresponding dipeptide.

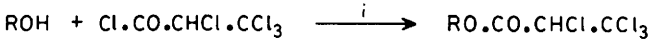


Reagents: i, NaH; ii, CO_2; iii, Z.Cl

Scheme 1



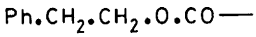
(2)



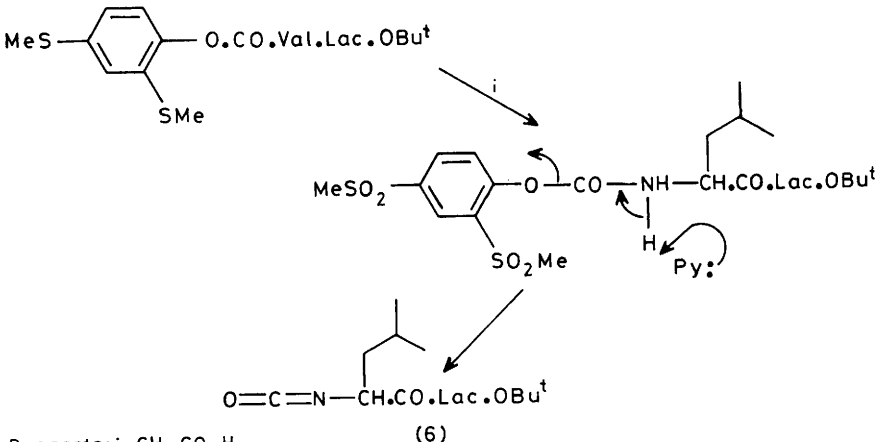
(3) (4)

Reagents: i, Pyridine

Scheme 2



(5)



(6)

Reagents: i, $CH_3.CO_3H$

Scheme 3

Alkyl-1-chloroalkyl carbonates have been described[8] as reagents for the introduction of urethane protecting groups. The reagents may be prepared by reaction of the acid chloride (3) with a suitable alcohol in pyridine. Appropriate alcohols may be used to prepare reagents for the introduction of the Boc, Fmoc, Troc and 2-trimethylsilyl-ethoxycarbonyl protecting groups (see scheme 2). In order to prepare the acylated amino acid derivative the chloroalkyl carbonate (4) was reacted with the amino acid in the presence of sodium hydroxide in aqueous dioxan. The reaction yields the urethane derivative of the amino acid and chloral hydrate as a by-product.

A study of the preparation of Fmoc amino acids using Fmoc-ONSu has been reported.[9] This work describes the facile synthesis of the mixed carbonate (Fmoc-ONSu) by reaction of hydroxysuccinimide with Fmoc-chloride in the presence of triethylamine. The chloroformate is prepared from 9-fluorenylmethanol at -78°C by reaction with phosgene. The overall yield of the hydroxysuccinimide mixed carbonate is between 85 and 90% starting from the corresponding alcohol. The normal method of acylation using Fmoc-ONSu involves reaction of the mixed carbonate with the amino acid in 10% sodium carbonate/dioxan. Under these conditions reaction may be prolonged and occasionally the product may precipitate. In order to improve this method the use of the mixed carbonate in acetonitrile/water in the presence of triethylamine is suggested; the reaction is complete in thirty minutes, during which time the pH of the reaction falls to approximately 8.5 and must be maintained by the addition of triethylamine. Under these conditions dipeptide formation and racemisation are prevented and yields of between 73 and 100% are obtained. The preparation of asparagine and leucine derivatives required slight modification.

9-Fluorenylmethyl-pentafluorophenyl carbonate has also been suggested as a useful reagent for the preparation of Fmoc amino acids and their pentafluorophenyl esters.[10] The mixed carbonate (Fmoc-OPfp) is prepared by reaction of Fmoc-chloride with pentafluorophenol in ethereal solution in the presence of triethylamine. At 0°C the reaction is complete in two hours and an 86% yield of the mixed pentafluorophenyl carbonate is obtained. The mixed carbonate may be reacted with amino acids in the presence of sodium bicarbonate in aqueous acetone, giving the Fmoc amino acid after acidification. If, after acidification, the Fmoc amino acid and resulting pentafluorophenol are extracted into ethyl acetate DCCI may be added to produce the Fmoc amino

acid pentafluorophenyl ester directly.

The formation of protected dipeptides during the preparation of N-acylated amino acids has been recognised as a problem. Accurate monitoring of dipeptide formation has been studied in detail,[11] and it has been found that using Bondapak C_{18} as the stationary phase with aqueous methanol or aqueous acetonitrile as the eluant, small amounts of contaminating protected dipeptides may be detected. Detection levels between 0.1 and 0.7% are generally achieved, and it was found that seven out of eight commercial samples of benzyloxycarbonyl amino acids were contaminated with the corresponding dipeptides. The authors point out that contamination with dipeptide is well known in the case of the introduction of the Fmoc group with Fmoc-chloride, but also draw attention to the fact that using dialkyl carbonates in place of the corresponding chloroformates does not guarantee the elimination of dipeptide formation, and indeed unless the conditions of acylation are carefully monitored it is difficult to avoid some degree of dipeptide formation.

2.1.2 New Methods of Amino Group Protection

The homo-benzyloxycarbonyl (hZ) (5) has been suggested as an amino protecting group.[12] The homo-benzyloxycarbonyl group may be introduced through the corresponding chloroformate, and interestingly may be removed by catalytic hydrogenolysis or by catalytic transfer hydrogenolysis using freshly precipitated palladium on charcoal using ammonium formate as the donor. There is a considerable rate differential between removal of benzyloxycarbonyl and homo-benzoxycarbonyl and this may be used to facilitate selective cleavage. For example when hZ-Gly-OBzl is subjected to hydrogenolysis over 10% palladium at atmospheric pressure only the ester function is cleaved, whereas catalytic transfer hydrogenolysis removes both protecting groups. In contrast to the benzyloxycarbonyl group, the homo-benzyloxycarbonyl group is stable to HBr in acetic acid and to trifluoroacetic acid. In some instances cleavage of the homo-benzyloxycarbonyl group may be slow, but this may be increased by heating to 50°C; also, it should be noted that the corresponding homo-benzyl ester is cleaved less readily than the corresponding urethane protection.

The conversion of benzyloxycarbonyl or allyloxycarbonyl protection to t-butyl-dimethylsilyloxycarbonyl protection has been reported.[13] The

transformation involves treatment of the benzyloxycarbonyl or allyloxycarbonyl compound with t-butyl-dimethylsilane in the presence of palladium(II) acetate using triethylamine as the base with dichloromethane as the solvent. Under these conditions the smooth conversion to the silyl based protecting group is achieved; the butyloxycarbonyl group is however stable to these conditions and Boc-Lys(Z)-OMe may be converted to the corresponding N^{ε}-t-butyldimethylsilyl-oxycarbonyl derivative.

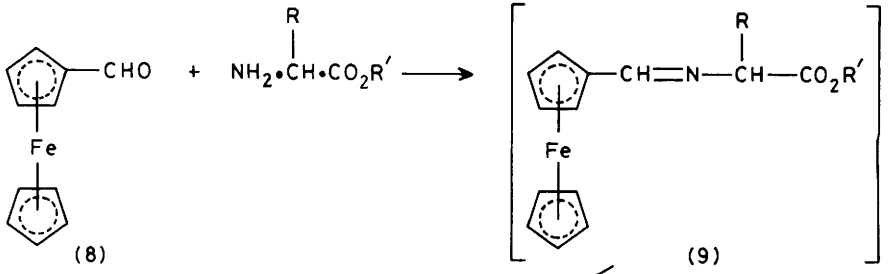
The 2,4-bis-(methylthiophenoxycarbonyl) (Bmpc) protecting group has been used in depsipeptide synthesis.[14] The protecting group was used to facilitate the formation of the cyclotetradepsipeptide cyclo(Val-L-Lac)$_2$. The protecting group is normally stable but after oxidation with peracetic acid may be removed by treatment with pyridine. This causes the generation of a terminal isocyanate (6) as indicated in Scheme 3. The isocyanate generated was eventually allowed to form a mixed carboxylic/carbamic mixed anhydride of the general type (7), which was allowed to decarboxylate with intramolecular formation of an amide bond; this reaction was used for the final cyclisation of the cyclodepsipeptide.

The protection of amide bonds in a peptide chain has also been examined, and this may be achieved through the introduction of the highly lipophilic ferrocenylmethyl protecting group.[15] The protecting group is introduced by reaction of the ferrocene aldehyde (8) with the amino acid ester according to scheme 4. The immediately formed Schiff's base (9) is then treated with palladium(II) phthalocyanine to reduce the carbon to nitrogen double bond liberating the Fem-protected amino acid (10). This derivative contains a nucleophilic nitrogen which can then be reacted with a protected amino acid such as Z-Ala-OH in the presence of DCCI and HOBt to give a protected dipeptide such as compound (11). The Fem protecting group may then be removed under similar conditions to those used for Boc group removal; for example treatment with TFA in the presence of β-thionaphthalene in dichloro-methane removes the group in two to four hours. Fem protected peptides are highly lipophilic and brightly coloured and thus behave well during chromatography.

Protection of amide bonds may also be achieved by treatment with Boc_2O in the presence of dimethylaminopyridine using acetonitrile as solvent.[16] Under these conditions a Boc group may readily be introduced; however, bulky substit-uents hinder the reaction substantially. Removal of a Boc group, which is protecting an amide bond, may be achieved by standard conditions.

R.CO.O.CO.NH.R′

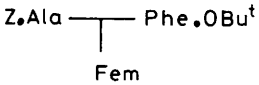
(7)



Fem.NH.CHR.CO_2R'

(10)

Scheme 4



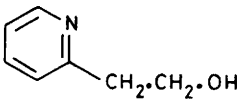
(11)

Ac.Asn.Gly.NHMe

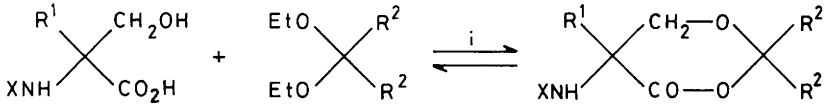
(12)

Ac.Isn.Gly.NHMe

(13)



(14)



(15) R^2 = Me, Et

$R^2—R^2=—(CH_2)_4—$

Conditions : i, H^+/Toluene

Scheme 5

2.1.3 Carboxyl Protection

As the trend towards solid phase synthesis continues there is a decreased requirement for new carboxyl protecting groups. Only a single paper has appeared which can be classified in this section and it relates to the preparation of benzyl ester hydrochlorides from the corresponding p-toluene sulphonates.[17] The normal method of preparation for benzyl esters involves heating the amino acid with benzyl alcohol in the presence of p-toluene sulphonic acid. The normal method of conversion to the corresponding hydrochloride salt is by dissolving the salt in an alkaline medium and extracting into organic phase; after drying and evaporation the free base is treated with hydrochloric acid. A new more satisfactory method involves dissolving the benzyl ester p-toluene sulphonate in sodium bicarbonate and extracting the resulting ester into ethyl acetate. Washing with saturated sodium chloride containing 1 mole of dry HCl followed by evaporation gives excellent yields of the corresponding hydrochloride salt.

2.1.4 Side-Chain Protection

Attempts to find aspartic acid esters which minimise succinimide formation has continued and the cycloheptyl and cyclooctyl side-chain esters have been described.[18] These esters were stable to TFA at $0^{\circ}C$ over two hours but were cleaved either by treatment with HF or with 1M trifluoromethane sulphonic acid in TFA in the presence of thioanisole at $0^{\circ}C$ over sixty minutes. They are less susceptible to succinimide formation than the corresponding benzyl esters on treatment with triethylamine, and during acid deprotection less than 7% of the β-peptide was observed in both cases. It is worth noting that the authors conclude that the cycloheptyl and cyclooctyl esters did not appear to be particularly superior to the corresponding cyclopentyl and cyclohexyl esters which have been described previously. The synthesis of human[19] and porcine[20] glucose-dependent insulinotropic polypeptide have been reported using cycloheptyl protection for the protection of the side-chain function of aspartic and glutamic acids. During the syntheses the cycloheptyl protected residues were added by the hydroxysuccinimide active ester method. The final deprotection using 1M trifluoromethane sulphonic acid in TFA in the presence of thioanisole cleanly removed the ester protecting groups along with the benzyloxycarbonyl group which had been used for the protection of the epsilon amino function of lysine and the Mts group

which had been used to protect tryptophan.

β-ℓ-Menthyl esters of aspartic acid have also been described as possible candidates for the suppression of succinimide formation.[21] The derivative may be prepared by reaction of Boc-Asp-OBzl with β-ℓ-menthol in the presence of DCCI and dimethylamino pyridine. The benzyl ester function can then be removed by hydrogenolysis over palladium. The protecting group is stable to trifluoroacetic acid but is again cleaved by hydrogen fluoride or 1M trifluoromethane sulphonic acid in the presence of trifluoroacetic acid using thioanisole as scavenger, sixty minutes being required for deprotection at $0^{o}C$. In a synthesis of tetragastrin diphenylsulphide was used as an alternative scavenger. It is claimed that the β-ℓ-menthyl esters are superior to other esters for the suppression of base catalysed succinimide formation, but they must of course suffer the disadvantage that they introduce further chirality. An investigation of the deamidation of the asparaginyl lysyl sequences shown in (12) and (13) has been carried out.[22] In this reaction the asparagine or isoasparagine becomes deamidated at alkaline pH, acting as a model of the situation pertaining in a protein. In both cases an identical mixture of products was produced containing 22% of the α-aspartyl peptide and 78% of the β-aspartyl peptide, the composition being determined by nuclear magnetic resonance spectroscopy. The authors postulate that the reaction proceeds through a cyclic intermediate and that, unlike peptides of aspartyl esters, the cyclisation does not occur under non-aqueous conditions or at low pH in aqueous solution. The α- and β-peptides were separated by ion exchange chromatography on Dowex I (acetate form) and the α-peptide was noted to be considerably less acidic than the corresponding β-peptide.

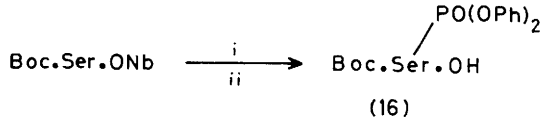
2-(2'-Pyridyl)-ethyl esters (Pet esters) have been described for protection of aspartic and glutamic acids.[23] In general the esters may be introduced by reaction of the Boc or Z amino acid with three equivalents of 2-pyridylethyl alcohol (14) in the presence of 1.1 equivalents of DCCI and 0.25 equivalents of hydroxybenzotriazole using dichloromethane or DMF as solvent. The γ-ester of glutamic acid may be prepared through the intermediacy of a copper complex which allows specific protection of the γ-carboxyl group. The β-aspartyl carboxyl group may be protected by opening of Boc aspartic anhydride with the alcohol (14), this gives a yield of 60% of the Boc β-2(2'-pyridyl)-ethyl ester. The protecting group may be removed by treatment with three equivalents of methyl iodide in DMF at $21^{o}C$ followed by

eight equivalents of diethylamine in dichloromethane or acetonitrile. The esters are thus semi-permanent and may be used for either terminal ester protection or for the protection of side-chain functions. The oily nature of these esters is potentially a disadvantage, but they have considerable stability and selectivity in removal. A major difficulty lies in the fact that they cannot be used in the presence of residues which are easily alkylated such as methionine and the second stage of the removal using diethylamine precludes the use of Fmoc for amino group protection.

A one-step protection has been developed which allows simultaneous protection of the hydroxyl and carboxyl functions in α-hydroxymethyl-α-amino acids.[24] The method, which is applicable to serine, but may be more generally used for other α-hydroxymethyl-α-amino acids, involves the formation of 5-acylamino-2,2-dialkyl-4-oxo-1,3-dioxanes (15) according to the procedure indicated in Scheme 5. A range of dioxanes (15) have been prepared by means of various aliphatic acetals. The group is stable to the usual coupling conditions and it was noted that it did not give rise to any racemisation during hydrolytic removal.

The preparation of phosphoseryl peptides has been reported,[25] employing phenylphosphorotriester protection during synthesis. The most useful derivative (16) has been prepared by the route outlined in Scheme 6. This involves reaction of diphenylchlorophosphite in pyridine with Boc-Ser-ONb, catalytic hydrogenolysis then yields the free acid which could be directly incorporated into peptides. A model tripeptide (17) was readily prepared using the mixed anhydride procedure and the intermediate Boc group was cleaved with TFA. Subsequent hydrogenolysis using one equivalent of PtO_2 /phenyl group in the presence of 40% trifluoroacetic acid/acetic acid gave the free phosphite containing peptide in fifteen minutes.

Frequently difficulties are encountered if attempts are made to synthesise homoseryl peptides without protection of the side-chain hydroxyl group.[26] When the hydroxyl group is unprotected lactonisation can readily occur, hence the route outlined in Scheme 7 was adopted to prepare the ether derivative (19). In this sequence trityl homoserine is reacted with trityl chloride in the presence of triethylamine to give the intermediate trityl ester (18) which rearranges to the corresponding ether (19). This derivative (19) may be coupled with a suitable amino component using DCCI/HOBt. The protected dipeptide (20) was prepared by this method and attempted



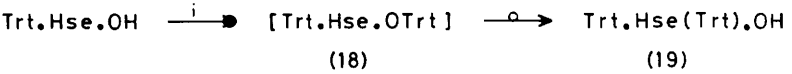
(16)

Reagents: i, $(PhO)_2PO.Cl$; ii, H_2/Pd

Scheme 6

Boc.Glu(OBzl).Ser[PO(OPh)$_2$].Leu.OBzl

(17)



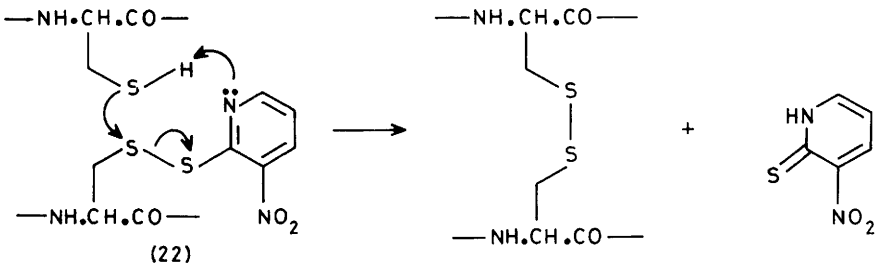
(18) (19)

Reagents: i, $Trt.Cl/NEt_3/CH_2Cl_2$

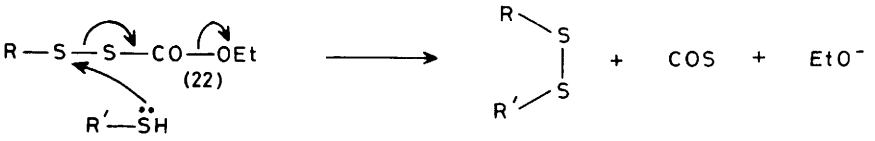
Scheme 7

Trt.Hse(Trt).Gly.NH_2 (20)

Z.Tyr(Dmp).OBzl (21)



Scheme 8



(24)

Scheme 9

deprotection using 25% TFA in dichloromethane over five minutes at $0^{o}C$ resulted in fragmentation of the peptide; however, treatment with 1.5% TFA in ethanol/chloroform (75:25) over five hours, gave a satisfactory deprotection of the trityl protecting groups. Subsequent modification of the homoserine residue was used to prepare a canaline or 1,4-diaminobutyryl peptide.

The dimethylphosphinyl (Dmp) protecting group has been applied to the protection of the hydroxyl function of tyrosine.[27] Z-Tyr-OBzl was reacted with dimethyl phosphinyl chloride in chloroform to give the fully protected derivative (21). This was then hydrogenolysed to give the dimethyl phosphinyl derivative of tyrosine. The protecting group is very stable to trifluoroacetic acid and hydrogenolysis, but is readily removed by fluoride ion. Generally, tetrabutylammonium fluoride trihydrate in acetonitrile may be used for the removal of the Dmp protecting group, but during solid phase synthesis HF may be used for deprotection. Treatment with HF for one hour at $0^{o}C$ did not completely remove the protecting group and it seems that direct use of fluoride ion, as suggested above, is a better method of removal. The applicability of the dimethylphosphinyl protecting group was illustrated by the synthesis of [D-Ala^{2}]-Leu-enkephalin.

An improved method for the synthesis of Fmoc-Arg(Adoc)$_2$-OH has been published.[28] In this procedure Z-Arg-OH is converted to Z-(Adoc)$_2$-OH by treatment with Adoc-F at pH 12. Catalytic transfer hydrogenation in the presence of 7% formic acid and palladium black gave the free zwitterion which was then treated with Fmoc-ONSu in the presence of triethylamine to give Fmoc-Arg(Adoc)$_2$-OH. The transfer hydrogenation is claimed to be more efficient than hydrogenolysis over palladium charcoal, which tends to cause cyclisation to a diazacycloheptane derivative. The use of Fmoc-ONSu for the preparation of the final derivative is important, as it is known that Fmoc chloride has a considerable tendency to bring about dipeptide formation and the zwitterionic Arg(Adoc)$_2$ has a particularly high tendency to oligomerisation under these conditions. The preparation of tri-Boc-arginine has also been reported,[29] this arginine derivative being prepared directly by acylation of arginine with di-butyl-pyrocarbonate.

The 9-fluorenylmethyl group has been suggested as a protection for the thiol function of cysteine.[30] This protecting group is stable to the action of HF, and has been used in a solid phase synthesis of oxytocin on a benzhydrylamine resin. The protecting group is also stable to iodine, but is

readily removed by treatment with 50% piperidine in DMF over two hours, or 10% piperidine in DMF overnight. The product of this reaction is not the free thiol but the corresponding disulphide; thus the fluorenylmethyl protecting group has some potential in the synthesis of molecules containing more than one disulphide bond.

The 1-adamantyl group has been studied for use as a thiol protecting group.[31] S-Adamantyl cysteine is more stable to trifluoroacetic acid than p-methoxybenzyl cysteine, but is cleavable by 1M trifluoromethane sulphonic acid in TFA in the presence of thioanisole at $0^{\circ}C$ in sixty minutes. Alternatively, the adamantyl group may be removed by treatment with thallium trifluoroacetate, and this method has been exemplified in a synthesis of human calcitonin gene related peptide.[32] In this case the thallium trifluoroacetate cleavage of the adamantyl group was carried out after the removal of the other groups by TFA/thioanisole. S-1-Adamantyl cysteine is less prone to spontaneous sulphoxide formation than the corresponding p-methoxybenzyl derivative; however, the sulphoxide may be prepared cleanly by the action of sodium borate. The sulphoxide, if present, may be reduced by treatment with phenylthiotrimethylsilane.

The S-(3-nitro-2-pyridinesulphenyl) derivative (22) has also been exploited in the synthesis of peptides containing cysteine.[33] The derivative is stable in trifluoroacetic acid/dichloromethane (1:1) and in anhydrous hydrogen fluoride. The effectiveness of using this protecting group in the formation of non-symmetrical disulphides was demonstrated in the synthesis of a fibrin related peptide. Non-symmetrical disulphide formation takes place through the mechanism illustrated in Scheme 8; the reaction is rapid at alkaline pH, but is useful over a considerable pH range.

Carboethoxysulphenyl chloride has also been used as a reagent for the formation of non-symmetrical disulphides.[34] Disulphide bond formation may take place in an aqueous medium between pH 4 and 7 and is mediated by the mechanism outlined in Scheme 9. The ethoxycarbonylsulphenyl derivative of a cysteine residue is reacted with a free cysteine residue (23), resulting in the formation of the non-symmetrical disulphide (24). In the intramolecular non-symmetrical disulphide formation involved in the formation of somatostatin the optimum yield was achieved at pH 7, using two equivalents of carboethoxysulphenyl chloride. Interestingly, 10% acetic acid was an extremely poor solvent for the reaction and the lowest yields were obtained at the highest

dilution. Other studies on urotensin IIA (6 - 12) and presenoic acid demonstrated the utility of the reagent, and it is claimed that the reagent is superior to the corresponding carbomethoxysulphenyl chloride.

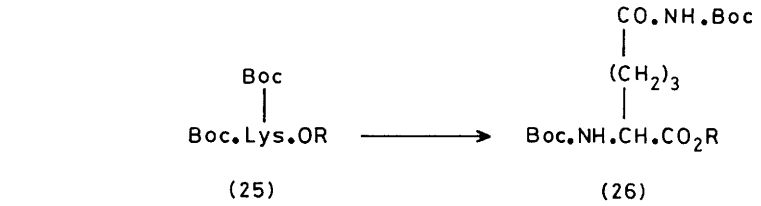
Methionine frequently does not require protection during synthesis; however, on occasions protection as the sulphoxide may be beneficial. An efficient method for the removal of the sulphoxide protection has been reported,[35] in which the methionine sulphoxide containing peptide is treated with phenylthiotrimethylsilane in the presence of trimethylsilyl-trifluoromethane sulphonate; the deprotection of the methionine sulphoxide should be carried out before the general deprotection which follows using 1M trifluoromethane sulphonic acid in trifluoroacetic acid in the presence of thioanisole.

An unusual synthesis of protected homoglutamic acid derivatives has been reported[36] which utilises the reaction of a bis-Boc-lysine ester (25) with ruthenium tetroxide as indicated in Scheme 10. The resulting Boc protected homoglutamine derivative (26) can then be modified in a number of ways depending on the ester protection which has been employed. Treatment of the trichloroethyl ester with zinc in acetic acid or treatment of the methyl ester with 1M sodium hydroxide in methanol gave the corresponding free acid, and treatment with trifluoroacetic acid in dichloromethane gave homoglutamine. Conversion to the benzyl ester could be effected by treatment with sodium benzoxide, and treatment with hydrazine in methanol gave the corresponding hydrazide. A number of derivatives were thus made available by this interesting oxidation.

The ruthenium tetroxide oxidation was also used in other unusual synthetic applications. In the first[37] a suitably protected proline derivative was converted to a pyroglutamic acid derivative through the intermediate formation of a 5-keto proline derivative. In the second case[38] a complex series of manipulations was carried out on the N^{α}-Boc-^{t}butyldimethylsilyl derivative of prolinol, ultimately giving an efficient synthesis of γ-carboxyglutamic acid.

2.2 General deprotection

1M Trifluoromethane sulphonic acid in trifluoroacetic acid has now become an established alternative to the use of HF for the removal of benzyl based protecting groups, and a further example of this is provided in the deprotection at the end of a solution synthesis of human calcitonin gene related peptide.[39] In this case both adamantyl cysteine and Arg(Mts) are

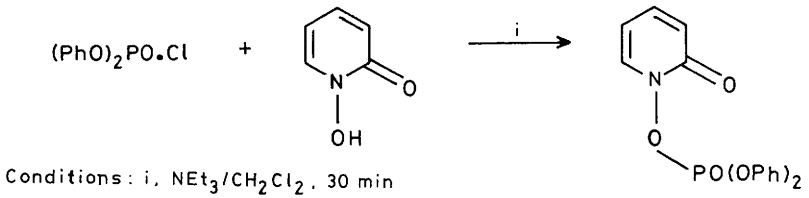


Reagent : RuO_4, where R = Tce, Me or Bu^t

Scheme 10

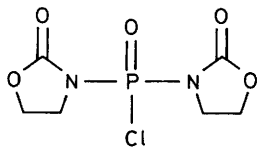
Boc.Asp(OBzl).Ala.Phe.Ile.Gly.OEt

(27)



Conditions : i, NEt_3/CH_2Cl_2, 30 min

Scheme 11



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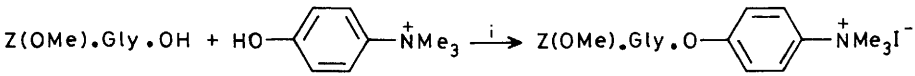


(30)

Reagent : i, DCCI

Scheme 12

deprotected at the same time as the benzyl protecting groups. The method is also now widely applied for deprotection at the end of solid phase synthesis, and a useful user bulletin has been assembled by Applied Biosystems which gives a number of examples and variations of the method which may be adopted for application in solid phase synthesis.[40]

On occasion deprotection of side-chain benzyl protecting groups can give rise to difficulties,[41] and in a synthesis of locust adipokinetic hormone by solution methods, using pentafluorphenyl esters, incomplete removal of benzyl protecting groups was achieved by hydrogenolysis over palladium.

In a synthesis of porcine growth hormone releasing factor[42] 2,2,2-trichloro-tert-butyloxycarbonyl hydrazides were used for intermediate protection of some of the subfragments. This group is readily cleaved by zinc in acetic acid and is significantly more easily removed than the corresponding 2,2,2-trichloroethyl protecting group which may also be used for hydrazide protection. The synthesis employed the assembly of nine subfragments, and ultimately final deprotection was by the trifluoromethane sulphonic acid in TFA method in the presence of thioanisole. Dimethylselenide was added to allow reduction of methionine sulphoxide to methionine.

The use of 10% sulphuric acid in dioxan for the removal of Boc protecting groups in solid phase synthesis has been examined as an alternative to the use of 50% TFA in dichloromethane.[43] A number of peptides were prepared by the solid phase technique and comparable yields and purity were obtained when 10% sulphuric acid in dioxan was used for deprotection. Traces of water were found to be non-detrimental, as the water appeared to act as a carbonium ion scavenger.

Removal of the benzyl side-chain protection from aspartic acid in compound (27) has proved to be difficult.[44] Both hydrogenolysis over palladium and transfer hydrogenation using ammonium formate and palladium, gave between 5 and 30% of the succinimide-containing peptide. FAB mass spectrometry proved to be an ideal method of analysis and from this it was concluded that any traces of base gave rise to a dramatic increase in the formation of the succinimide. Succinimide formation was also sequence and solvent dependent, but could be completely eliminated by the addition of a few equivalents of acetic acid or formic acid.

Fast Atom Bombardment mass spectrometry has been proposed as a real time method of analysis for deprotection.[45] FAB mass spectrometry was thus used

to optimise reaction conditions for the removal of benzyl based protecting groups by HBr in acetic acid, hydrogenolysis over palladium, and treatment with methane sulphonic acid in trifluoroacetic acid. It is claimed that the method is superior to tlc or hplc, but it should be noted is considerably more expensive! In another paper relating to the use of FAB mass spectrometry[46] it was observed that the spectra of glycopeptide derivatives were often suppressed when the peptide chain was of a hydrophobic nature. The problem was resolved, to some extent, by permethylation and/or acylation.

2.3 Formation of Peptide Bonds

The coupling agent 2-pyridone-1-yl diphenylphosphate (28) has been prepared by the route shown in Scheme 11.[47] The reagent (28) may be used as a one-pot coupling reagent so that the carboxyl and amino components may be mixed in its presence along with triethylamine using DMF as the solvent to give the peptide product. The examples quoted give yields between 85 and 94% and the Young test shows no racemisation.

bis-(2-Oxo-3-oxazolidinyl)phosphinic chloride (29) has been used in the assembly of the cyclosporin (8-11) sequence.[48] The sequence which contains two N-methylated residues was efficiently assembled using the reagent employing either Fmoc or benzyloxycarbonyl protection and virtually no racemisation was observed. Comparison with standards showed that the level of racemisation was less than 1%. This reagent was also shown to be particularly efficient in the cyclisation reaction which was used in the preparation of clamydocin and HC-toxin analogues.[49]

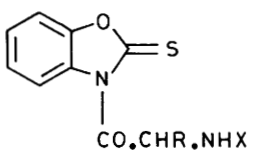
Diimide mediated couplings are still extremely popular, and the search for additives for use with diimides continues. In hindered couplings, such as the coupling of Z-Val-Aib-OH or Fmoc-Pro-Aib-OH to a simple amino component, it was found that excellent results were obtained by using zinc chloride as an additive to the dicyclohexylcarbodiimide mediated reaction. In this coupling the corresponding oxazol-5-(4H)-one is implicated as the actual coupling species.[50] The use of N-hydroxysulphosuccinimide as an additive[51] for use with water soluble carbodiimides has also been proposed. The application of the water soluble p-dialkylsulphoniophenyl esters[52] and p-trimethylammoniophenyl esters[53] has been reported. In the latter case the active esters (30) are prepared by the route exemplified in Scheme 12. The active esters were used in a synthesis of leucine enkephalin and due to their

water solubility excess acylating species was easily removed after each coupling step. The yields were comparable with that obtained by a conventional mixed anhydride synthesis of leucine enkephalin. Attempts to increase the activation of these esters by the introduction of a nitro function adjacent to the phenolic group resulted in rather unstable derivatives and purification of the intermediate esters was sometimes difficult.

Benzoxazolethiol derivatives of the type (31)[54] have been prepared by the reaction of N-protected amino acids with 2-benzoxazolethiol in the presence of DCCI. The active esters thus formed (31) couple in a similar manner to other active esters and do not show racemisation. The high solubility of the resulting thiol frequently permits easy work-up after coupling, however there is a slight problem if any residues remain, as there is a possibility of poisoning hydrogenolysis catalysts.

An unusual application of t-butylpyrocarbonate has been its use in the preparation of active esters.[55] In this work it was shown that if a Boc amino acid is treated with t-butylpyrocarbonate in the presence of pyridine, and then nitrophenol added, the product is the resulting Boc-nitrophenyl active ester.

3-Hydroxy-4-oxo-dihydrobenzotriazine active esters have recently found considerable utility as self-indicating active esters for use in solid phase peptide synthesis.[56] The active esters are easily prepared by reaction of DCCI with the compound (32), although contamination with the compound (33) may occur. The rigorous purification which is occasionally required may be minimised by carrying out the preparation in THF as opposed to the more polar DMF. The side product (33) is virtually eliminated by preformation of the adduct between the Fmoc amino acid and DCCI prior to the addition of (32). The rate of reaction in solid phase synthesis was found to be very similar to symmetrical anhydrides and five times greater than the corresponding pentafluorophenyl ester. The liberated triazine (32) acted as a very useful indicator, as when a free amino group was present on the resin a bright yellow colouration was produced, however this colouration fades as the acylation is completed. The new method was examined in a synthesis of acyl carrier protein as a test peptide using the macroporous resin. The first residue was attached using the pentafluorophenyl active ester in the presence of dimethylaminopyridine, and all other residues were attached using the DHBT esters.



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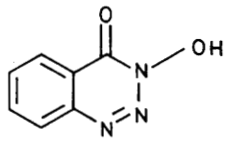

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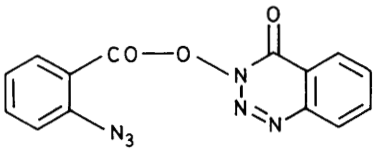

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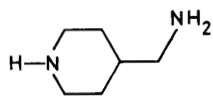

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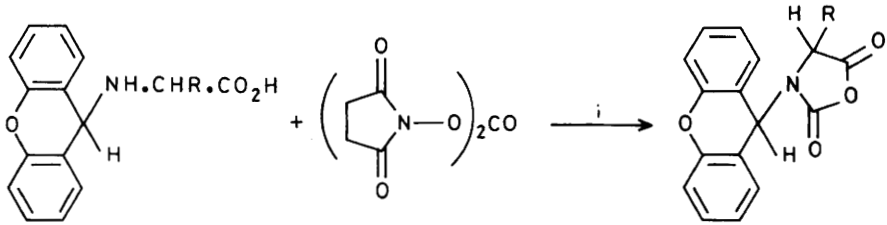
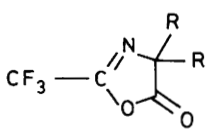


(35) (36)

Reagent : i, NEt_3/THF

Scheme 13



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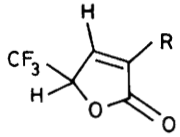


(38)

Intermediate deprotection of the Fmoc group was carried out by 20% piperidine in DMF and the very long reaction time for the final valine residue was similar to previous syntheses using other methods of activation. Deprotection with 95% aqueous TFA gave a high yield of product.

Until recently amino acid chlorides were considered to be unsuitable for the synthesis of polypeptides due to the high risk of racemisation attendant in the use of this method of activation. Recently Fmoc amino acid chlorides have been prepared[57] by reaction of the Fmoc amino acid with thionyl chloride. The resulting acid chloride could be recrystallised from dichloromethane in hexane and analytically pure samples could be obtained which were indefinitely stable under dry conditions at room temperature. The stability of the acid chlorides was tested by the addition of methanol which allowed differentiation between the methyl ester (produced by reaction with the acid chloride) and the free acid which might have been produced prior to reaction by hydrolysis. The method was applied to the synthesis of model peptides and a racemisation test using hplc indicated that less than 0.1% racemisation had occurred. Intermediate deblocking of the Fmoc function was carried out with 4-amino-methyl-piperidine (34) as on reaction with the fulvene liberated from Fmoc cleavage an adduct is produced which may be extracted into pH 5.5 phosphate buffer.

Xanthenyl-N-carboxy anhydrides have been prepared by the route outlined in Scheme 13.[58] The xanthenyl amino acid (35) is reacted with bis-(succin-imidyl)carbonate in the presence of triethylamine to give the xanthenyl-N-carboxyanhydride (36). This route alleviates the dangers associated with the use of phosgene and eliminates the acid conditions which would remove the highly labile xanthenyl group. The weakly acid hydroxysuccinimide which is liberated in this reaction does not give rise to any difficulties. Attempts to use carbonyl-diimidazole led to racemisation, but the corresponding bis-N-succinimidyl carbonate did not cause racemisation. The procedure was used to prepare 2,4-dimethoxybenzyl and 4,4-dimethoxybenzhydryl NCA derivatives. The resulting NCA derivatives could then be used in peptide synthesis with the amino protecting group being removed by acidolysis. Attempts to prepare the corresponding trityl NCA from trityl alanine resulted in the generation of Trt-Ala-ONSu, this result being due to steric hindrance of the bulky trityl protecting group.

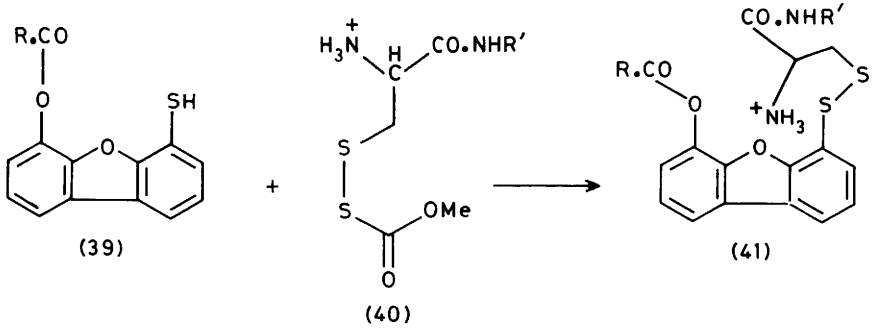
The syntheses of peptides containing dialkyl glycine is difficult due to the problems associated with activation.[59] Recently, however, it has been shown that oxazolinones of the type (37) are useful for the incorporation of dialkylglycine residues. These compounds may be coupled with amino acid esters or amides to give trifluoroacetyl peptides. The trifluoroacetyl group may eventually be removed by treatment with sodium borohydride. A range of oxazolinones of type (37) are available, as the trifluoromethyl-4-alkyl oxazolin-5-(2H)-ones (38) may readily be alkylated by active alkyl halides in the presence of mild base. The efficiency of this procedure was demonstrated by the synthesis of dibenzyl glycine peptides.

A detailed account of peptide synthesis using prior thiol capture has been published.[60-62] In this work 4-hydroxy-6-mercapto-dibenzofuran is used as a template precursor to bring about intramolecular disulphide bond formation according to the general route outline of Scheme 14. The acylated benzofuran (39) may be reacted with an activated cysteine derivative (40) to give an intermediate (41) which may be used to form an amide bond by intramolecular acyl transfer. The resulting peptide may be cleaved from the template by disulphide bond reduction.

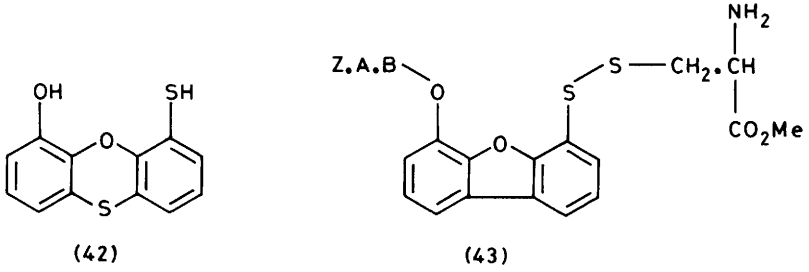
A number of linker templates has been investigated[61] including the 4-hydroxy-6-mercapto-dibenzofuran and 4-hydroxy-6-mercapto-phenoxythiin (42). It was found that the geometry of the dibenzofuran compound produced an effective molarity which was two orders of magnitude greater than the compound (42), and that the effective local concentration was approximately 5M. A Hammet ρ-value of 2.6 was observed for the intramolecular acyl transfer. The effect of varying the steric bulk of the acyl component on the rate of amide bond formation was also studied,[62] from the rates of intramolecular isomerisation of methyl-S-[4-(N-benzyloxycarbonyl-L-aminoacyloxy)-6-dibenzofuranylsulphenyl]-L-cysteines (43) to methyl-(N-benzyloxycarbonylaminoacyl)-S-(4-hydroxy-6-dibenzofuranylsulphenyl)-L-cysteines. It was found that Ala, Asn, Asp, Arg, Gly, Lys and Thr had a $t_{\frac{1}{2}}$ in DMSO at 25°C between two and four hours, and that Pro and Val were an order of magnitude slower, also aspartic acid showed intramolecular general base catalysis.

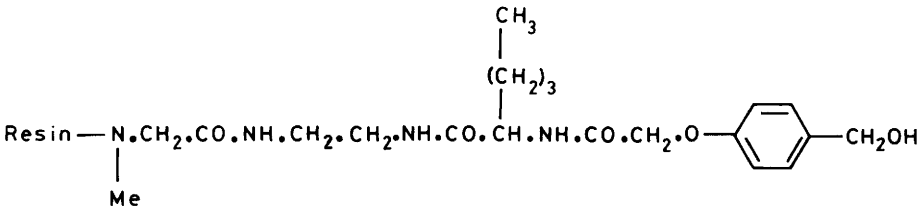
2.4 Racemisation

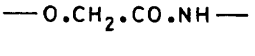
The effect of tertiary amines, anions and cations on the yield and optical purity in DCCI mediated couplings has been studied.[63] The synthesis



Scheme 14







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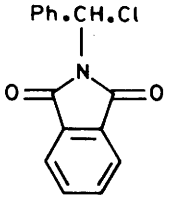


(46)

of Ac-Phe-Leu-OMe was used as a model system, and the production of side products and racemisation levels were monitored by hplc. It was found that the salts of tertiary amines supppress N-acyl urea formation and that the ability of hydroxybenztriazole to suppress racemisation was blocked by the presence of tribenzylamine. A kinetic investigation showed that racemisation was not linked to the production of 5-(4H)-oxazalone formation, and other activated forms of Ac-Phe-OH were suspected as being implicated in the racemisation, although the presence of such compounds was not demonstrated. Interestingly N-trityl amino acid hydroxybenzotriazole esters were found to resist racemisation at relatively high temperatures (between 30 and 80°C), even in the presence of excess triethylamine.[64]

The addition of polyacrylic acid and its derivatives to DCCI couplings has been studied as a means of suppressing racemisation.[65] It was found that polyacrylic acid has the ability to suppress racemisation in the coupling of Z-Ala-Phe-OH to valine methyl ester, and following these results with model systems it is proposed as a useful additive for more general use in DCCI mediated couplings.

The racemisation of π-benzyloxymethyl histidine during synthesis has been evaluated.[66] In this work the (3-10) sequence of [D-Trp^6]-LHRH was assembled by solid phase synthesis on a 1% cross-linked chloromethyl polystyrene resin. Boc group protection was used throughout and Boc-His(π-Bom)-OH was coupled as the final residue to give D-Trp^6-LHRH (2-10). The final cleavage from the resin was using liquid ammonia and total deprotection was effected using HF/anisole. The coupling of Boc-His(π-Bom)-OH was studied using isobutyl-chloroformate, carbonyldiimidazole/HOBt and DCC/HOBt. Enzymic digestion of the cleaved product and hplc using the D-histidine derivative as a standard, demonstrated that practically no racemisation had occurred during the synthesis. It should be noted, however, that approximately 1% of D-histidine was present due to initial racemisation during the preparation of the derivative.

2.5 Repetitive Methods of Peptide Synthesis

Both Fmoc/polyamide and Boc/polystyrene based solid phase procedures are widely used. There is a slight degree of overlap in that some advocates of the Boc/polystyrene method have adopted the Fmoc strategy for protection using a variety of resin linkages.

An improved method for the deprotection of cysteine-containing peptides using anhydrous HF has been developed.[67] In this procedure, with cysteine protected as the 4-methylbenzyl derivative, cleavage under conditions of low acidity (S_N2) using HF/DMS (1:3) gives little or no deprotection of 4-methylbenzyl cysteine, however other benzyl based protection is removed. High (90%) HF/anisole gave a 79% cleavage but when p-cresol and p-thiocresol were used in place of anisole (90:5:5) at 0°C deprotection was virtually quantitative. The formation of sulphoxide from S-4-methylbenzyl cysteine was also evaluated and it was found to be at the level of approximately 0.15% per residue added. As this sulphoxide derivative is resistant to high HF cleavage, two methods for the reduction were studied. In the first an intermediate concentration of HF was used (40 - 60%) at 0°C for four hours and in the second a mixture of TFA/dimethylsulphide and dichloromethane (45:10:45) containing tetraethyl ammonium chloride monohydrate, gave reduction of both free and resin-bound sulphoxide with a $t_{\frac{1}{2}}$ of approximately thirty-five minutes. Both procedures also reduced methionine sulphoxide to methionine. It should be noted that reduction of sulphoxides should be carried out prior to the high HF deprotection procedure.

The efficiency of the low HF (S_N2) method was demonstrated by the elegant synthesis of the (41) residue peptide thymosin-β_9.[68] In this case HF/dimethylsulphide/p-cresol (25:65:10) at 0°C was used to cleave the protecting groups and linkage to the phenylacetamidomethyl resin. In this instance a four hour cleavage was required as a time-course study showed that the usual two hour procedure at 0°C was not adequate. Treatment under high HF conditions (90%) with or without scavengers gave an extremely heterogeneous product, whereas the modified low HF procedure gave analytically pure material after a one-step purification.

In a synthesis of Cerebratulus lacteus toxin AIII (1-16) and (63-95)[69] both the benzhydrylamine and PAM resins were studied. Final deprotection by HF gave a good yield with an excellent level of purity by hplc. Efficient HF deprotection was also observed in a synthesis of squash seed trypsin inhibitor CMTI (III)[70] in which a PAM linkage was again used.

A simplified procedure for carrying out simultaneous multiple hydrogen fluoride cleavages on protected peptide resins has been developed.[71] By this method fifty individual cleavages per day may be carried out. A specially designed apparatus containing many compartments allows 2 x 25 cleavages per

day to be carried out. Hplc comparison of the deprotections with those obtained by single HF deprotection showed the products to be virtually identical, and thus it is now possible to carry out multiple deprotections without the risk of loss of purity.

Solid state deuterium nuclear magnetic resonance has been used to study the nature of swollen Merrifield resin extended with a pendant glycine chain.[72] The nature of the polystyrene matrix with pendant glycine chains is basically that of a comb-type graft copolymer. Partial aggregation of glycine residues occurs when more than five residues are present, and at that stage overlap may occur allowing the formation of a helical polyglycine II structure. The polystyrene backbone is immobilised due to the additional effective cross-linking produced by aggregation of the glycine chains.

An interesting comparison between the Boc/polystyrene and Fmoc/polyamide methods in the synthesis of α-human atrial natriuretic factor has been made.[73] In this study a conventional Boc/benzyl synthesis was carried out on a PAM polystyrene resin using HF/p-cresol for final deprotection. Under these conditions some partial removal of the acetamidomethyl protection may be observed, however this may be eliminated using the low/high procedure. The alternative synthesis employed Fmoc/t-butyl protection, and employed a Kieselghur-polydimethylacrylamide flow support. In this case piperidine was used for intermediate deprotection and TFA was used for final deprotection from the resin and cleavage of side-chain protecting groups. Purification in both cases was carried out by hplc and in the latter assembly using the polyamide method it was noted that the Arg(Mtr) cleavage was rather slow and sixteen hours were required in order to bring about complete deprotection. The final result was that the yield and activity of the material obtained from both syntheses was very similar.

In a hybrid synthesis employing Fmoc for amino protection and a chloromethyl polystyrene (1% divinyl benzene) support,[74] a portion of the Herpes simplex glycoprotein D antigenic domain was synthesised. In this synthesis several difficult couplings were encountered and six triple acylations were required at various points in the chain assembly. Deprotection from the resin was achieved using HBr/TFA/thioanisole over forty-five minutes; under these conditions cleavage of the Mtr group from Arg(Mtr) was satisfactory and the crude product was purified by chromatography on Sephadex G25.

As mentioned above, trifluoromethane sulphonic acid has now become a popular method of cleavage both in solution and solid phase synthesis.[40] An interesting mechanistic study of the removal of benzyl based protecting groups by the trifluoromethane sulphonic acid/trifluoroacetic acid/dimethylsulphide has been carried out.[75] Under different conditions both the S_N1 and S_N2 mechanisms were observed when employing TFMSA/TFA/DMS, and the kinetic study showed a sharp changeover point from S_N2 ($A_{al}2$) to S_N1 ($A_{al}1$) when the concentration of TFMSA in TFA increased. The activity of dimethylsulphide required for S_N2 deprotection was determined from ^1H proton nuclear magnetic resonance, and the acidity calculated from the Yates McClelland equation. Changeover points occurred when the activity of DMS approached zero. The S_N1 mechanism predominates at high acidity with low DMS activity and S_N2 at moderate acidity with high DMS activity. A composition diagram showing regions of S_N1 and S_N2 mechanism was established. From these considerations it was deduced that TFMSA/TFA/DMS/m-cresol (10:50:30:10) is a practical mixture for cleavage. However, when Trp(For) is present ethanedithiol must also be added (10:50:30:8:2). Under these conditions the formyl group is removed with other groups and methionine sulphoxide is reduced to methionine. The presence of Arg(Tos) or Cys(Mbz) requires a separate S_N1 deprotection.

Two definitive papers relating to Fmoc/polyamide synthesis under flow conditions have been published.[76,77] The first[76] describes the solid phase synthesis of adeno virus pentadecapeptide using polydimethylacrylamide which had been polymerised within the pores of a macroporous Kieselghur support. The paper describes both manual and semiautomated synthesis and the hardware and monitoring procedure for using the flow conditions are described. The support is a fine free flowing powder which permits rapid diffusion of solvent and negligible back pressure. Pentafluorphenyl active esters were investigated as an alternative to the symmetrical anhydride procedure and a spectrophotometric record of the acylation and deprotection cycles was obtained. The deprotection liberates the piperidine adduct of dibenzylfulvene, the area of which may give some measure of ther overall efficiency of the coupling cycle, particularly when used in conjunction with the record from previous cycles. The profile of such peaks also gives information regarding the kinetics of the deprotection. The resin, as prepared, is functionalised with sarcosine methyl ester, this function is reacted sequentially with ethylene diamine, Fmoc-norleucine symmetrical anhydride and the trichloro-

phenyl active ester of the linkage agent in the presence of HOBt, to give the assembled matrix shown (44). The first residue is then introduced as the symmetrical anhydride in the presence of dimethylaminopyridine. The remaining residues were generally introduced as symmetrical anhydrides and on occasion double acylation was used following either the Kaiser test or testing with trinitrobenzene sulphonic acid. There was little loss of peptide from the resin during the synthesis, as indicated by amino acid ratio to the marker residue norleucine, and final cleavage was achieved with 95% TFA, giving an 82% recovery. The hplc of the crude product was excellent. Computer modelling of the acylation kinetics with curve fitting is described and it is probably best to regard this procedure as being an attractive qualitative or semiquantitative procedure which provides reassuring information, however there are many pitfalls to quantitative use.

A related procedure was used for the synthesis of melanin concentrating hormone.[77] The same linker was used and the acylation cycles were checked using the Kaiser test and trinitrobenzene sulphonic acid monitoring. The first residue was introduced as the Fmoc symmetrical anhydride in the presence of dimethylaminopyridine and subsequent residues were again introduced as symmetrical anhydrides. On some occasions, however, repetition of the coupling cycle was required and the molar excess was increased four-fold. Test deprotections indicated that a five hour TFA treatment was required in the presence of anisole and ethane dithiol. This gave 90% cleavage from the resin and all the protecting groups, with the exception of the acetamidomethyl group were removed. It should be noted that this acid treatment should completely cleave all the peptide from the resin but on this occasion there was an indication that there was a residual peptide content which remained attached to the resin. It is believed that a new linkage had been formed through the intermediacy of the benzyl carbonium ion which had in turn been generated from the resin during deprotection; the carbonium ion reacting with either tryptophan, Cys(Acm) or methionine. In this synthesis $\underline{N}^{G}$-methoxytrimethylbenzene sulphonyl arginine was used, and it was noted that when arginine and tryptophan occur together in the same peptide then on deprotection major by-products may occur; however, if Arg(Mtr) and tryptophan are present separately no problems occur.

In another Fmoc based synthesis a polyacrylic resin was used as the matrix.[78]. In this synthesis a glycolamidic ester link (45) was used for

linking the peptide to the resin. The acrylic copolymer was prepared by copolymerisation of N-acrylyl-β-alanine, ethylene-bis-acrylamide and N-acrylyl-β-alanine methyl ester. The latter component allows functionalisation and may be subsequently reacted with ethylene diamine and then treated with bromoacetic anhydride to give the glycolamide linkage. Functionalisation at the level of 0.42 mM/g was achieved, and the first residue was attached as the corresponding caesium salt. The linkage is stable to piperidine solutions and the Fmoc procedure was therefore adopted for the synthesis of the (32-36) sequence of thymopoietin. Deprotection of acid labile groups may be achieved on the resin and final saponification under basic conditions gives the free peptide.

A synthesis of the (44-45) sequence of toxin II from Androctonus australis hector[79] has been carried out on the bromomethyl-Nbb resin. With this resin the experimental conditions of the photolysis are critical in order to obtain a high yield of the final product. In this case precipitation from DMF with water, followed by hplc purification gave sufficiently pure product. The synthesis starts from Boc-Pro-oxymethyl-Nbb resin which was prepared through the caesium salt procedure. The best solvent for photolysis was found to be 20% 2,2,2-trifluoroethanol in dichloromethane, however, other combinations of dichloromethane, DMF, methanol and trifluoroethanol can be used. A photograph shows the large swollen spheres of resin in the ideal solvent system and it should be noted that the photolytic yield varies considerably with solvent composition due to the swelling of the beads, a 76% yield was achieved on photolysis. In another synthesis of toxin II fragments (35-43) and (32-34)[80] the p-alkoxybenzyl resin was used in conjunction with the Fmoc procedure, Tyr(cHex), Cys(Acm), and Ser(Bzl) were used for side-chain protection. On this occasion the first residue was incorporated using the symmetrical anhydride over one hour in the presence of 10% dimethylaminopyridine, and the remaining residues were incorporated as their symmetrical anhydrides. Acidolysis of the peptide resin, using TFA/ anisole, gave an 80-85% cleavage, and hplc purification on Ultrasphere C_{18} with DMF/CH_3CN/H_2O/propionic acid (24.5:45.5:30:0.1) gave an excellent purification.

On a number of occasions diketopiperazine formation has been observed at the dipeptide stage during solid phase synthesis. In a recent paper[81] which employs Fmoc amino acids for synthesis on a p-alkoxybenzyl resin, piperidine

(50% piperidine in DMF) was found to be an extremely efficient catalyst for the formation of diketopiperazine at the dipeptide stage. The reaction was more rapid in DMF than in dichloromethane and the reaction could be used preparatively; the use of the very acid labile Bpoc protecting group for the second residue during an Fmoc based synthesis on the p-alkoxybenzyl resin prevents diketopiperazine formation at the dipeptide stage.

The novel and ingenious thiocarboxyl segment coupling procedure has been employed on two occasions.[82,83] In the first paper a synthesis of α-inhibin-92 was described; the synthesis proceeds through the assembly of three fragments, the [GlyS^{34}]-α-1B-(1-34) sequence, the CF_3.CO[GlyS^{35}]-(35-65) sequence and the Msc (66-92) sequence. The free peptides were each reacted with citraconic anhydride and the Msc group removed from the citraconylated Msc (66-92). Combination of the second and third fragments was then achieved using silver nitrate in the presence of hydroxysuccinimide. Following purification the trifluoroacetyl function was removed with 10% hydrazine hydrate and the resulting peptide coupled to the first peptide in the sequence again by use of silver nitrate in the presence of hydroxysuccinimide in 50% DMF/water. The final deblocking to remove the citraconamide function was achieved under mild acidic conditions, and a product of high purity was obtained.

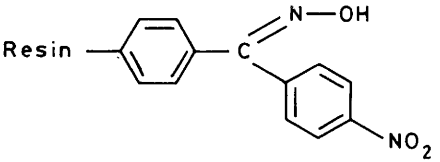
Three fragments and the total 104 residue sequence of S-carbamoyl-methyl bovine apocytochrome-c were prepared.[83] The synthesis of the fragments was carried out according to the usual procedure using Boc-Glu(OBzl)-resin or Boc-Gly-S-resin. Boc symmetrical anhydrides were used throughout and the aspartic acid side-chain was protected by the cyclopentyl group in order to minimise succinimide formation. Intermediate protection with citraconic anhydride and condensation, using silver nitrate in the presence of hydroxy-succinimide, were again used and a product of high purity was obtained. An improved preparation of the benzhydrylamine resin has been carried out.[84] In this modified synthesis N-(α-chlorobenzyl)phthalimide (46) was reacted with polystyrene in the presence of a Lewis acid catalyst. On reaction with 1% styrene/divinylbenzene $SnCl_4$ gave 0.92 mM/g of the benzhydralamine polymer after deprotection of the initial product with hydrazine. The choice of the Lewis acid catalyst is critical and it is interesting that the commonly used $AlCl_3$ gave no product. By varying the stoichiometry precise control of the functionalisation may be achieved between the limits 0.1-1 mM/g.

Phosphoseryl peptides have been prepared on a solid phase support,[85] using a conventional synthesis commencing from Boc-Leu-polystyrene resin. The phosphoserine residues were incorporated using the Boc-Ser[PO.$(OPh)_2$]-OH derivative which was mentioned earlier,[25] employing 40% TFA in dichloromethane for intermediate deblocking of the Boc functions. Cleavage from the resin was achieved using $Pd(OAc)_2/H_2$ at 60 Psi over twenty-four hours using 40% TFA/DMF as the solvent, these conditions also removed benzyl-based side-chain protection. Hydrogenolysis in the presence of PtO_2 in 40% TFA/DMF then efficiently removed the two phenyl ester groups from phosphoserine. A novel solid phase synthesis of dehydropeptides has also been reported[86] which uses Boc-dehydroamino acid N-carboxyanhydrides for chain extention.

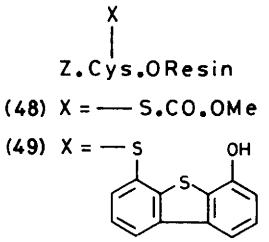
Chloroalanyl antibiotic peptides have been synthesised using the oxime resin (47).[87] The first Boc amino acid was incorporated using DCCI then the Boc-aminoacyl support was deprotected using 4 M HCl in dioxan and coupled with a second Boc amino acid anhydride. The resin linkage was then cleaved by aminolysis with the t-butyl ester of the carboxy terminal amino acid residue giving the fully protected tripeptide, making use of the activation provided by the oxime linkage.

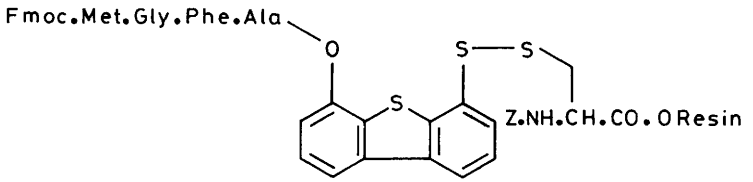
As mentioned previously[60], prior thiol capture has now proved to be a viable method of peptide synthesis. In order to adapt this procedure to solid phase synthesis N-benzyloxycarbonyl-S-methoxycarbonylsulphenyl cysteine was attached to a polystyrene resin to give the support (48). This activated resin was then reacted with 4-hydroxy-6-benzo-dibenzofuran to give the modified resin (49) which has an exposed phenolic hydroxyl group. This hydroxyl group was then acylated by the symmetrical anhydride procedure and chain extension carried out using the Fmoc group for amino group protection. The resin linked peptide (50) was then cleaved by reduction of the disulphide bond, and the methoxycarbonylsulphenyl derivative of Boc-Cysteine methyl ester reacted with the resulting thiol. Removal of the Boc function then allowed acyl transfer and preparation of the C-terminal cysteine peptide.

Little work has been published on the liquid phase method in the past year, although it should be noted that the initial reaction of Boc amino acid potassium salts with p-bromomethylbenzoyl polyethylene glycol methyl ether may be catalysed by 18-crown-6. By this method benzyl linked polyethylene glycol supports may be used in liquid phase synthesis.[88]

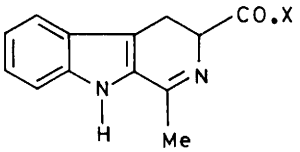


(47)





(50)



(51)

H.Arg.Gly.Phe.Leu.X

(52) X = NH_2

(53) X = OBu^t

2.6 Enzyme-mediated synthesis and semisynthesis

An excellent general review of the use of enzymes in organic synthesis has been published.[89] This review covers both peptide bond formation and the resolution of D,L amino acids in addition to a large number of other applications, a total of 388 references are cited. A more specific review of the use of enzymes in peptide synthesis has been published[90] and a general discussion of catalysis in peptide synthesis including enzymatic synthesis has also appeared.[91]

The use of chymotrypsin in enzyme catalysed synthesis has continued to receive attention.[92-94] Acetyl tryptophan and acetyl tyrosine ethyl esters have been used as donors in aqueous ethanol and under these conditions the enzyme was fairly stable over one hundred hours, it was also observed that chymotrypsin maintains its conformation even in 90% ethanol.[92].

Chymotrypsin has also been used in the preparation of fluorescent peptide derivatives[93] based on the use of 1-methyl-3,4-dihydro-β-carboline-3-methyl carboxylic acid (MDC) (51). If MDC methyl ester and glycine were condensed using chymotrypsin as catalyst at a pH between 6.5 and 9 a ten-fold excess of glycine amide was required. The most efficient synthesis was achieved when the D-isomer of MDC was used, but D-amino acid amides were either inactive or very poor as incoming amino components.

Occasionally protecting groups which are removed by proteolytic enzymes are employed, and on one occasion carboxamidomethyl esters have been studied.[94] Z or Boc amino acid or peptide carboxyamidomethyl esters are able to act as substrates for either papain or chymotrypsin, leading to removal of the carboxamidomethyl ester. The kinetics of enzymatic synthesis using $Z\text{-Phe}_2\text{-OMe}$[95] and Z-Asp-Phe-OMe[96] have been studied in aqueous/organic biphasic systems, the effects of varying pH and the organic solvent composition were monitored. The effect of introducing inhibitors such as phosphoramidon during thermolysin catalysed synthesis has also been studied[97] in the enzymic synthesis of Z-Asp-Phe-OMe.

The specificity of both the amino component and the carboxyl acceptor have been studied[98] and the pH dependence of thermolysin catalysed synthesis has been examined. The initial rates of synthesis were found to be identical to that of hydrolysis, and the overall rate of synthesis was found to be independent of the pK_a of the amino function of the acceptor amino component. Deprotonation of the attacking nucleophilic component was found to be non-rate

limiting. The effect of glycerol on thermolysin has been studied[99] both in relation to its hydrolytic activity and to its efficiency in peptide bond formation. In this study the effect on hydrolysis was measured by degradation of azacaesine or furylacryloyl glycyl leucine amide; increasing the glycerol concentration reversibly inhibited hydrolytic activity with both substrates. Introduction of glycerol to the synthetic medium in the condensation of Z-Asp-OH with phenylalanine methyl ester facilitated production of Z-Asp-Phe-OMe, however the initial rate of reaction was reduced. It was noted that the glycerol apparently stabilised the thermolysin against thermal denaturation.[99]

Thermolysin has also been used as a catalyst in the enzymatic synthesis of asparagine containing peptides,[100] benzyloxycarbonyl and p-methoxybenzyl-oxycarbonyl protection being used for asparagine. In general a low enzyme concentration (4×10^{-6} M) was used and reaction time, pH and organic solvent composition were varied. Leucine-benzyl ester, phenylalanine-benzyl ester and valine-benzyl ester were employed as attacking nucleophiles and in general the yield exceeded 85% over five hours. It was of interest to note that the tripeptide formation proceeded more rapidly than dipeptide formation.

Trypsin is also frequently used as a catalyst in peptide bond formation, and in DMSO/DMF using immobilised trypsin[101] it was observed that acylation with phenylalanine generally required a relatively high concentration of nucleophile in comparison to when the non-immobilised enzyme was used. A number of amino acid amide, methyl ester and t-butyl esters were employed in addition to two dipeptides, and on all occasions a high concentration of nucleophile was required.

The stereochemical preference of enzymes for various substrates is usually taken to be rather rigid, however, recently the enzyme catalysed synthesis of peptides containing D-amino acids has been examined.[102,103] Z-Tyr-OMe and Z-Phe-OMe were coupled to a number of D-amino acid esters and amides using α chymotrypsin in soluble and immobilised forms. The reaction was examined by monitoring the Z amino acid ester by hplc and isolation could be carried out by gel filtration on Sephadex LH20. In both misible or immisible organic/aqueous systems, the reaction is kinetically controlled and virtually irreversible. No side reactions or racemisation of the D-amino acid esters was observed and the rate of peptide bond formation was one hundred times that of hydrolysis by water. The efficiency of D-amino acid

esters as nucleophiles was only 10% of that of the corresponding L-amino acid esters, however two synthetically useful systems were developed.[102] From X-ray analysis it appears that at the active site of chymotrypsin there is little possibility of variation in the configuration of the acyl component, however the site which is occupied by the amino component is more flexible, allowing accommodation of D-amino acid esters. Reaction yields were generally high except when sterically hindered amino acids were used, this being similar to the findings using L-amino acids. In order to improve the long term stability of the enzyme an imobilised form was produced in which the Met^{192} was oxidised to the corresponding sulphoxide. The irreversibility of the synthetic reaction makes the isolation of products very straightforward and starting materials do not need to be of a high level of purity; indeed inorganic residues and amino acids may be tolerated as impurities.

The serine endoprotease, alcalase, has been used in the highly enantioselective hydrolysis of racemic amino acid methyl and benzyl esters.[104] The enzyme has the advantage that it has a high turnover rate and a low cost of immobilisation. It is stable at high temperatures and tolerates the presence of organic solvents, thus a high concentration of substrate (0.5 M) and a temperature of 45°C may be used.

Both trypsin and chymotrypsin have been immobilised by conjugation to alumina using glutaraldehyde.[105] The alumina bound enzyme is capable of synthetic use in organic solvents, and the synthesis of Ac-Trp-Leu-NH_2 was demonstrated. Polyethylene glycol modified chymotrypsin has also been prepared[106] and it was observed that the enzyme had considerable solubility in organic solvents, and could be used as a catalyst for amide bond formation using such solvents.

A new approach to peptide bond formation using enzymes has been developed using aminoacyl t-RNA synthetases (ARS).[107] Aspartic acid, histidine, leucine and tyrosine aminoacyl t-RNA synthetases where purified from thermophilic bacteria and the tyrosine (ARS) was condensed with leucine amide in the presence of ATP to give the dipeptide H-Tyr-Leu-NH_2. The tyrosine (ARS) did not have very strict specificity requirements and even D-amino acids could be accepted as nucleophiles. More interestingly it was observed that D-tyrosine may be converted to the corresponding adenylate and thus using H-D-Leu-NH_2, the dipeptide H-D-Tyr-D-Leu-NH_2 could be prepared. The absence of stereospecificity was thought to be due to the lack of the transfer RNA in

this system. This unfortunately means that the rate of reaction is rather low, but it is advantageous in that it allows preparation of peptides containing D residues. Unfortunately the lifetime of the enzyme is not particularly great and a relatively high concentration of the incoming amino acid amide is required.

Semisynthesis has now become an established method for the preparation of larger peptides and proteins. Using transpeptidation [Asp^{B30}]-insulin[108], [Thr^{B30}]-insulin (human insulin)[109] and [Leu^{B30}]-insulin[110] have been prepared. In these three examples trypsin[108], immobilised trypsin,[109] and both Achromobacter protease I and trypsin[110] were used. In the synthesis of [Leu^{B30}]-insulin, [des-Ala^{B30}]-insulin was isolated after treatment with trypsin. Leucine butyl ester was then introduced, again by the use of trypsin, using a large excess of the amino component. The concentration of organic solvent was adjusted to between 35 and 50% and a neutral pH was employed, giving a maximum product yield of 90% under optimal conditions. Both Achromobacter protease I and trypsin were studied, and it was found that the first enzyme gave a more efficient reaction at 37°C and that at 25°C trypsin was lower in efficiency.

A synthesis of [Ala^{B5}]-insulin[111] has been reported, in which $\underline{N}^{\alpha A1}$, $\underline{N}^{\varepsilon B29}$, Msc_2 insulin was degraded by Edman degradation at the amino terminus of the B chain. Degradation was carried out to leucine-6 and subsequently Boc-Asn-Gln-Ala-OOBt was coupled. After TFA removal of the Boc group, Boc-Phe-Val-OOBt was then coupled giving the protected [Ala^{B5}]- insulin. Deprotection by acidic and basic treatment gave the free insulin analogue. It was found that this analogue could not be straightforwardly prepared by coupling of the modified Boc-(1-5)-OOBt sequence, as coupling to leucine-6 gave very poor yields .

The [des-B^{23-30}]-insulin formed by tryptic digestion of insulin has been used by a number of workers. Unprotected Des-octapeptide insulin has been coupled with Gly-Phe-Phe in order to prepare the Des(B^{26-30}) insulin. The synthesis was carried out using DMSO/1,4-butandiol/water.[112] The reaction was faster and simpler than when di-Boc-desoctapeptide insulin was used, as evidenced by monitoring by hplc. Some oligomerisation of the Gly-Phe-Phe was observed and an additional product containing an extra bond between Gly^{A1} and Arg^{B22} was observed. This compound which may be regarded as porcine des-(23-63)-pro-insulin was also independently studied in some detail.[113]

[Leu^{B25}]-des-B(26-30)-insulin has also been synthesised.[114] Digestion of insulin with trypsin gave the des-B(23-30)-insulin; the A^1 and B^1 residues were protected as their Boc derivatives for the synthesis of the [Leu^{B25}]-insulin amide and with the Msc protecting group for the synthesis of [Leu^{B25}]-insulin t-butyl ester. Trypsin catalysed coupling of the suitably protected des-octapeptide insulin with the tetrapeptide amide (52) or the tetrapeptide t-butyl ester (53) were carried out using trypsin employing DMF/glycerol containing 20% water. In both cases a coupling efficiency in the order of 90% was obtained. TFA deprotection of the coupled product using (53) gave the corresponding deprotected [Leu^{B25}]-analogue of Msc_2-[Leu^{B25}]-, Des-B(26-30)-insulin.

Relaxin has also been modified semisynthetically;[115] in this work native relaxin was treated with 4.2 equivalents of citraconic anhydride at pH 7. During this reaction the pH fell to 4.5 causing partial hydrolysis of the citraconyl functions on the side-chain ε-amino function of lysine. The resulting product was the $\underline{N}^{\alpha A1}$ citraconyl relaxin. This compound was reacted with Msc-ONSu giving the $\underline{N}^{\varepsilon A7,\varepsilon A16,\varepsilon B8}$ Msc_3, $\underline{N}^{\alpha A1}$ citraconyl insulin. Subsequent removal of the citraconyl function from the A^1 position then allowed selective modification at the amino terminus of the A chain by Edmann degradation. Modification of residues in the region of the A chain amino terminus could then be made by incorporation of modification through active esters. The methylsulphonylethoxy carbonyl (Msc) group was advantageous as its hydrophilic nature and its stability under acidic conditions made the Tris-Msc derivative particularly useful in this work.

Norvaline, glutamine and lysine have been substituted for the glutamic acid residue at position-66 in cytochrome c, using a semisynthetic approach, which utilises the condensation between the (1-65) and (66-104) fragments.[116] Related work has also been carried out[117] on the synthesis of [Hse^{65}]-cytochrome c analogues, in this work modification at a number of positions was considered and the (66-104) fragment, which was required for the synthesis was assembled through the intermediacy of protected hydrazides.

3.0 SYNTHESIS

A number of syntheses have been reported in the previous section (2.0) and in the main, large peptides are now generally produced by solid phase synthesis. Semisynthesis has also played an important part, but solution synthesis is certainly declining.

Some peptides have been synthesised for specific purposes using solution methods. For example, a conventional solution synthesis using minimal protection with benzyl-based side-chain protection was used for the synthesis of the cyclic eicosapeptide ($Gly^{88,90}$)-(82-101)-β-subunit of human chorionic gonadotropin.[118] Deprotection using HBr/TFA/m-cresol was employed and the resulting bis-Acm peptide was oxidised to give the cyclic product.

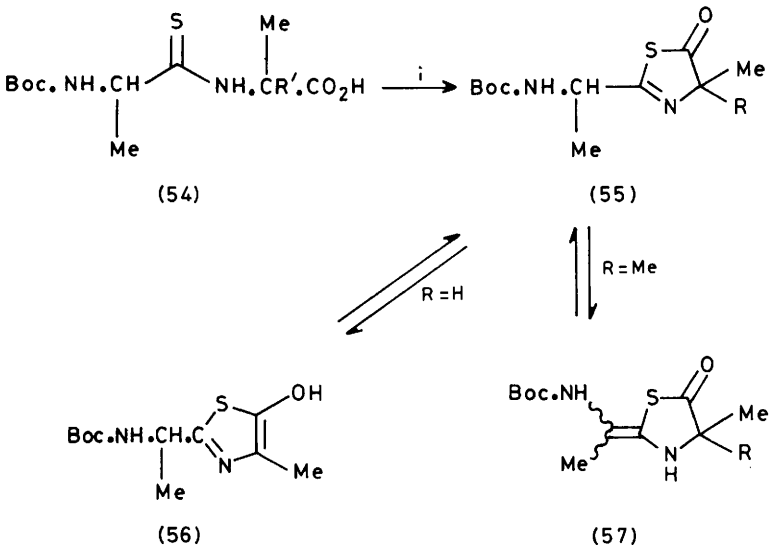
The syntheses of S-cerevisiae cytochrome c fragments (71-108) and (70-108)[119] were also carried out by solution synthesis, employing benzyloxycarbonyl for amino protection with side-chain t-butyl based protection. The fragment condensations were effected using the azide procedure.

Many modifications of the peptide backbone have been made, and details of the majority are provided in the Appendices; additional details are provided in Chapter 4. One rather unusual structure, however, was the analogue of cyclo(Gly-D-Phe-Pro) containing the thiomethylene isostere.[120] The compound was prepared by solid phase synthesis employing Boc-Pro-$\psi(CH_2S)$-Gly-OH, which permitted the introduction of a thiomethylene linkage as an amide bond isostere. Following assembly on the resin HF/anisole/methylethylsulphide was used for final cleavage, and cyclisation in 85% yield was achieved using DPPA/HOBt/DMAP.

Interest in thionated peptides has also increased since the introduction of Lawessons reagent, and an interesting study of Aib peptides which contain thioamide bonds has been made.[121] In this synthesis the compound (54) was activated to give the thiazlactone (55), which was then aminolysed to give the modified tripeptide. When R' was equal to methyl, isomerisation to the structure (57) took place and the chirality of the first residue was lost, and when R was equal to H, tautomerisation to the structure (56) took place and the chirality of the terminal residue was lost as indicated in scheme 15.

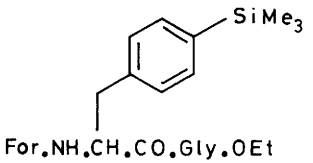
The modification of amino acid residues previously incorporated into a peptide sequence is also of interest, particularly when it permits the introduction of isotopic labels. In a simple case a (4-trimethylsilyl)-phenylalanine peptide has been converted to the corresponding 4-iodo peptide.[122] The peptide (58) was treated with silver tetrafluoroborate and iodine at $0^{\circ}C$ to achieve replacement of the trimethylsilyl group by iodine. This method has the potential for the specific introduction of a radiolabelled iodine at the 4-position of a phenylalanine residue.

The introduction of tritium has also been demonstrated in a synthesis of [^{3}H-Nle^{11}] substance P (5-11).[123] In this work L-2-amino-4-hexynoic acid was



Reagent: i, Piv.Cl

Scheme 15



(58)

incorporated in the synthesis, coupling by the mixed anhydride procedure. The alkenyl peptide was then transformed into a tritiated norleucine peptide by catalytic tritiation.

4 Appendix I : A List of Syntheses Reported in 1986

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

Peptide	Ref.
4.1 Natural Peptides, Proteins, and Partial Sequences	
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Adenovirus pentadecapeptide	76
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Ahpatinin	125
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Angiotensin II amino terminal extended analogue	133
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Apolipoprotein CII/fragments	134
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[$Ala^{14,38}$]-Aprotinin	135
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Ascamycin	136
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Aspartame	138

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Atrial natriuretic peptide (96-126) 142
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Bleomycin analogues (BYML6) 147
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Bradykinin potentiating peptide 148
Bradykinin potentiating peptides 149
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Human calcitonin 152
Salmon calcitonin fragments 153
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Fragments of human calcitonin gene related peptide 39
Human calcitonin gene related peptide 32
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Calmodulin; Ca binding domains II and III 155
Calmodulin/melittin complex 156
Calmodulin binding peptide
Calmodulin binding peptide 157
Canaline
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Carpetimycin A
Carpetimycin A 159
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$\underline{N}^{\alpha 1}$-acetyl, $\underline{C}^{\alpha 25}$-methylamide of the $\underline{N}$-terminal pentacosapeptide of Cowpea Chlorotic Mottle Virus 165
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α-$\underline{N}$-Hydroxysulphonyl-(Nle^{28,31})-CCK-PZ (26-33) 170
[^{3}H-Nle^{28,31}]-CCK-PZ (27-33) 171
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[Met(O)^{192}]-Chymotrypsin 103
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(Phe^4, Arg/Lys^8]-Vasopressin 365

4.2 Sequential Oligo- and Poly-peptides

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5 Appendix II : Amino Acid Derivatives Useful in Synthesis

The list of derivatives is divided into two groups, the coded and the uncoded amino acids. The following unusual abbreviations are employed:

1-Adamantyl	Ad
L-Amino adipic acid	Aha
L-Amino-hexynoic acid	Aha
9-Anthryl alanine	Anl
Cycloheptyl ester	OChp
Cyclooctyl ester	OCoc
Cyclopentyl	OCpe
Dimethylphosphinyl	Dmp
homo-Glutamic acid	Hgu
β-l-menthyl ester	Men
1-and 2-Naphthylalanine	Nal
2-(2'-Pyridyl)-ethyl ester	OPet
Selenohomocysteine	Shc
Selenomethionine	Set
p-Trimethyl-ammoniophenyl ester	OTAP

*1 Measured between 12 and 33°C

Compound	M.P./°C	$[\alpha]_D^{*1}$	Conc./g 100cm^3	Solvent	Ref.
5.1 Coded Amino Acids					
Alanine					
Boc-Ala-OPet	Oil	-32.9	1.5	MeOH	23
Boc-Ala-OTAP	159-161	-40.0	1	H_2O	53
Fmoc-Ala-Cl	112-114	+4.03	1	CH_2Cl_2	57
Fmoc-Ala-NH.NH.Boc	65	-44.9	1	MeOH	165
Arginine					
Fmoc-Arg(Adoc)$_2$-OH	154-156	+19.2	1	$CHCl_3$	28
H-Arg(Adoc)$_2$-OH	174-177	-30.3	1	$CHCl_3$	28
Z-Arg(Adoc)$_2$-OH	118-120	-17.7	1	$CHCl_3$	28
Aspartic acid					
Boc-Asp(OCpe)-OH	112-114	+27	1	MeOH+ 1.2≡DIPEA	83

Boc-Asp(OBzl)-OPet	Oil	-12.6	3.2	MeOH	23
Boc-Asp(OChp)-OBzl	52-54	- 3.2	1	MeOH	18
Boc-Asp(OChp)-OH	96-98	- 3.4	1	MeOH	18
Boc-Asp(OCoc)-OBzl	40-41	-17.5	1	MeOH	18
Boc-Asp(OCoc)-OH	84-86	- 2.0	1	MeOH	18
Boc-Asp(OH)-OPet.DCHA	Oil	- 1.7	1.0	MeOH	23
Boc-Asp(OMen)-OBzl	90-91	-48.4	1	MeOH	21
Boc-Asp(OMen)-OH	135-137	-17.7	1	MeOH	21
Boc-Asp-OPet	Oil	-16.1	1.0	MeOH	23
Fmoc-Asp(OBzl)-Cl	50-53	+ 7.9	1	CH_2Cl_2	57
Cysteine					
Fmoc-Cys(Bzl)-Cl	104-105	-13.5	1	CH_2Cl_2	57
Z(OMe)-Cys(Ad)-OH.DCHA	146-148	- 7.1	0.9	MeOH	31
Z(OMe)-Cys(Ad)(O)-OH	81-84	-30.0	0.8	MeOH	31
Boc-Cys(Npys)-OH	153-155	-	-	-	33
$Cl^-H_2^+$-Cys(Npys)-OH	188-190	+140.5	1	MeOH	33
Glutamic acid					
Boc-Glu(OBzl)-OPet	Oil	-15.1	1.0	MeOH	23
Boc-Glu(OChp)-OH.DCHA	117-119	0	1	MeOH	19
Boc-Glu-OPet	Oil	-21.4	1.0	MeOH	23
H-Glu(OChp)-OH	184-186	0	0.7	MeOH	19
Msc-Glu(OBu^t)-OTcp	116-118	-24.9	1.43	MeOH	117
Z-Glu(OChp)-OBzl	38-40	-18.9	1.6	MeOH	19
Z-Glu(ODnp)-OBzl	102.5-103.5	- 2.6	1	$CHCl_3$	487
Z-Glu(OMe)-OBzl	49-51	+ 1.2	1	$CHCl_3$	487
Z-Glu(ONSu)-OBzl	105-107	+ 4.7	1	$CHCl_3$	487
Z-Glu(OPfp)-OBzl	60-70	+ 2.8	1	$CHCl_3$	487
Z-Glu(OTcp)-OBzl	118-119	+ 0.3	1	$CHCl_3$	487
Z-Glu(SPfp)-OBzl	58-61	+ 7.6	1	$CHCl_3$	487
Z-Glu(SPy)-OBzl	84-85	+ 6.3	1	$CHCl_3$	487
Z(OMe)-Glu(OChp)-OH.DCHA	127-129	- 1.8	0.5	MeOH	19
Z(OMe)-Glu(OChp)-ONb	43-45	-15.3	0.6	MeOH	19
Z(OMe)-Glu-ONb.DCHA	145-147	0	0.2	MeOH	19
Glutamine					
Boc-Gln-OPet	Oil	+22.4	1.4	MeOH	23
Z(OMe)-Gln-OBzl	120-124	- 5.3	0.8	DMF	19
Glycine					
Boc-Gly-OPet	Oil	-	-	-	23
Fem-Gly-OH	198	-	-	-	15
Fem-Gly-OMe	Oil	-	-	-	15
Fmoc-Gly-Cl	108-109	-	-	-	57

Trt-Gly-ONSu	138-139	-	-	-	117
Z(OMe)-Gly-OTAP	167-168	-	-	-	53
Isoleucine					
$Cl^-H_2^+$-Ile-OBzl	51-52	- 4	2	1M HCl	17
Fmoc-Ile-Cl	103-104	+ 2.43	1	CH_2Cl_2	57
Leucine					
$Cl^-H_2^+$-Leu-OBzl	135	- 8	2	1M HCl	17
Fmoc-Leu-Cl	80-81	- 4.24	2	CH_2Cl_2	57
Lysine					
Boc-Lys(Z)-OPet	Oil	-16.2	1.0	MeOH	23
$Cl^-H_2^+$-Lys(Me_3,Cl^-)-OH	143-147	+10.4	0.07	H_2O	610
$Cl^-H_2^+$-Lys(Me_2)-OH	-	+16.7	0.05	0.1M HCl	610
Fmoc-Lys(Msc)-OH	113-116	-10.7	1	DMF	9
Fmoc-Lys(Tos)-OH	134-136	-11.2	1	DMF	10
Fmoc-Lys(Tos)-OPfp	113-118	- 5.9	1	$CHCl_3$	10
Fmoc-Lys(Z)-Cl	67-68	+ 0.53	4	CH_2Cl_2	7
H-Lys(Msc)-OBzl	94-95.5	- 1.5	1	MeOH	117
H-Lys(Msc)-OH	222-224	+15.0	1.04	1M HCl	117
Z-Lys(Boc)-OPet	Oil	-11.6	2.0	MeOH	23
Z-Lys(Fmoc)-OH	86-88	- 6.7	1	DMF	10
Z-Lys(Fmoc)-OPfp	127-128	- 8.0	1	$CHCl_3$	10
Z-Lys(Me_2)-OH	-	- 0.6	0.25	AcOH	610
Z-Lys(Msc)-NH.NH.Boc	110	-13.6	0.8	Dioxan	117
Methionine					
Fmoc-Met-Cl	118-119	- 9.76	1	CH_2Cl_2	57
Msc-Met-NHNH.Boc	105.5-106	-29.5	1	MeOH	117
Phenylalanine					
Boc-Phe-OPet	Oil	- 4.4	1.0	MeOH	23
Fmoc-Phe-Cl	120-121	+15.7	1	CH_2Cl_2	57
Z(OMe)-Phe-OTAP	110-115	-50.5	1	DMF	53
Proline					
Boc-Pro-OBu^t	Oil	-35.5	0.9	$CHCl_3$	37
Boc-Pro-ONb	Oil	-45.1	1.24	$CHCl_3$	37
Boc-Pro-OPet	Oil	-30.4	2.0	MeOH	23
Fmoc-Pro-Cl	93-94	-39.9	1	CH_2Cl_2	57
H-Pro-NH.NH.Boc	117-118	-	-	-	117
Troc-Pro-OMe	Oil	-52.5	1	EtOH	37
	b.p.124°/1mm Hg				
Z-Pro-NH.NH.Boc	71-74	-87.0	1	MeOH	117
Z-Pro-OPet	Oil	-32.3	2.0	MeOH	23
Z(NO_2)-Pro-OMe	Oil	-39.8	1	EtOH	37

Serine					
Boc-Ser-OPet	Oil	-14.6	2.0	MeOH	23
Fmoc-Ser(Bzl)-Cl	96-98	+11.1	1	CH_2Cl_2	57
Fmoc-Ser(Bu^t)-OPfp	67-71	- 3.7	1	Dioxan	10
Z-Ser-OPet	Oil	+ 5.7	0.3	MeOH	23
Threonine					
Boc-Thr-OPet	Oil	-19.9	1.2	MeOH	23
Tryptophan					
$AcO^-H_2^+$-Tyr(Dmp)-OH	180(dec)	- 6.8	1	AcOH	27
Boc-Trp-OPet	Oil	- 3.4	2.0	MeOH	23
Tyrosine					
Boc-Tyr(Bzl)-OPet	Oil	+ 1.6	1.3	MeOH	23
Boc-Tyr(Dmp)-OBzl	117-118	- 8.0	1	EtOH	27
Boc-Tyr(Dmp)-OH	75	+18.5	1	EtOH	27
Boc-Tyr-OPet	Oil	- 1.06	1.3	MeOH	23
Fmoc-Tyr(Bzl)-Cl	114-115	+16.5	1	CH_2Cl_2	57
Fmoc-Tyr-OH	98-107	-19.9	1	DMF	10
Fmoc-Tyr-OPfp	152-156	-15.7	1	$CHCl_3$	10
Z-Tyr(Bzl)-OTAP	187-190	- 7.3	1	DMF	53
Z-Tyr(Bu^t)-$NH.NH_2$	117-118	- 2.4	1.33	MeOH	117
Z-Tyr(Dmp)-OBzl	98-99	-14.4	1	MeOH	27
Valine					
$Cl^-H_2^+$-Val-OBzl	142-143	-10.6	2	1M HCl	17
Fmoc-Val-Cl	111-112	+ 5.5	1	CH_2Cl_2	57
Z-Val-OPet	Oil	-13.6	4.0	MeOH	23

5.2 Uncoded Amino Acids

Alanine					
$TosO^-H_2^+$-Ala-(ol)	98-100	+ 9.3	2	MeOH	493
Aminoadipic acid (Aad)					
Z-Aad-OH	133-135	-10.2	1	MeOH	350
4-Aminocrotonic acid					
(Z)-4-Aminocrotonic acid	144-145	-	-	-	535
(Z)-4-Phthalimidocrotonic acid	179-181	-	-	-	535
L-Amino-hexynoic acid (Aha)					
Boc-Aha-NH_2	140-141	+26.2	1	MeOH	567
Boc-Aha-OBzl	Oil	-26.0	1	MeOH	567
Boc-Aha-OCH_3	Oil	+ 3.7	1	MeOH	567
Boc-Aha-OH	83-84	+34.2	1	MeOH	567
Boc-Aha-OH.DCHA	161-163	+47.5	1	MeOH	567

Boc-Aha-ONp	83-84	-38.0	1	MeOH	567
Boc-Aha-OSu	123-124	-39.5	1	MeOH	567
Nps-Aha-OH	131-133	+ 9.3	1	MeOH	567
Nps-Aha-OH.DCHA	184-185	+20.2	1	MeOH	567
Z-Aha-OH	107-108	+43.5	1	MeOH	567
Z-Aha-OH.DCHA	145-146	+27.3	1	MeOH	567
Z-Aha-ONp	107-108	-34.6	1	MeOH	567
9-Anthrylalanine (Anl)					
Ac-D-Anl-OMe	208-209	-	-	-	495
D-Cl$^-$H$_2^+$-Anl-OMe	229	-	-	-	495
D-Anl-OH	215-218	-	-	-	495
γ-Carboxyglutamic acid (Gla)					
Boc-Gla-OH	-	-20.3	1	MeOH	38
Homo-glutamic acid (Hgu)					
D-Boc-Hgu(NH_2)-OH	161-162	+ 4.5	1	MeOH	36
L-Boc-Hgu(NH_2)-OH	162-163	- 4.9	1	MeOH	36
L-Boc-Hgu(N_2H_3)-OBu^t	Oil	-13.9	1	MeOH	36
L-Boc-Hgu(N_2H_3)-OH	59-64	- 1.4	1	MeOH	36
D-Boc-Hgu-OBu^t	75-77	+23.9	1	MeOH	36
L-Boc-Hgu-OBu^t	75-77	-24.8	1	MeOH	36
D-Boc-Hgu-OH	126-127	+ 7.6	1	MeOH	36
L-Boc-Hgu-OH	126-127	- 7.8	1	MeOH	36
D-Boc-Hgu(OBzl)-OH.DCHA	120-122	-10.0	1	MeOH	36
L-Boc-Hgu(OBzl)-OH.DCHA	122-124	+ 9.9	1	MeOH	36
Homoserine					
Trt-Hse(Trt)-OH.DCHA	172-174	+18.6	2	MeOH	26
Isoleucine					
TosO$^-$H$_2^+$-Ile$^-$(ol)	134-136	+ 4.8	2	MeOH	493
Leucine					
TosO$^-$H$_2^+$-Leu$^-$(ol)	116-117	+ 4.7	2	MeOH	493
Methionine					
TosO$^-$H$_2^+$-Met$^-$(ol)	138-139	+ 4.7	2	MeOH	493
1- and 2-Naphthylalanine (Nal)					
Cl$^-$H$_2^+$-1-Nal-OH	159-161	-	-	-	526
Cl$^-$H$_2^+$-1-Nal-OH	143-145	-	-	-	526
Z-1-Nal-OH	150-152	-	-	-	526
Z-2-Nal-OH	165-170	-	-	-	526
Phenylalanine					
Boc-L-β-Phe-OH	118-119	+44.7	1	MeOH	174
Boc-L-β-Phe-OPac	92-93	+24.0	1	MeOH	174
Cl$^-$H$_2^+$-β-Phe-OPac	169	-19.0	1	MeOH	174

Fmoc-D-Phe-Cl	119-120	-15.5	1	CH_2Cl_2	57
$TosO^-H_2^+$-Phe^-(ol)	132-134	- 6.9	2	H_2O	493
Proline					
Boc-Pro-(ol)	57-58	-	-	-	120
$Br^-H_2^+$-cis-Pro(4Cl)-OMe	157-158	- 2.8	1	MeOH	174
Pyroglutamic acid					
Boc-Glp-OBu^t	Oil	-35.1	0.9	$CHCl_3$	37
Boc-Glp-OMe	72-72.5	-44.3	1	EtOH	37
Boc-Glp-ONb	Oil	-193	1	$CHCl_3$	37
Troc-Glp-OMe	90-91	-52.8	1	EtOH	37
Z-Glp-OMe	Oil	-41.3	1	EtOH	37
$Z(NO_2)$-Glp-OMe	Oil	-32.8	1	EtOH	37
Selenohomocysteine (Shc)					
Boc-Shc(Bzl)-OBzl	84.5	-45.5	1	MeOH	595
Selenomethionine (Set)					
Boc-Set-OBzl	Oil	-34	1.1	MeOH	595
Tryptophan					
Tfa-Trp(2Br)-OH	150-152	-	-	-	603
Tfa-Trp(2Br)-OMe	154.5-155.5	- 5.5	0.69	MeOH	603
Tfa-Trp(2Cl)-OH	148-150	+27	0.52	H_2O/1.5 ≡$NaHCO_3$	603
Tfa-Trp(2Cl)-OMe	150-152	- 4.3	0.63	MeOH	603
Valine					
$TosO^-H_2^+$-Val-(ol)	100-101	+ 7.1	2	MeOH	493

6 Appendix III : Purification Methods

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

Technique	Ref.
6.1 High-Performance Liquid Chromatography	
Activated carbamates for amino acid derivatisation for hplc	611
Hplc of amino acid derivatives on chiral phases	612
Hplc of amino acid enantiomers	613
Hplc of amino acids and peptides with crown ether mobile phase	614
Hplc separation of amino acid enantiomers on a chiral stationary phase	615

Hplc of Dns amino acid enantiomers on a chiral phase 616
Hplc of phosphorylated amino acids 617
Separation of o-phthalaldehyde amino acid derivatives by hplc on C_8 columns 618
Hplc of angiotensin analogues 619
Hplc purification of angiotensin II 620
Hplc of aspartame 621
Hplc of alpha- and beta-aspartyl peptides 622
Hplc of bradykinin 623
Hplc of betaine esters 624
Hplc on chemically bonded chiral phases 625
Hplc on chiral stationary phases 626
Hplc separation of phenyl hydantoin enantiomers on a chiral stationary phase 627
Chiral adsorbents (cyclodextrin) for hplc 628
Hplc separation of enantiomers using copper complexes 629
Hplc of peptides on charged cyclodextrins 630
Hplc of Ac-cysteine 631
Hplc separation of dipeptides 632
Hplc of dopamine derivatives 633
Hplc enantiomer separation 634
Hplc of beta endorphin 635
Hplc of enkephalin pseudopeptides 636
Hplc fractionation of peptides on Asahipak GS320 637
Hplc of bovine growth hormone 638
Hplc of bovine growth hormone 639
Hplc of human growth hormone 640
Hplc of GRF 641
Prediction of Hplc retention time from hydrophilicity scale 642
Hplc of LHRH and GRF analogues 643
Hplc separation of abnormal haemoglobins 644
Hplc of dialkylglycine enantiomers on reverse phase 645
Hplc of indican and tryptophan 646
Hplc analysis of bovine, porcine and human insulins 647
Hplc separation of mono-iodoinsulins 648
Hplc of isopeptides 649
Hplc for microsequencing 650
Separation of neuropeptides on hplc 651
Hplc of oxytocin 652
Hplc of peptides on an adsorbed reverse phase 653

Hplc separation of phenylthiohydantoins 654
Hplc of PTH derivatives on Nova Pak C18 655
Quantitation of Pro and Hyp by hplc 656
Retention times of peptides on hplc 657
Retention times of peptides on hplc 658
Hplc of phosphoserine and tyrosine sulphate 659
Hplc purification of human somatomedin-C 660
Hplc of substance P fragments 661
Hplc of TRH analogues 662
Hplc of tyrosine derivatives 663
Large scale hplc of urogastrone 664
Hplc of enantiomers using urea derivatives of valine 665

6.2 Gas-Liquid Chromatography
GC of amino acid enantiomers on heliflex/chirasil-Val 666
GC determination of asparagine 667
Capillary GC for separation of Asp enantiomers 668
GC of difluoromethylornithine 669
GLC of peptides on chirasil Val 670
GLC of peptides using a nitrogen sensitive detector 671
GC separation of tert-butyl-dimethylsilyl derivatives 672
Glc of tert-butyldimethylsilyl derivatives of Glp, Glu, Asp, Gln and Asn 673

6.3 Other Chromatographic Methods
Chiral stationary phases for enantiomer separation 674
GC/MS of tert-butyl dimethylsilyl derivatives of amino acids 675
Gel permeation chromatography on poly-Glu(OMe) or poly-Glu(OBu^t) 676
Liquid chromatography of endorphins 677
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Resolution of 2,4-dinitrophenyl and dansyl amino acid enantiomers 682
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3
Analogue and Conformational Studies on Peptide Hormones and Other Biologically Active Peptides

BY J. S. DAVIES

1 Introduction

A significant increase in activity under this category has appeared this year, with some augmentation coming from the 9th American Peptide Symposium[1] although overlap with full papers in Journals necessitated careful selection from this source. New pseudo-peptide bond analogues have again appeared this year and are a continuing field of interest.

There is clear evidence for the increasing impact of solid-phase synthesis on structure-activity studies, and a wide variety of substitutions into peptide sequences are now routinely made by this technique. The availability of a larger number of analogues increases the precision of the deductions but everyone working in the field should heed the advice[2] of the Glaxo group on problems associated with the quality and purity of peptide products. The scope of the "bislactim ether method" for the asymmetric synthesis[3] of non-proteinogenic amino acids promises to provide interesting new amino acids for analogue preparation. A useful update of the methods available for synthesising N-hydroxyamino acids and peptides has appeared[4].

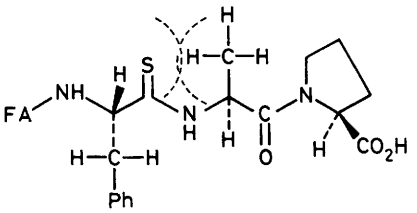
2D Nuclear magnetic resonance techniques provide improved precision for assessing interatomic distances and have contributed greatly to conformational analysis of peptides in solution. The developments of the 1980's in this field have been reviewed[5]. Biological structure-activity relationships (QSAR) combined with the use of molecular graphics are slowly gaining insights for working models of receptor sites of unknown structures, and could be useful in drug research[6].

2 Peptide-backbone Modifications

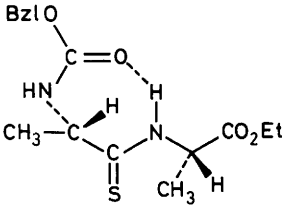
2.1 *ψ*[CSNH]-Analogues. - Regioselective thiation of selective amide bonds using the Lawesson reagent has produced[7] the

monothiated analogues Boc(S)Ala-Aibψ[CSNH]-(S)-Ala-OMe, Acψ[CSNH]-(S)-Ala-Aib-(S)-Ala-OMe, Ac-(S)-Ala-Aibψ[CSNH]-(S)-Ala-OMe and the dithiated analogue Boc-(S)-Alaψ[CSNH]-Aibψ[CSNH]-(S)-AlaOMe. But Boc-(S)or(R)-Alaψ[CSNH]-Aib-(S)-Ala-OMe had to be obtained by coupling H-(S)-Ala-OMe to (S)-2(1-Boc-amino)ethyl-4,4-dimethyl-3,3-thiazol-5(4H)-one. A more soluble form of the Lawesson reagent has provided[8] a number of thiated analogues which have provided some interesting information on the features of the Angiotensin Converting Enzyme (A.C.E) active site. In this work the N-furylacryloyl(FA) derivatives FA-Pheψ[CSNH]-Gly-Pro and FA-Pheψ[CSNH]-Ala-Pro show quite different hydrolysis rates in the presence of A.C.E. with the latter derivative not being hydrolysed over extended time periods. It is suggested that the increased length of the thiocarbonyl bond together with the larger size of the S atom precludes the side-chain methyl in (1) from lying in the plane of the sulfur atom. A C-terminal thioamide analogue of TRH appears[9] to have biological properties very similar to TRH itself. Physical methods, including circular dichroism and nuclear magnetic resonance techniques using different solvents, on N-Z-endothiodipeptide esters containing Ala, Val, Phe and Pro residues suggest[10] that conformer (2) predominates in non-polar solvents while a more extended form (3) exists in dimethyl sulfoxide. A trans configuration of the thioamide bond in dipeptide esters has also been confirmed[11] using nuclear Overhauser enhancement (n.O.e.) studies.

2.2 ψ[NHCO]-Retro-inverso Analogues. - The retro-enantio analogue of AM-Toxin 1 has been synthesised[12] but shows no toxic effects on apple leaf. A similar lack of biological response was found in analogues of the Toxin with an extra methyl group on the dehydroalanine residue. Glutathione analogues having either the C-terminal, or both amide bonds reversed[13] have been shown to be substrates for several enzymes that participate in the biochemistry of the sulfhydryl group. Two diastereoisomers of the cyclic retro-inverso enkephalin analogue (4) have been subjected to high-field n.m.r. and computer simulation studies[14] which show that the γ-NH of the D-diaminobutanoic acid residue is H-bonded in both isomers.



(1)



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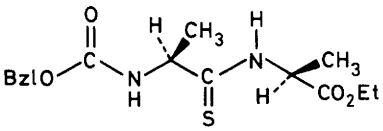

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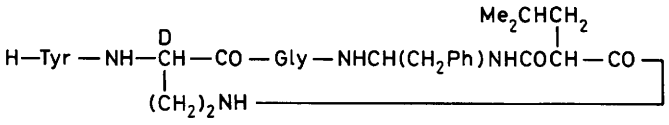

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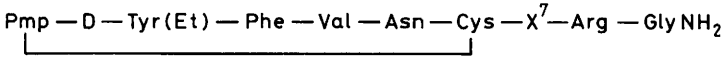
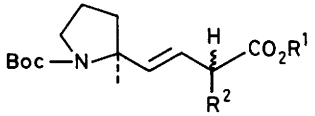


Pmp = ββ—cyclopentamethylene—β—mercaptopropionic acid

(5)



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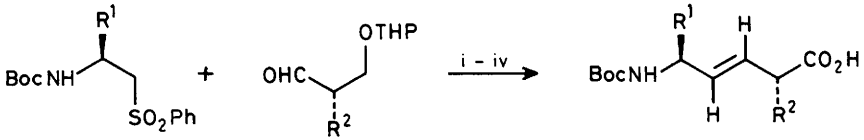


i, CH_3Li/Et_2O, -78°C ii, $CH_3OAl—Bu^i_2$ iii, Na(Hg), Na_2HPO_4

iv, $PyrH^+OTs^-$; $CrO_3 \cdot H_2SO_4$

Scheme 1

2.3 ψ[CONR]-N-Alkylated Analogues. - Substitution of either proline, N-methylalanine or sarcosine into position 7 of the vasopressin antagonists (5) gave analogues[15] which were equipotent with their alanyl or glycyl counterparts. Thus the conformational constraints of an N-alkyl bond at position 7 is not a necessary factor for potent V_2- receptor antagonism, and it is confirmed that position 7 could be deleted altogether without loss of antagonism. A spectroscopic investigation[16] on a series of N – methylated model dipeptides has compared data with non-methylated analogues and reveals a strong preference in homochiral sequences for the β-VI folded conformation with a middle cis-amide bond while heterochiral sequences retain a β-II folded conformation with a trans amide bond.

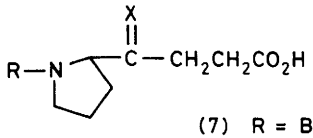
2.4 ψ[CH_2NH] - Amino Methylene Analogues. - In a comprehensive study[17] on the effect of residue substitutions on the biological activity of the C-terminal tetrapeptide of gastrin, enzymatic stability advantages have been seen by replacing the Trp-Leu amide bond by [CH_2NH]. The aminomethylene unit was also inserted at various positions in the gastrin tetrapeptide sequence, as for example in Boc-Trpψ[CH_2NH]-Met-Asp-PheNH_2, Boc-Trp-Metψ[CH_2NH]-Asp-PheNH_2. Substitution between Trp and Met gave compounds which stimulated gastric secretion in the rat in vivo, but substitution between Met and Asp did not bring about any activity. The bonds between Pro-Leu or Leu-Gly in Pro-Leu-GlyNH_2, the C.N.S.-active hormone, have been replaced[18] by [CH_2NH] by condensation of the appropriate protected aldehyde with the amine and reducing the resulting Schiff base in situ with sodium cyanohydridoborate, with a benzyloxycarbonyl group affording further protection of the secondary amine. ^{1}H and ^{13}C N.m.r. studies on the Pro-Leuψ[CH_2NH]-GlyNH_2 analogue confirmed a β-turn conformation similar to the parent hormone, which also coincides with the biological activity results.

2.5 ψ[CH=CH] - Ethylenic Isosteres. - The challenge of formulating convenient methods of making stereochemically pure ethylenic isosteres has been taken up by three groups. Units such as (6) have been prepared[19] via selective hydroboronation and oxidation of conjugated en-ynes followed by selective α-alkylation

of the resulting β,γ-unsaturated acid. It has been suggested[20] that the alkylation step to make (6)(R^1=H,R^2 = $PhCH_2$) can be carried out very efficiently using the trimethylsilyl (TMS) or tetrahydropyranyl (THP) esters (i.e. with R^1 = TMS or THP at the precursor stage) because of the ease of hydrolysis of the intermediates. Separation of the diastereoisomeric forms of (6) was possible using h.p.l.c. and confirmation of the configuration at the introduced asymmetric centre was made by hydrogenation and cyclisation to cyclic analogues whose configuration could be confirmed by high-field n.m.r. A general synthetic route has been used[21] to prepare isosteres of Tyr-Ala, Phe-Phe and Leu-Leu, the key step in the synthesis being that summarised in Scheme 1. This permits the fully stereocontrolled preparation of these isosteres in optically active form.

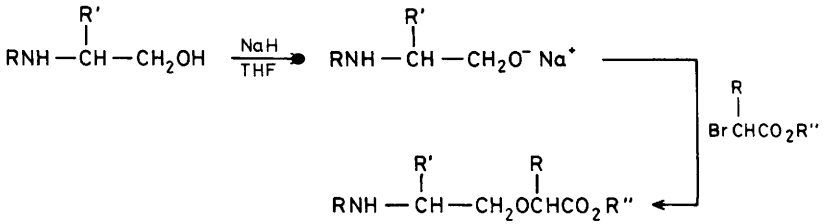
2.6 ψ[$COCH_2$]—Keto Methylene Analogues. - Two papers[22,23] describe the use of synthetic schemes based on a Dakin-West reaction to produce analogues. N $^{\alpha}$-Acyl amino acids, dipeptides and tripeptides possessing a free carboxyl function at the C-terminus are heated[22] at 40-50°C with Et_3N/4-dimethylaminopyridine and the appropriate anhydride. A decarboxylation step then yields carboxy terminal ketones. Ketomethylene and α,β-dehydroketo-methylene units have been incorporated[23] in syntheses to produce analogues of substance P such as [pyroGlu^6(RS)Phe^8ψ[$COCH_2$]Gly^9] substance P_{6-11}, which is a full agonist with 70% potency relative to [pyroGlu^6] substance P_{6-11} in guinea pig ileum assay. The analogue is also a potent inhibitor of substance P degradation. Ketomethylene analogues (7) and (8) of Pro-Gly have been attached[24] to benzhydrylamine resin and used in solid-phase synthesis.

2.7 ψ[CH_2O]—Methyleneoxy Analogues. - This group offers a polar, flexible, proteolytically resistant surrogate whose geometrical correspondence to the amide bond appears to be better than the [CH_2S] group[25]. A choice of at least two methods[25] of synthesis, depending on the need for preservation of chirality, are now available as summarised in Schemes 2 and 3. Such units have been incorporated into substance P(SP) and enkephalin analogues, which display considerable biological activity in vitro. Preliminary

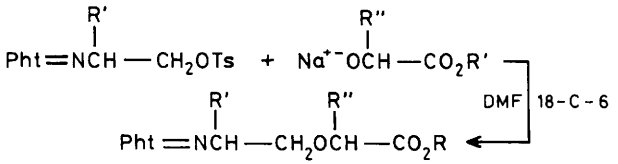


(7) R = Boc , X = O

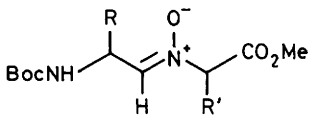
(8) R = Fmoc , X = OCH_2CH_2O



Scheme 2



Scheme 3



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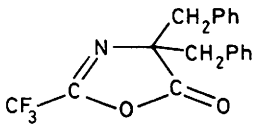

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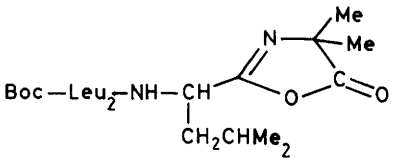

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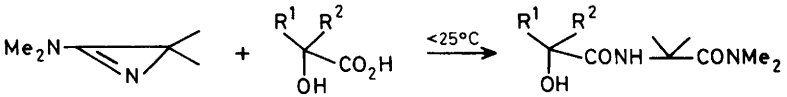


Scheme 4

details of an alternative approach to $\psi[CH_2O]$ insertion have also appeared[26]. The activity of [pyroGlu^6-trans Hyp(Bzl)$^8\psi[CH_2O]$-Gly^9] SP_{6-11} is five times[27] that of [pyroGlu^{6}trans Hyp(Bzl)^8]SP_{6-11}.

2.8 ψ[CON(OH)]-N-Hydroxy Analogues. - N-Hydroxyenkephalin analogues[28] with the sequence H-Tyrψ[CON(OH)]-X-Gly-Phe-LeuOH where X = Gly, L-Ala or β-Ala have been shown by n.m.r. studies to possess a type I β-turn in DMSO. The analogues are resistant to aminopeptidase M, and the Ala-analogue seems to have a more lasting potency than Leu-enkephalin.

2.9 ψ[C≡C] - Acetylene Isostere. - Prospects of incorporating an alkyne unit have been improved by the synthesis[29] of a Leu-Gly alkyne isostere. A key step involved a Mitsunobu reaction of $Me_2CHCH_2CH(OH)C{\equiv}CCH_2CH_2OTHP$ with phthalimide to give $Pth\text{-}NCH(CH_2CHMe_2)C{\equiv}CCH_2CH_2OTHP$ followed by a Jones oxidation of the tetrahydropyranyl (THP) ester to the acid.

2.10 $\psi[CH{=}N^+\text{-}O^-]$ - Nitrono Isostere. - The interesting series of isosteres (9) where R is Me, $CHMe_2$ or CH_2Ph and R^1 is Me, $CHMe_2$, CH_2CHMe_2 or CH_2Ph have been made[30] by treating aldehydes L-BocNHCH(R)CHO with L-HONHCH(R^1)-CO_2Me in ether in the presence of calcium chloride.

2.11 ψ[CH_2S] - Thiomethylene Analogues. - A full n.m.r. study[31] has been carried out on cyclo-[D-Phe-Pro-Gly-Pro$\psi[CH_2S]$-Gly-] formed by cyclisation of its linear precursor using diphenylphosphoryl azide/1-hydroxybenzotriazole. The cyclic pseudopeptide adopts both β- and γ-intramolecular bonds in $CDCl_3$ but in contrast to its all-amide counterpart it exhibits cis/trans proline bond isomerism on addition of DMSO.

2.12 Phosphono-Peptides. - A large-scale synthesis[32] of the potent antibacterial agent alafosfalin, L-Ala-L-AlaP(O)$(OH)_2$, has been reported and, during studies on other analogues, several more potent analogues such as L-Nva-L-AlaP(O)$(OH)_2$ and L-Ala-L-Ala-L-AlaP(O)$(OH)_2$ have been found. More stability and a broader spectrum of activity is shown by more stabilised analogues such as

H-Sar-L-Nva-L-Nva-L-AlaP(O)$(OH)_2$. A comprehensive in vitro test study[33] of a series of synthesised alafosfalin analogues has also revealed that C-terminal norvaline, or an α,α-dimethylglycyl phosphonic acid residues give analogues with antibacterial activity comparable to alafosfalin. Synthesis of Boc-NH-$CH(CO_2H)CHROP(O)(OPh)_2$, with R=H or Me, has provided[34] intermediates for use in solid-phase synthesis of phosphorylated serine- and phosphorylated threonine-containing peptides related to the C-terminus of rhodopsin. Diphenylmethyl has been used[35] as a phosphorus-protecting group in the synthesis of P-terminal phosphonodidepsipeptides.

2.13 Aza-Peptides. - Well established carbazate technology has yielded[36] an elastase inhibitor, $HO_2CCH(Me)$-NHCO-Val-Gly-Azala-OBzl which is 60-fold more potent than an earlier reported inhibitor, Ac-Ala-Ala-AzalaONp. Human leucocyte elastase inhibitors carrying azanorvaline at the C-terminus and a number of analogues also containing amide bonds replaced by CH_2S have been prepared[37]. Both types inhibited elastase, the most potent being $MeOCOCH(CH_2CHMe_2)$NHCO-Val-Pro-Aznva-OPh with IC_{50} = 0.28μM, K_i 0.02μm). The results have enabled some broad conclusions about the specificity of the enzyme to be drawn:- (a) the P_1 binding site can accommodate both linear α-azanorvaline and branched chain amino acid residues, (b) a Pro residue in P_2 appears to be better than Gly, Ala or Val, (c) P_3 to P_5 seems to require the larger hydrophobic groups for interaction with enzyme and (d) amide bonds between P_1 and P_2, and P_2 and P_3 may be replaced by CH_2S without greatly affecting potency. Stereospecific amination of chiral enolates promises to be a novel and interesting source[38] of α-hydrazino acid derivatives incorporation into peptides, and high optical purity has also been reported[39] during the formation of these acids by electrophilic amination.

2.14 Replacement of L- by D-Residues. - D-Isomeric replacements at the His^6, Arg^8 and Trp^9 residues within the 6-9 core sequence of Ac-[Nle^4]-αMSH_{4-11}-NH_2 and Ac-[Nle^4,D-Phe^7]-α-MSH_{4-11}-NH_2 have been evaluated[40]. Insertion of D-residues in positions 6 and 8 in either series was detrimental to biological potency, but D-Trp in position 9 gives analogues with up to 1900 times more potency in

the frogskin bioassay. Proton n.m.r. studies on the analogues indicated a non-hydrogen bonded β-like structure as the predominant solution conformation. Biological and conformational data suggest that high melanotropic potency requires a close spatial arrangement of the His^6,Phe^7 and Arg^8 side-chains. Proton n.m.r. and CD spectra show[41] that the molecular conformation of [D-$Ala^{4,4'}$]-gramicidin S is similar to that of gramicidin S(GS), with the trans form about the D-Ala-Pro peptide bond. This contrasts with the [L-$Ala^{4,4'}$]-GS in d_6-DMSO or TFE solution when the cis form predominates. Dissimilar properties are also shown by the two analogues when they interact with the phospholipid membrane. The D-analogue interacts with the membrane while the L-form does not. When D-residues are substitued in at least two positions in substance P(SP) antagonists the resulting peptides show agonist or receptor selective antagonist effects[42]. Two types of antagonists have been obtained. The first type [N^{α}Z-Arg^1,-N^{ϵ}Z-Lys^3,D-$Trp^{7,8}$D-Met^{11}]SP-OMe antagonised the SP and SP_{6-11} hexapeptide in the tests on guinea pig ileum, but only SP_{6-11} on the rat spinal cord. The second type, [N^{α}Z-Arg^1,-N^{ϵ}Z-Lys^3,D-$Pro^{9,10}$]SP-OMe were inactive on the ileum but were potent antagonists of the hexapeptide on the spinal cord. Two antagonists tested in vivo, [N^{α}-Z-Arg^1,-N^{ϵ}Z-Lys^3,D-$Trp^{7,8}$D-Met^{11}]SP and [D-$Trp^{7,8,9}$]SP, both depressed hypotensive responses to SP and SP_{6-11} in rabbits. Further modifications of substance P antagonists by substitution of D-residues in positions 7,9, or 7, 8 and 11, can be made with retention of antagonist activity.

Practical procedures for the formation of a series of tyrosyl dipeptides containing D-amino acids by using α-chymotrypsin and the $Met(O)_{192}$-modified enzyme as catalyst have been reported[43].

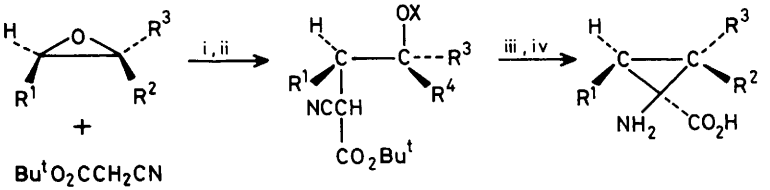
2.15 α,α-Di-Alkylated Glycine Analogues. - The extra steric hindrance at the α-position in these residues demands special activation strategies. Successful application of oxazolinone chemistry seems to overcome the problems, and reagents such as (10) have been well utilised[44] in the synthesis of highly hindered analogues of enkephalin and substance P. When phenylalanine was replaced by α,α-dibenzylglycine(Dbg) in substance P analogues, the analogue [Glp^6,Dbg^7]-SP_{6-11} showed no agonist or antagonist activity, yet [Dbg^4,Leu^5]-enkephalinamide was 8.4 times as potent

as Leu-enkephalinamide and proved highly δ-selective. [N,N-DiallylTyr^1,Dbg^4,Leu^5]-enkephalinamide was a moderately potent opioid antagonist but showed little μ or δ selectivity. During the synthesis of aminoisobutyric acid (Aib) containing peptide oxazolone intermediates such as (11) again play an important role[45] and tend to form preferentially to active esters when DCCI activation is used. Coupling of (11) with other peptides is catalysed by N-hydroxysuccinimide but not N-hydroxybenzotriazole. A novel approach[46] to making Aib derivatives is the use of an azirine precursor as depicted in Scheme 4. In toluene/HCl the terminal dimethylamido group is selectively hydrolysed to the acid (Ac-Aib) which can then be incorporated into peptides.

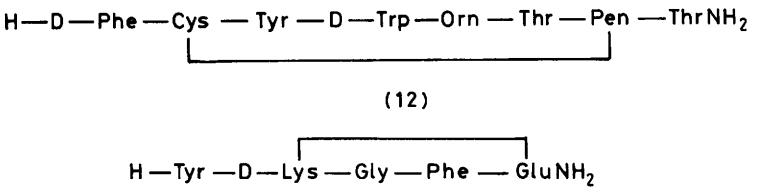
Full details of binding affinities and CD spectra of a series of diastereoisomeric cyclopropylphenylalanines, reviewed last year, have now appeared[47]. An inexpensive and chirally specific synthesis (Scheme 5) of 1-amino-1-cyclopropane carboxylic acids, starting from epoxides, should provide interesting analogues in future[48]. The α,α-disubstituted analogues continue to be a source of conformational intrigue A first unequivocal observation[49] of the oxyanalogue of a β-bend has been made in the X-ray diffraction of Z-Aib$_3$-OH. Intramolecularly H-bonded NH-groups consistent with a 3_{10}-helical conformation have been identified[50] in solutions of Boc-Aib-L-Val-(Aib)$_2$-(L-Val)$_3$-Aib-L-Val-AibOMe and Boc-Aib-L-Leu-(Aib)$_2$-(L-Leu)$_3$-Aib-L-Leu-AibOMe. ^{1}H N.m.r. studies[51] also establish a β-turn conformation in Boc-Acc^5-Acc^5-NHMe where Acc^5 = 1-aminocyclopentanecarboxylic acid.

3 Conformationally Restricted Bridged Analogues

3.1 Somatostatin. - Solid-phase synthesis has provided[52] a number of analogues conformationally constrained by virtue of a disulphide link between Cys and penicillanic acid(Pen). A typical example (12) displayed both high affinity (IC_{50}= 3.5 nM) and exceptional selectivity ($IC_{50}\delta/IC_{50}\mu$ = 4000) for μ-opioid receptors. Analogue(12) showed very low affinity for somatostatin receptors in the rat brain. A large-scale synthesis of a cyclic hexapeptide analogue of somatostatin, cyclo-(MeAla-Tyr-D-Trp-Lys-Val-Phe-) has been achieved by solution methods[53].

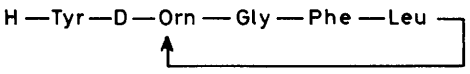


Scheme 5



(12)

(13)



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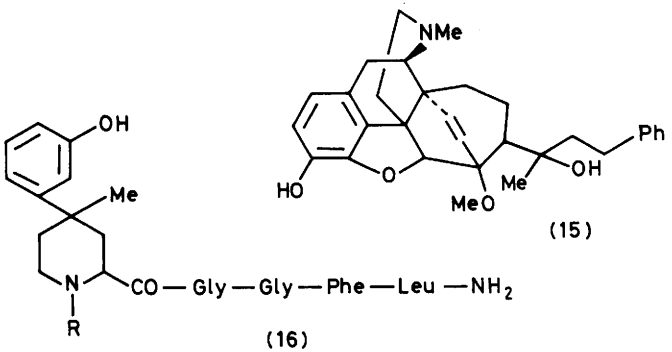

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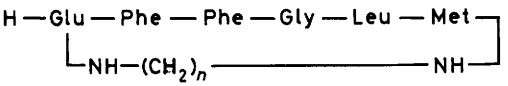


(17) n = 3 - 10, 12

3.2 Enkephalins. - Contrary to recent trends in bridging analogues between residues 2 and 5, cyclic enkephalin analogue (13) showed worse receptor selectivity than structurally related open-chain analogues[54]. The size and relative rigidity of the ring structures might account for this anomaly. Computer graphics have been used[55] to compare the vector map of (14) with that of a potent opiate alkaloid (15). Several low-energy conformers of (14) were identified that permitted a good fit with (15), provided that the tyramine moiety of the respective molecules does not coincide. The side-chain of leucine has no structural correlate in the alkaloid. A full ^{1}H, ^{13}C and ^{15}N nmr study[56] of (14) and analogues where Phe is replaced by either MePhe or a 1,2,3,4-tetrahydroisoquinoline carboxylic acid residue(Tic) revealed that only the MePhe analogue showed conformational flexibility (due to cis/trans rotation) and appears to correlate with that analogue exhibiting the highest activity in the guinea pig ileum assay. A depsipeptide analogue of (14) was also investigated in the study. Conformational restriction around the tyrosine moiety, e.g. as in (16), continues to give products lacking in activity[57]. The addition of lysine to the N-terminal position to allow cyclisation to form cyclo-[Lys-Tyr-Gly-Gly-Phe-Leu] also reduced[58] interaction with opiate receptors, although the analgesic potency remained similar to Leu^{5}-enkephalin. A cystamine-enkephalin dimer containing two molecules of [D-Ala^{2}, Leu^{5}]-enkephalin crosslinked with $(-NH-CH_2CH_2-S)_2$ showed[59] high affinity for δ-opiate receptors (5 times more potent than the cysteamine monomer).

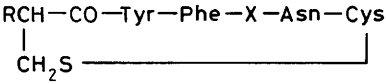
3.3 Substance P and Neurotensin Analogues. - Similar themes have been used in bridging linear precursors in this area. Using the side-chain of glutamic acid and a polymethylene link gave the substance P_{6-11} analogue(17)[60] with the analogues with n=3,7 and 12 showing hypotensive effects in rats. Cyclo-(Leu-Met-Glu-Phe-Phe-Gly-) showed[61] only a small kinin activity on guinea pig ileum compared to substance P, but was a full agonist.

Cyclic analogues of C-terminal neurotensin(NT) such as H-Phe-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu have high depressor activity with a selectivity with respect to smooth vasal muscles[62]. CD spectra of aqueous solutions show that cyclisation

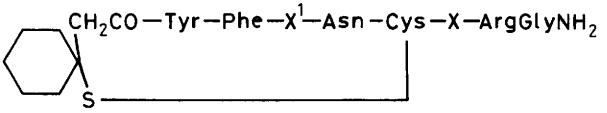
of NT_{6-13} leads to a dramatic restriction in conformational mobility.

3.4 ACTH, Vasopressin and Bradykinin Analogues. - Cyclo-[$Glu^{\gamma \rightarrow \epsilon}Lys(Gly)$]ACTH 5-14 undecapeptides have been synthesised[63] using diphenylphosphoryl azide for cyclisation. Melanotropic activity was increased by two orders of magnitude but the steroidogenic activity decreased. Two covalently linked dimers of $ACTH_{11-24}$ using either N-acetylglutamic acid or lysine amide as spacers have been synthesised[64]. Increased inhibition potency upon steroidogenesis was recorded. Three analogues (18)-(20) of [Arg^4]-vasopressin(AVP) all had low pressor effect when compared with AVP[65]. Other vasopressin analogues[66] (21) and (22) lacking a Pro-residue retained a high degree of antidiuretic activity, so neither Pro nor Gly are essential for antagonism of F_2-receptor. A detailed n.m.r. study[67] of the bradykinin analogue H-Lys-Pro-Pro-Gly-Phe-Gly-Pro-Phe-Arg confirms a type II β-bend in the sequence Pro^2-Pro^3-Gly^4-Phe^5.

3.5 Other Cyclic Analogues. - It has been shown[68] that a dicyclohexylcarbodiimide(DCCI)/4-dimethylaminopyridine combination gave higher yields than the azide method in the synthesis of cyclic analogues of thymopoietin. It is implied that the cyclisation is easier when an N-terminal or C-terminal D-residue is present, and when an all-L precursor is used racemisation or inversion of one residue occurs during cyclisation. In the series of cyclic analogues investigated(23)-(26), high-field n.m.r. studies indicated a βII' conformational structure for all the examples. Exchanging aromatic amino acid residues did not change the spectral parameters but replacing Asp by Glu decreased the backbone flexibility. In another study[69] the homodetic cyclic $eledosin_{6-11}$ hexapeptide cyclo-(Ala-Phe-Ile-Gly-Leu-Met) gave agonist and antagonist effects in guinea pig ileum. Interesting developments aimed at conformational restriction via non-peptidic insertions have been reported. In (27) the phenoxathiin S-dioxide unit has been used[70] as a successful simulator of the i+1 and i+2 positions of a β-turn. The unit(28) has been used[71] successfully to mimic the β-turn in gramicidin S, while the indolizidine(29) has been prepared[72] from cyclo-octadiene as a non-peptide mimic of



(18) R = NH_2, X = Arg, X^1 = Pro
(20) R = H, X = Arg, X^1 = Pro
(22) R = H, X = Val, X^1 = deleted.



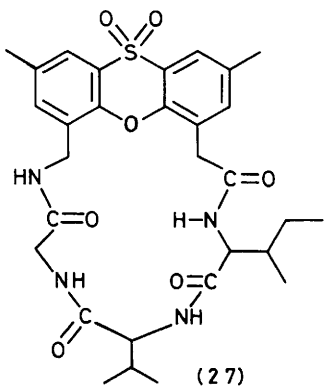
(19) X = Pro, X^1 = Arg
(21) X - deleted, Tyr replaced by Tyr(Et) X^1 = Val

cyclo - [Arg(NO_2) - Lys(Z) - X - D - Val - X^1]
(23) X = AspOBzl or GluOBzl
X^1 = Tyr or Trp.

cyclo - [D - Lys(Z) - Arg(NO_2) - X - Val - Tyr]
(24) X = AspOMe or GluOBzl

cyclo - [Arg(NO_2) - Lys(Z) - X - Val - X^1]
(25) X = GluOBzl, X^1 = D - Phe
or X = AspOBzl, X^1 = D - Tyr

cyclo - [Arg(NO_2) - D - Lys(Z) - X - D - Val - Tyr]
(26) X = AspOBzl or GluOBzl



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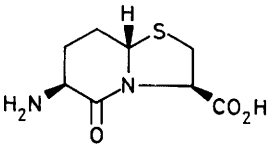

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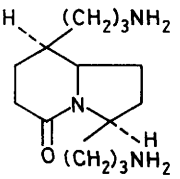

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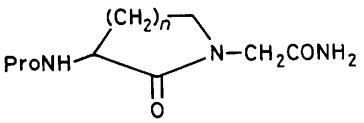
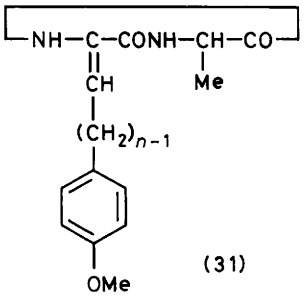


(30) (*n* = 1,2)



(31)

immunosuppressant H-Lys-Pro-Arg-OH. The unit (30) has been used[73] as a conformationally restricted analogue of Pro-Leu-Gly-NH_2, and the analogues showed a greater ability than the natural form to enhance the binding of dopamine agonist ^{3}H-ADTN to striated dopamine receptors.

Disulphide bridging stabilises[74] γ-turn conformations in model systems such as Boc-Cys-Ala-Cys-NHMe and there might be a role for carboethoxysulfenyl chloride[75] in disulfide bond formation when other methodology proves difficult.

4 Dehydroamino Acid Analogues

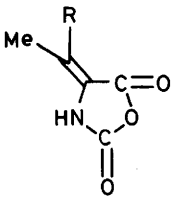
Two reviews, on dehydropeptides[76] and dehydroproline-containing peptides[77], have been published, but not in sources readily available for the Reporter to review. A 3,4-dehydroproline $^3\Delta$-Pro residue in [D-Thr^2,Δ^3Pro^5]-enkephalinamide gives a compound less active[78] in tests than [D-Ala^2,MePhe^{4}Met(O)ol]enkephalinol. The full story of the [E and Z-Δ-Phe^4] enkephalins has now appeared[79] and a monodehydro Leu-enkephalin analogue, Z-Tyr(OTs)-Gly-Gly-ΔPhe-LeuOMe, has been used[80] as a substrate for asymmetric hydrogenation studies. A 93% diastereoisomeric excess in the hydrogenated compound when (Rh-dipamp COD)$^+BF_4^-$ was used as chiral catalyst gives hope for the stereoselective incorporation of tritium into enkephalins. The steric course of such reductions has been studied[81] using model compounds Ac-Δ-Phe(Gly)$_n$-LeuOR with n=0-2 and the first picture which emerges is a moderate but definite contribution of the (S)-leucine, whatever its location, to the steric course of the reaction. Aspartame with an L-L:L-D ratio of 90:10 has been prepared[82] by the asymmetric hydrogenation of HCO-L-Asp-ΔPheOMe using Rh-(R)-prophos. Very low stereoselectivity, 60:40 DL/LL and 60:40 LD/DD, was obtained[83] when Bz-Δ-Val-L-PheOMe and Bz-Δ-Val-D-PheOMe respectively were hydrogenated over 5% Pd/C. Yet for the model compound(31), hydrogenated over Pd in DMF, the hydrogenated cyclic peptide[84] had an L,L content of >94%. Insertion[85] of Δ^Z-Phe, or a D-Phe residue in position 3 and/or 5 of dermorphin (1-5) H-Tyr-D-Ala-Phe-Gly-TyrNH_2 has been carried out using Stammer's techniques. Results from a guinea pig ileum preparation study showed that the analogue insertion of D-Phe^3 was barely tolerated,

and Δ^Z-Phe in positions 3 and/or 5 was very detrimental to μ-activity. A similar result was found on inserting Δ-Phe in position 3 and/or 5 of dermorphin, H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-SerNH$_2$. The conformations of these dehydrodermorphin analogues seem[86] to be very solvent dependent; in methanol, trifluoroethanol, CD evidence exists for a folded form with intramolecular H-bonds, while in water extended flexible conformations are present. Coupling experiments explored[87] with Δ^Z-Phe and Δ-Ala residues have brought to light competing reactions. Thus when R-X-ΔX^1-OH, (X=Gly, Phe or Val, ΔX^1 = ΔPhe or ΔAla) was coupled with GlyOR^1 by activation with DCCI/HOBt, diphenylphosphorazidate (DPPA) or mixed anhydride techniques, in general only moderate yields of the desired tripeptides were obtained. In the DPPA method involving the Δ-Ala analogues, considerable amounts of DPP-Gly-OR^1 and azido tripeptides, R-X-NHCH(CH_2N_3)CO-Gly-OR^1, were found. Tripeptides Boc-L-Phe-Δ^Z-Phe-X-OMe(X=Val, Leu or Ala) when studied[88] in $CDCl_3$ solution by 270 MHz ^{1}H n.m.r. spectroscopy show evidence of a type II β-turn with the Phe and Δ^Z-Phe occupying the i+1 and i+2 positions respectively.

5 Enzyme Inhibitors

5.1 Angiotensin Converting Enzyme (A.C.E) Inhibitors. - Crystal structures of enalapril (MK-421)(33) and two potent A.C.E. inhibitors (35) and (36) confirm[89] that they have a common conformation. A phosphoric acid group in the MK-422 analogue (34), instead of a carboxy group causes loss of binding affinity[90], but postulated binding functions for the active site of A.C.E. derived earlier, together with molecular graphics have made possible[91] improved inhibitors such as cilazapril(37). Potential transition state A.C.E. inhibitors based on peptide-derived amino alcohols have been synthesised[92] optically pure, and in an *in vitro* assay showed inhibitory potencies of 28 and 10,000 mM respectively for (2R,3S)(38) and (2S,3S)(38).

The first evidence that there might be alternative binding modes for the two major classes of small molecule A.C.E. inhibitors comes from the observation[93] that analogues (39), which contain the tetrahydro-3-isoquinoline carboxylic acid residue, possessed equivalent *in vitro* and *in vivo* efficacy to enalapril.



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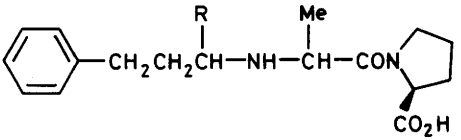
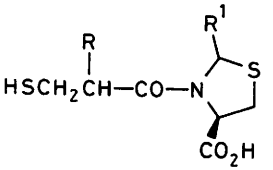
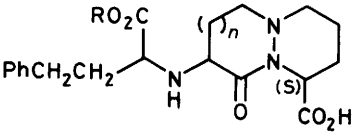


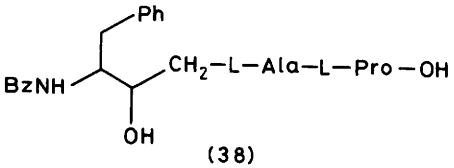
(33) R = CO_2Et(MK421)
(34) R = PO_3H_2



(35) R = CH_3, R^1 = H(YS-980)
(36) R = H, R^1 =  (SA-446)



(37) *n* = 2, R = H



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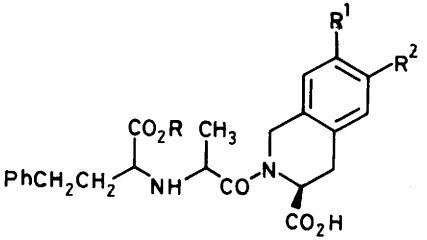
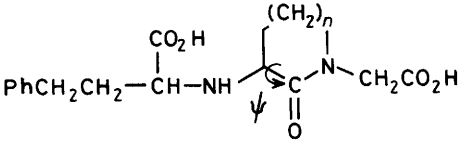


(39) R^1 = R^2 = H, or R^1 = R^2 = OMe

Sulfhydryl analogues with the same structure variation were also highly potent. In contrast, tetrahydro-1-isoquinoline carboxylic acid and homologous isoindoline-1-carboxylic acid analogues showed a divergence in potency between the two types, the sulfhydryl analogues being essentially inactive, while the others were equipotent with the prototype. Monocyclic lactams (40, n = 1-5) contain the required recognition and binding elements[94] for inhibition. A correlation was found between the inhibitor potency IC_{50} and the computed ψ angle, as defined in (40), for the lowest-energy conformation of the model compounds. The data serve to define a window of ψ angles between 130 to 170° which seems to be acceptable to the A.C.E. active site. In a series of peptidic A.C.E. inhibitors studied, with structures ranging from derivatives such as RNH-$(CH_2)_n$-Phe-Ala-Pro-OH (n = 0-5) and t-Boc (or trifluoroacetyl)-Lys(Z)-Phe-Ala-Pro-OH[95] to bradykinin potentiating peptide analogues[96] pyroGlu-Lys-X-X^1-Pro-OH (X-X^1 = Trp-MeAla, Phe-MeAla or Trp-D-Ala) or pyroGlu-Trp-Pro-Arg-Pro-X^2-X^3-MeAla-Pro-OH(X^2-X^3=Gln-Ile,Lys-Phe), the former series possessed high inhibitor activity, while the latter two peptide types showed a sharp decrease in comparison with natural peptides. It has also been reported[97] that the best inhibitor in a series of dipeptides was $NH_2(CH_2)_4CH(CO_2H)NHCH(Me)CONHCH(Me)CO_2H$ with an IC_{50} of 10 nmol/L.

5.2 Renin Inhibitors. - Through the solution-phase synthesis[98] of a large number of pepstatin analogues of the general formula R-X-X^1-Sta-Ala-Sta-R^1 where R was varied with groups such as isovaleryl Iva, Boc or Z, with X=Phe, D-Phe, MePhe etc, and X^1= Phe, D-Phe, Trp, His, Nva, Nle etc, it has been possible to study ways of improving the inhibitory potency of pepstatin (R = Iva, X=X^1=Val, R^1=OH). Boc and Iva were the most effective R-groups, with X=Phe, X=His or an aliphatic amino acid giving inhibitory activity. Ester or amide groups at the C-terminal position did not change potency. Stereochemically pure samples of amino-statine(Asta)(41) derivatives have been prepared[99] for X-ray crystallographic comparisons and incorporation into substrates. The data confirm that the 3S stereochemistry was the preferred form for Asta as it is for statine(Sta). In each case the (S,S)Asta analogue was of similar potency to (S,S)Sta, whereas the



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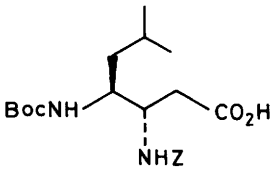

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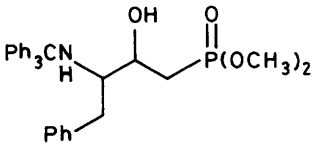

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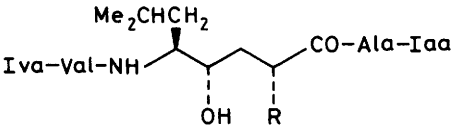

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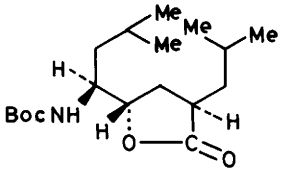

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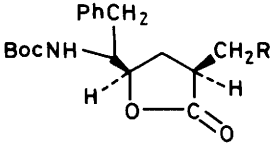

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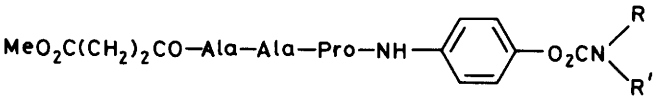


(46)

less potent (S,R) Asta conferred much higher potency than (S,R)Sta. Potent inhibitors have also been derived[100] from the incorporation of a phosphostatine residue into an appropriate peptide sequence, as a result of the availability of (42) after synthesis from N-trityl-L-phenylalinal. In a series of substrates containing difluorostatine and difluorostatone, the 3S epimer of Boc-Phe-His-NH-CH(CH_2CHMe$_2$)CH(OH)CF_2-CO-IleNH-CH_2-(2-pyridyl) exhibited a 60-fold lower inhibition activity than the 3R form[101]. Longer *in vivo* action has been derived from incorporation of the [$CHOHCH_2$] isostere as exemplified[102] by Boc-Pro-Phe-MeHis-Leuψ[$CHOHCH_2$]Val-Ile-NH-CH_2-(2-pyridyl)(IC_{50}= 0.26 mM) and Boc-αMePro-Phe-His-Leuψ[$CHOHCH_2$]Val-Ile-NH-CH_2-(2-pyridyl)(IC_{50}= 1.8 mM) in studies with human renin. 3-Aminodeoxystatine(Ads) has been shown[103] to be a comparable replacement for 3S-statine when incorporated into N-terminal fragments of human angiotensinogen. Synthesis of leucine α-amino alcohols could provide possible analogues of statine[104].

5.3 Other Inhibitors. - A hydroxyethylene isostere and its ketomethylene analogue substituted into a pepstatin sequence (43) inhibited[105] porcine pepsin with a potency equal to that of a statine-containing analogue. Lysyl and ornithinyl side-chain analogues of statine when substituted into appropriate sequences e.g. Iva-Val-Val-NHCH[$(CH_2)_nNH_2$]CH(OH)CH_2COR (where n=4 for lysine and n=3 for ornithine) gave inhibition of penicillinopepsin, which has been correlated[106] with the ability for binding to the Asp-77 on the enzyme. The lactone (44) has provided[107] a stereochemically pure precursor to the corresponding (2R,4S,5S)-hydroxyethylene dipeptide isostere, and in the same manner modern synthetic methods[108] starting from α-amino-aldehydes have produced (45) which is useful for further conversion to the isostere. Peptidyl carbamates of the structural type (46) specifically inhibited[109] elastase without affecting trypsin and chymotrypsin. The kinetic studies implied that there was a strong initial formation of an enzyme–inhibitor complex, followed by a slow acylation of active site serine. Substrate analogue Boc-Phe-Leu-Ala-Sta-Val-Leu-OMe proved[110] to be the best inhibitor (k_i= 1.1 mM) of bovine cathepsin D, while an assessment of known pepstatin analogues as inhibitors of this enzyme showed

that the P_4 substituent was more important for cathepsin D inhibition than other aspartic proteinases. A chymotrypsin-like proteinase, cathepsin G, from human leukocyte interacts[111] with the p-nitroanilide group of inhibitor succinyl-Ala-Leu-Phe-p-nitroanilide and analogues.

A solid-phase synthesis[112] of a linear 29-residue sequence, followed by oxidation to give 3-disulphide bridges, has confirmed the structure of the trypsin inhibitor CMTI III from squash seeds (Cucurbita maxíma). A full paper[113], following up last year's preliminary report, has been published on the synthesis of a naturally occurring inhibitor of glutamine synthetase. The cyclobutanol (47), produced by Streptomyces species X-1092, has been synthesised[114] and its mode of action explained in terms of the cyclobutanol moiety acting as a 'suicide substrate' for the pyridoxal-based enzyme cystathionine-γ-synthetase.

The effect of various enkephalin analogues on enkephalinase B from calf brain striatum has been studied[115]. A free carboxylic acid group is needed for good interaction but enkephalinase B has low affinity for [D-Ala^2]-Leu-enkephalin. The relationship between stereochemistry and enzyme recognition has been studied[116] as a result of stereospecific syntheses of thiorphan (48) and its retro-inverso analogue (49). In the thiorphan series, affinity for enkephalinase was independent of the stereochemistry of the N-terminal moiety but in the retrothiorphan a 100-fold difference in inhibitory activity of the two enantiomers was observed. The recognition that N^3-fumaramoyl-L-2,3-diaminopropanoic acid was a strong inhibitor of glucosamine-6-phosphate synthetase has initiated[117] a search for analogues. Analogue (50) was the most powerful inhibitor synthesised. Carboxyl-modified amino acids and peptides have inhibitory effects on various proteases[118], as shown by the following summary (R represents $CONH_2$, $CSNH_2$, CN, trans-$CH{=}CHCO_2Me$ or trans-$CH{=}CH{-}SO_2Me$):

Type	Inhibitory effect
$PhCH_2CH(R)NH_2$	All except CN analogue were competitive inhibitors of microsomal and cytosolic leucine aminopeptidase.
$PhCH_2CH(R)NHAc$	Also competitive inhibitors of chymotrypsin.

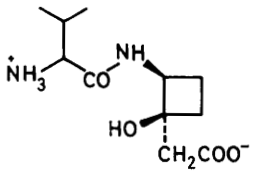
$NH_2CH_2CONHCHRCH_2Ph$ When R is first three in list, competitive inhibition of dipeptyl aminopeptidase takes place.

$PhCH_2CH(NHAc)-CONHCH_2R$ First three members in the R list were competitive inhibitors of papain, the latter members proved to be irreversible affinity labels.

The potency order of a series of dipeptide aldehydes as inhibitors of prolyl endopeptidases has been reported[119] to be Z-L-Val-L-Pro-H≈Z-L-Ile-L-Pro-H>Z-L-Phe-L-ProH>Z-L-Ala-L-Pro-H with IC_{50} values in the 10^{-8}-10^{-6}M range. It has also been reported[120] that amongst a series of analogues studied, Z-Gln-Val-Val-Ala-Gly-OMe not only inhibits papain but also protects it from T kininogen.

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

Contemporary strategies and methodologies for the synthesis of opioid peptides have been briefly reviewed[121]. Quaternisation of the N-terminal amino group in [D-Ala^2, Leu^5]-enkephalin using $CH_3I/KHCO_3$ does not seem to affect[122] the interactions of the peptide with its receptors. Similarly [Orn$^+Me_3^{2,2^1}2I^-$] gramicidin S exhibits identical antimicrobial activity to the parent gramicidin S. It suggests that binding to receptor is electrostatic rather than involving H-bonds. Enkephalin tripeptide amides such as (51) have shown[123] good binding and analgesic activity, and drastic modifications at the C-terminal position using compounds such as H-Tyr-Ala-NH-CH(Me)CH_2CH_2Ph or H-Tyr-Ala-NH-CH(Me)CH=CHPh have been explored[124]. [D-Ala^2,Met(O_2)^5]-Enkephalin dimethylamide has been shown[125] to exhibit stronger analgesic effects in mice than its Gly^2 analogue. Incorporation of a β-chloroethylcarbamoyl group ($ClCH_2CH_2NHCO$) into N-, C-terminal and side-chain positions in enkephalin did not give rise[126] to analogues with greatly improved activity. Binding data for μ, κ and δ-receptors have shown[127] that the peptide portions of the alkaloid-peptide hybrids (52) did modulate the receptor selectivity of the attached alkaloid pharmacophore.



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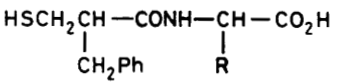

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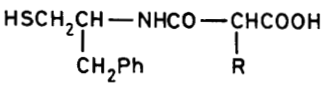

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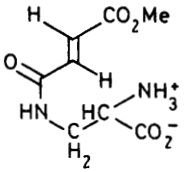

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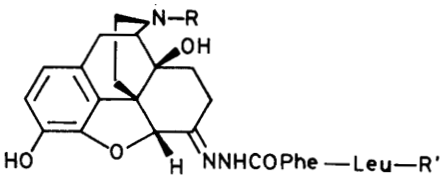
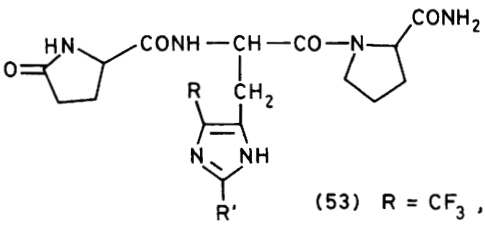


(52) R = Me , CH_2CHMe_2 ;

R' = OH , Arg — Arg — Ile — OMe



(53) R = CF_3 , R' = H

(54) R = H , R' = CF_3

(55) R = NO_2 , R' = H

Attempts to map out in more detail the molecular entities associated with the various receptor sub-types have been made[128] by synthesising photoaffinity probes based on DAGO(Tyr-D-Ala-Gly-MePh-Gly-ol) a μ-selective analogue and DTLET(Tyr-D-Thr-Gly-Phe-Leu-Thr) a δ-selective probe. Substitution of a 4-NO_2 group in the Phe-residue of each analogue preserved the receptor selectivity, but the inserted group was not photoactivable by u.v. irradiation. Likewise a p-azido group inserted into the Phe-residues does not change the selectivity, but the azido derivative of DAGO has less affinity than DAGO itself. Azido-DTLET has more affinity than the parent compound and both azido derivatives were photoactivable, thus providing a tool for characterisation of the receptor sub-types. The insertion of a ^{11}C-label, with its short half-life, into a peptide demands the synthesis of a peptide analogue which can very swiftly be converted in one step to the final compound. This has been achieved[129] efficiently by taking the homocysteine(Hcy) enkephalin analogue Z-Tyr-Gly-Gly-Phe-Hcy-(CH_2Ph)-OCH_2Ph, deblocking it with Na/NH_3(ℓ) and S-alkylation with $^{11}CH_3I$ giving a labelled Met-enkephalin.

[Ala^5,Orn^9]-Somatostatin has been shown[130] to inhibit somatotropin, insulin and glucagon but shows no inhibition of the secretion of prolactin. Oxidation of the D-Trp residue of the cyclic somatostatin cyclo-(MeAla-Tyr-D-Trp-Lys-Val-Phe-) to a 2-oxindolyl derivative has been reported[131], and when X = trans-4-aminocyclohexyl glycine in the somatostatin cyclohexapeptide analogue cyclo-(D-Trp-X-Tyr-Phe-Pro-Phe) the analogue is ten times more active[132] than the case where X=Lys.

In a series of residue replacement studies on substance P analogues a number of structure-activity deductions have been made. Thus substitution[133] of a homoglutamine(Hgn) residue into positions 5 or 6 enhanced activity in the guinea pig ileum, but substitution of D-Hgn in the same positions gave reduced activity, with D-Hgn^6-SP(4-11) acting as an antagonist. Although substituting fluorine atoms in the para position of the rings in Phe^7 and Phe^8 of substance P (H-Arg-Pro-Lys-Pro-Gln-Glu-Phe-Phe-Gly-Met-NH_2) did not affect[134] the biological activity or CD spectra, unequivocal differences in the ^{19}F n.m.r. chemical shifts of three analogues were observed. Substitution of Orn, Thr, Thr(Me) or Val for the N-terminal Gln of $SP_{(6-11)}$ gave

analogues[135] with little effect on the rat colon and reduced activity in guinea pig ileum, so the carboxamide side-chain may be needed as a H-bonding link in biological activity. [pyroGlu^6,Pro^9]SP(6-11) has been shown[136] to have selective agonist activity towards the SP-P receptor sub-type, with reduced activity on SP-E systems. The central role played by 'spantide' [D-Arg^1, D-Trp^{7,9},Leu^{11}]-SP as a reference antagonist has been emphasised[137] in the design of a further 47 peptide antagonists. Twenty-one peptides showed superior potency to spantide with the best, [D-Arg^1, D-Nal^5, D-Trp^{7,9}, Nle^{11}]SP, showing 5 times the potency of spantide.

The recent observation that [D-Pro^{10}] dynorphin(1-11) has extremely high affinity for the κ-receptor has prompted[138] modifications to be made to positions 8 and 10 of dynorphin(1-13) and the effects analysed on guinea pig ileum, together with competitive binding studies against the traditional sub-type ligands, [^{3}H]-ethylketocyclazocaine (κ), DAGO(μ), DSLET(δ). [L-Ala^8]Dynorphin(1-13) showed a decreased activity in the ileum (50% of parent sequence), but induced a 2.22 fold increase in its affinity for the κ-receptor. Binding to the δ-receptor was decreased by a factor of 1.7 without affecting its μ-receptor affinity. Replacement of position 8 by D-Ala or position 10 by D-Pro did not greatly affect the binding characteristics. [D-Trp^8]-Dynorphin(1-13) and [D-Trp^{10}]-dynorphin(1-13) both showed decreased affinity for all the receptor types. When a series of D-Trp derivatives of dynorphin(1-11) are synthesised[139] by solid-phase techniques, e.g. [DTrp^8,D-Pro]- [D-Trp^{n,8},D-Pro^{10}]- with n=2,3,4,5 and [D-Trp^{2,4,8}, D-Pro^{10}]-dynorphin(1-11), the analogues showed antagonist properties in the κ-specific rabbit vas deferens preparation. Peptide hybrids in which the dermorphin and dynorphin A(1-13) sequences have been substituted into β-endorphin from camels (β_c-EP) and humans(β_h-EP) have been investigated.[140] Dermorphin(1-7)β_cEP was 120 times more potent than β_c-EP in guinea pig ileum, 49 times in mouse vas deferens and 4 times in rat, while replacement of the first 1-13 segment of β_h-EP with dynorphin A(1-13) caused an increase in opiate potency in both the ileum and vas deferens.

There has been interest in adding and deleting sequences in dermorphin. Based on the observation that many hormones possess a

terminal glycine amide, biosynthesised from precursor hormones containing a longer C-terminus, a study[141] on the biological activity of the dermorphin sequence having Gly, Sar or Gly-Arg added to the carboxyl end. However, additional residues were detrimental to μ-activity in the guinea pig ileum test but the analogue with extra Gly-Arg residues was 2.5 and 120 times more potent than dermorphin and morphine respectively in the *in vivo* tail flick test. Maybe this is due to the improved pharmokinetic properties of the analogue. Shorter dermorphin analogues[142], such as H-Tyr-D-Arg-Phe-GlyNH_2, showed 31 times more activity than morphine on a molar basis, while tetrapeptides bearing D-Met S-oxide at position 2, such as H-Tyr-D-Met(O)-Phe-GlyNH_2 showed higher central activities than those of dermorphin but less effect at the periphery[143].

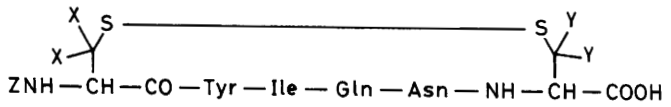
To overcome the instability towards oxidation of two methionine residues in C-terminal cholecystokinin (CCK) derivatives, these residues have been systematically replaced by norleucine in four analogues of Z-CCK(27-32)-NH_2. All the analogues[144] antagonised the action of gastrin with ED_{50} figures between 0.5 and 3 mg/kg. Analogues of various lengths centred on CCK(27-32) amide have been synthesised and evaluated[145]. A heptapeptide (CCK-7) proved to be one of the shortest CCK fragments to retain analgesic effect. Model probes for investigating the cholesystokinin receptors, in the form of a series of analogues, Boc-Trp-NH-$(CH_2)_n$-CO-Phe-NH_2 (n = 1-4), and Boc-Trp-X-X^1-Phe-NH_2 with X-X^1 = Gly-Gly, Met-Gly and Gly-Asp have been synthesised using solution-phase techniques.[146] A 'one-pot' sulfonation process using DCC and sulfuric acid has been developed for O-sulfonation to give a tyrosine O-[^{35}S]-sulfated CCK-octapeptide[147].

Solid-phase synthesis of a number of analogues of the amino-terminal region of LH-RH, gave rise[148] to a compound [N-Ac-D-Nal^1,D-$Phe^{2,3}$,D-Arg^6-Phe^7-D-Ala^{10}]-LH-RH with Nal = 3-(2-naphthyl)-D-Ala, which caused a 56% blockade of ovulation, equipotent with the parent sequence. Reductive alkylation[148] of the D-Lys^6 side-chain with a number of aldehydes and ketones had little effect on histamine-releasing activity as long as basicity was retained. Five p-azido phenylalanyl-containing analogues of LH-RH have been iodinated with ^{125}I for photoaffinity labelling

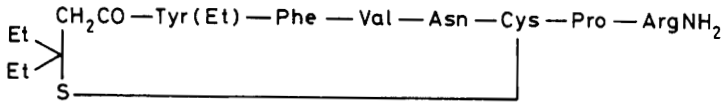
studies[149]. All the imidazole-substituted TRH analogues (53-55) raised the blood pressure and heart rates in rats when compared[150] with normal TRH. Seven out of seventeen analogues of TRH which have greatly different structures[151] to the parent hormone had stronger anticataleptic effect than TRH, but with negligible hormonal potency. Amongst the active ones synthesised were L-pyro-2-aminoadipyl-Leu(or Nva)-$ProNH_2$, pyroGlu-Leu-$PipNH_2$ and L-pyro-2-aminoadipyl-Nva-$TcaNH_2$ (Tca = L-thiazolidine-4-carboxylic acid).

Influences of increased side-chain length and of hydrophilicity on the potencies of oxytocin analogues have been monitored as a result of the synthesis[152] of [3-O-methyl homoserine]-, [3-O-ethylserine]-, and [3-O-methylthreonine]-oxytocin. The longer side-chains produced an increase in all activities but moving the oxygen atom further away from the backbone decreased vasopressin-like activities. Two neurohypophysial hormones, mesotocin and vasotocin from the ostrich Struthio camelus, have been identified[153] as [Ile^8]-oxytocin and [Ile^3]-vasopressin respectively. In order to check if the tripeptide tail (Pro-Leu-Gly-NH_2) of oxytocin was necessary for inhibition, and to investigate further the role of bulky substituents at the Cys positions, penicillamine(Pen) derivatives have been synthesised[154]. Thus the tocinoic acid derivatives (57) and (58) when tested proved to be strong inhibitors of the uterine activity of oxytocin, whereas (56) was a mild agonist. So the end tripeptide does not seem to be necessary for antagonistic activity.

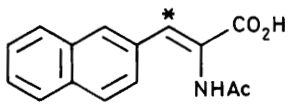
Enzymatic semisynthesis of the larger peptides has found probably its most fertile ground in making useful insulin analogues. [$Leu^{B.30}$]-Insulin has been obtained[155] via this methodology by coupling [$desAla^{B.30}$]-insulin with $Leu\text{-}OBu^t$ using Achromobacter protease I and trypsin as catalyst. Previous studies had shown that this analogue had the least immunoreactivity to anti insulin sera from beef/pork insulins. [$Gly^{B.30}$]-Insulin a new structural analogue of human insulin has been produced enzymatically[156] and shown to be as active as the human insulin. Unprotected (B23-30) insulin has been shown[157] to be a satisfactory substrate for the trypsin-catalysed synthesis of despentapeptide (B26-30)-insulin and a partial synthetic route has



(56) X = H , Y = Me
(57) X = Me , Y = Me
(58) X = Me , Y = H



(59)



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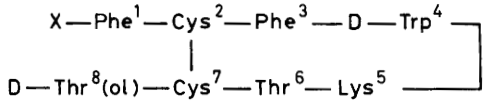
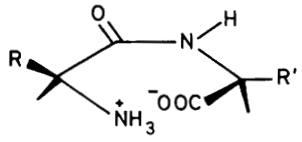
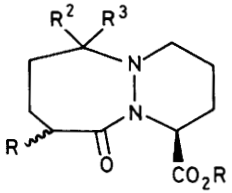


(61) X = L (62) X = D



(63)



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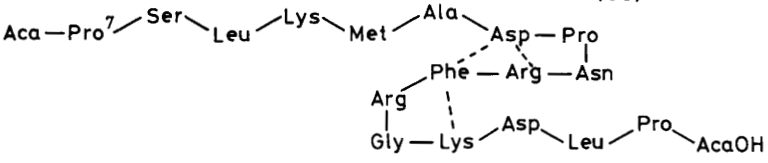
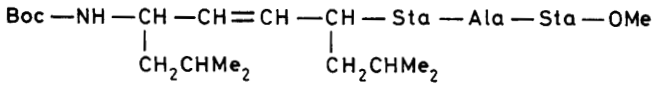


(64) Aca = 6 - aminocaproyl



(66)

produced[158] [$Ala^{B.5}$]-insulin and an analogue in which the N-terminal tetrapeptide of the B-chain has been replaced by a tripeptide sequence H-Gly-Pro-Glu. The importance of the 10-13 region of glucagon for its receptor interactions has been studied[159] using glucagon analogues synthesised by solid-phase techniques. Biological activity results on the analogues, [Phe^{13}]-, [Phe^{10}]-, [Pro^{11},Gly^{12}]-glucagonamide and [Phe^{10}]-, [$Phe^{10,13}$]-, [Pro^{11}]-, and [Ala^{11}]-glucagon indicated that the 10-13 region has multiple roles in the glucagon-glucagon receptor interaction. Other analogues[160] based on changes at the C-terminal end of glucagon, e.g. [$Lys^{17,18}$, Glu^{21}]-glucagon, designed to enhance α-helix character, had 500% and 700% greater potency than glucagon in standard binding and adenylate cyclase assays respectively. No direct relationship has been found[161] between the hydrophobic moment of the amphipathic helix in the central portion of salmon calcitonin and its biological activity, from studies carried out on [des Leu^{19}]- and [des Ser^{13}]-calcitonin.

Solid- and solution-phase synthesis has enabled morphiceptin (H-Tyr-Pro-Phe-ProNH_2) analogues to be assessed[162] in terms of their μ-opiate receptor selectivites. It was concluded that Tyr^1 and Phe^3 are very important for activity since elimination or methylation of the tyrosyl phenolic hydroxyl and reduction of the Phe phenyl ring caused dramatic loss of activity. Each replacement[163] of amino acid residue in a series of bombesin analogues, H-Gly-Asn-X-Trp-Ala-X^1-Gly-His-X^2-MetNH_2 where X = Leu, His, X^1 = Thr, Val, X^2 = Leu, Phe, induced a lowering of the contracting activity. A large decrease in potency has also been experienced[164] in analogues of α-MSH containing para-substituted aromatic, and non-aromatic acids in position 7 of the hormone, or when Phe^7 was replaced by Gly.

Although a large number of analogues of angiotensin II have been synthesised, they have only served to show how stringent the structure-activity relationship is. All three analogues of $RR^1N(CH_2)_5CO$-Arg-Val-Tyr-Ile-His-Pro-IleOH with R=H, R^1=H,Me; R=R^1=Me did not change[165] the blood pressure of rats at the 50ng/μg level. Changes[166] at the C-terminal position based on the analogues Ac-Asn-Arg-Val-Tyr-Val-His-Pro-R, with R in turn being Phe-ol, Phe-Me, Phe-OMe, or MePhe-OMe, only gave rise to one

analogue (R = PheOMe) which had significant biological activity (20% oxytocic and 13% pressor activity in rats). Substitutions in the 1, 4 and 8 positions of the angiotensin antagonist [Sar^1, $\underline{O}MeTyr^4$]-angiotensin II only produced antagonist activity[167]. First examples have been reported[168] of potent vasopressin V_2-antagonists that do not possess a β,β-cycloalkyl moiety at position 1. Analogue (59) was a fairly potent example of a V_2-antagonist in this work, and also reported was a convenient synthesis of an $\underline{S}$-protected β,β-dialkyl-β-mercaptopropanoic acid. In a search for more selective agonists of Arg-vasopressin (AVP), ten analogues of [Sar^7]- and [$MeAla^7$]-AVP with additional substitutions at the 1, 2, 4, 7 and 8 positions have been prepared[169]. Generally the analogues retained high binding affinities to renal vasopressin receptors, but a large decrease in binding affinities to hepatic vasopressin receptors.

A direct correlation between the ability to inhibit [^3H-Arg^4] tuftsin specific binding to mice peritoneal macrophages and their capacity to inhibit tuftsin-mediated phagocytosis by the cells and to potentiate the cell's immune responses has been derived[170] from a systematic replacement of individual residues in the tuftsin sequence H-Thr-Lys-Pro-ArgOH. When Gly residues were substituted[171] in positions 4, 5, 6 and 7 of neurokinin B, H-Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-$MetNH_2$, the intrinsic activity on smooth muscle implied an essential need for the Phe^6 and Val^7-residues. Solution methods of peptide synthesis still seem to be the preferred route when the more unusual amino acids are incorporated in the sequence. Thus mixed anhydride couplings were used to incorporate[172] D-Pro, pyroGlu, L-4-thiazolidine-carboxylic acid(Thz), L-2-piperidine-carboxylic acid(Pip), L-azetidine-carboxylic acid(Aze) and D or L-$\Delta^{3,4}$-proline($\Delta^{3,4}$-Pro), into the $\underline{N}$-terminal position of Pro-Leu-$GlyNH_2$. Most of the analogues showed dopamine receptor modulation comparable to the parent sequence, but the Thz- and D-$\Delta^{3,4}$-Pro-containing analogues were inactive. However, the $\underline{N}$-terminal proline does not seem to be essential for modulation. When similar substitutions were made at the $\underline{C}$-terminal position a prolyl and thiazolidine-2-carboxylic acid enhanced the binding of the dopamine agonist to the receptor. The tyrosyl residue in the neuropeptide proctolin, Arg-Tyr-Leu-Pro-Thr, has been substituted[173] by 4-NO_2, 4-NMe_2, 4-OMe and an

analogue prepared with the aromatic residue completely saturated. The 4-substituted analogues exhibited higher cardioexcitatory effects in insects than proctolin itself, and the results also confirmed the need for an aromatic system in the 2-position. In order to minimise the adverse effect of histamine release of some gonadotropin releasing hormone(GnRH) antagonists new structures with modifications at positions 1, 2, 3, 5, 7 and 10 have been synthesised[174] by solid phase. [Ac-D-2Val^1, D-4-ClPhe^2, D-3-Pal^3, Arg^5, D-(p-methoxybenzoyl)-2-aminobutyric acid,^{6} D-Ala^{10}]-GnRH, (D-3-Pal=3-(3-pyridyl)-D-Ala, 2-Nal=3-(2-naphthyl-D-Ala)) was one of the most potent analogues causing 100% inhibition of ovulation at 5 μg/kg with a lowered potency to release histamine.

Substitution of proline in position 1 of tentoxin, cyclo-[L-Leu-NMe(Z)Δ-Phe-Gly-MeAla] showed no effect[175] on chlorosis inducing activity compared with tentoxin itself. Substitution of 4-chloroproline in place of 3,4-dichloro-proline in cyclochlorotine, cyclo(L-Ser-β-Phe-L-Ser-L-ProCl$_2$-L-Abu), gave an analogue[176] with similar conformational characteristics but none of the biological activity (toxicity towards microorganisms and mice). So although the 3,4-dichloroproline moiety is difficult to handle synthetically it seems to have an essential role biologically. The synthesis of [D-lactic acid^2]- and [L-2-hydroxy-4-methylpentanoic acid^2]- AM Toxin I, and an assessment of their biological activity, has shown[177] the importance of bulky side-chains in position 2. The influence of basic residues on the biological activity of gramicidin S has been investigated[178] through the synthesis of analogues containing four ornithinyl residues. Cyclo-(Orn-Leu-Orn-Phe-Pro)$_2$ showed substantial activity against Gram-negative bacteria but other analogues containing D-residues showed negligible activity. Past problems with incorporation of homoserine into peptides because of lactonisation have been overcome[179] through the synthesis of N, O-ditritylhomoserine.

Incorporation of specific labels into peptides has become an important part of the armoury for monitoring pharmacological effects and metabolism studies. Asymmetric reduction of dehydroamino acids has provided a useful entry into specifically labelled LHRH analogues. 2-N-Acetylamino-3-(2-naphthyl)-3-^{14}C-acrylic acid(60) has been prepared[180] at 52.8 mCi/mmol from

$Ba^{14}CO_3$ and subjected to asymmetric reduction in the presence of a Wilkinson's chiral catalyst (S,S)-$BPPMRh^+$. The reduced derivative N-acetyl-D-3(2-naphthyl)-3-^{14}C-alanine(Nal) (98% optically pure) has been incorporated into the ^{14}C-labelled compounds, [N-Ac-D-3[^{14}C]-Nal^1, D-pClPhe^2, D-Trp^3 D-hArg(Et$_2$)^6, D-Ala^{10}]LHRH and [D-3[^{14}C]-Nal^6]LHRH having specific activities of 50mCi/mmol. Using the same route tritium-containing analogue[181] [Ac-D-(2,3-^{3}H)Nal^1, D-pCl-Phe^2, D-Trp^{3}D-hArg(Et$_2$)^6-D-Ala^{10}]LHRH has been obtained. Reductive dehalogenation in the presence of tritium has been used[182] to make a number of tritium-labelled somatostatin analogues, e.g. cyclo[4-^{3}H-Phe^7, D-Trp^8, Pro^{12}]-somatostatin (7-12), [4-^{3}H-Phe^6, D-Trp^8, D-Cys^{14}]-somatostatin and [D-Trp^8, ^{3}H-Phe^{11}, D-Cys^{14}]-somatostatin.

7 Conformational Information Derived from Physical Methods

Methodology and expertise continue to expand in this area and accurate intermolecular distance values are now being accumulated for the solution phase. Again 2D n.m.r. techniques show the greatest expansion area, and whenever these are referred to, it is assumed that all the technology associated with the technique, n.O.e. studies, amide bond temperature dependence etc, will have been used in deducing conformational forms. Cyclic and conformationally restricted forms tend to be rich territory for the application of these techniques. Such situations will tend to receive only brief comment as the area may overlap with material covered in Dr. Hardy's Chapter in this Report.

7.1 Nuclear Magnetic Resonance Techniques. - High-field n.m.r. at 500 MHz has been used[183] to study somatostatin analogues (61) and (62), intermediate in activity between SMS-201-995 and somatostatin. Both analogues seem to have a type II' β-turn involving Phe^3 to Thr^6. The presence of exocyclic residues Phe^1 and Thr^8(ol) plays a major role in stabilisation of the active conformation. In order to clarify a situation where other workers had found stacking of the Phe^6 Phe^{11} residues of somatostatin in methanol solution, and a bioactive conformation different to the conformers in H_2O/D_2O, a complete interpretation of the low-

temperature n.m.r. spectrum has been carried out[184]. The predominancy of one conformer already present in water was demonstrated with no evidence of interaction between Phe^{11} and other phenylalanine residues. Substance P has been shown[185] to adopt a rather extended form in DMSO and pyridine, but a complex conformational equilibrium was present in water solutions. For methanol solution it was concluded (i) that the N-terminal Arg-Pro-Lys conformation is flexible, (ii) Pro^4 to Phe^8 represents a sequence of residues with α-helical character and (iii) C-terminal carboxamide interacted with both glutamine side-chains. Spectra of substance P(SP) fragments, $[p\text{-}Glu^5]SP_{5-11}$ and $[p\text{-}Glu^6]\text{-}SP_{6-11}$ when compared[186] to simulated spectra showed no evidence of a preferred conformation for the backbone. SP_{1-4}, Arg-Pro-Lys-Pro, existed as a stretched molecule. A complete assignment[187] of exchangeable and non-exchangeable proton resonances of neurotensin (1-13) in aq. solution supports the conclusion that neurotensin exists mainly as a flexible random coil in agreement with previous c.d. studies. No significant population of β-type structures as proposed from potential energy calculations could be identified. Spectroscopic data[188] on the chemotactic peptide HCO-L-Met-L-Leu-L-Phe show that the three hydrophobic side-chains extend in divergent directions with respect to the backbone, and weak intermolecular H-bonds involving the formamide end group have been detected. A related sequence Boc-Met-Aib-PheOMe has been studied[189] in the solid state by X-ray diffraction and in solution by n.m.r. The results established that this peptide adopted a Met-Aib type II β-turn in the solid state, and there was evidence of a similar form in $CDCl_3$ and $(CD_3)_2SO$.

Dynorphin(1-13) has been studied[190] in aqueous solution and no major preferred secondary conformations emerged with no significantly large populations of χ^1 rotamers. N.O.e. measurements[191] between the ring protons of Tyr^1 and Phe^4 in Met^5-enkephalin have shown that a pair of protons, one from each ring are as close as 3.3Å. This could be intepreted in terms of a $Gly^2\text{-}Gly^3$ β-bend which could bring Tyr^1 and Phe^4 rings spatially close together as implied by the crystal structure of Leu^5-enkephalin. Comparisons of the n.m.r. spectra of Met-enkephalin dissolved in an aqueous solution of sodium dodecylsulfate to those obtained for the same peptide in lyso-phosphatidylcholine micelles

have indicated[192] that the peptide backbone was not remote from micelle surface. A H-bond involving the Met-5 amide proton and N-terminal residue interactions with Phe-4 and Met-5 side-chains have been detected. N.m.r. experiments and fluorescence transfer measurements have indicated that the ionisation state of the peptide has a great influence on conformational behaviour[193]. Under neutral conditions cholecystokinin fragments CCK_5, CCK_6 and CCK_7, the latter in sulfated or unsulfated form, adopt a β-turn involving residues Gly-Trp-Met-Asp. The presence of stable folded conformations is supported by evidence of through-space effects during titration of the ionisable groups. Analogues of the neuropeptide Ac-Asp-Glu have been studied[194] by ^{13}C n.m.r. Evidence of zwitterionic species such as (63) able to stabilise a cis-amide bond was accumulated, and these were not present when β-Asp residues were involved. A predominance of type I β-turn around Pro^6-Ser^7 at the C-terminus and an extended structure in the central sequence Phe^3-Gly^4-Tyr^5 has been suggested for the solution conformation of dermorphin[195]. Conformations ascribed to β-turns have been found in desGly^9[Arg^8]-vasopressin (β-turn in the 2-5 fragment)[196] and in aqueous solutions of Boc-Gly-Lys-Asp-Gly-OMe and Boc-Asp-Lys-Asp-Gly-OMe where β-turns are stabilised[197] by interactions between Lys and Asp side-chains. Following up a hypothesis that the receptor site conformation of gastrin is similar to a conformation deduced from previous chiroptical studies for the hormone in trifluoroethanol, high-resolution n.m.r. studies[198] have been carried out in d_6DMSO and in trifluoroethanol on three gastrin analogues, tetragastrin, octagastrin and minigastrin. Proof for some ordered secondary structure was found in trifluoroethanol even at the tetrapeptide level, but in DMSO only a random conformation was found.

The transformation of 2 Zn insulin dimer to 4 Zn hexamer by the addition of thiocyanate ion, already studied by X-ray methods, has now been monitored by 270 MHz n.m.r.[199] Aromatic interactions with sulfur atoms have been detected[200] through chemical shift differences in a series of deamino-oxytocin analogues. A *Herpes simplex* virus glycoprotein D antigenic domain (64) has been studied[201] with the result that a schematic H-bonded secondary structure as depicted in (64) was put forward. Fully assigned proton n.m.r. spectra of the repeating glycotripeptide unit in

antifreeze glycoprotein show[202] the presence of a hydrophobic surface of disaccharide side-chains wrapped closely against a 3-fold left-handed helical peptide backbone. N.m.r. parameters and molecular modelling have revealed[203] that in the structure of the ristocetin-pseudo-aglycan Ac_2Lys-D-Ala-D-Ala complex the Ala^1 residue is situated between the B and G aromatic rings of ristocetin with Ala^2 near the E-ring.

Excellent examples of the versatility of n.m.r. techniques can be seen from the work[204] on the tricyclic octapeptide moroidin from Laportea moroides, on antamanide[205], the cyclic pentapeptide[206] cyclo-(Pro-Pro-Phe-Phe-Gly), the cytostactic cyclic tetrapeptide[207] cyclo-[L-Leu-L-Pip-L-(2-amino-8-oxo-9,10 epoxydecanoyl)-D-Phe], and the bacterial lipopeptide[208] iturin A. Measurements[209] using 2D n.O.e. confirm previously deduced absolute interatomic distances in gramicidin S, and accurate backbone distances for [Val^3]-HC-toxin are identical with the parent toxin[210]. Four diastereoisomers of cyclo-(Gly-Pro-Phe-Ala-Asn-Ala-Val-Ser) have been studied[211] with the result that it appears that a single Pro residue in the ring is insufficient constraint to stabilise the conformation established for cyclo-$(Gly\text{-}Pro\text{-}Phe\text{-}Ala)_2$. Conformational analyses of bicyclic A.C.E. inhibitors in the series (65)[212], and on a series of N(α)-t-BocN(π)benzyloxymethylhomo-oligo-L-histidyl methyl esters[213] have been reported. Lanthanide ion complexes of aspartame α-Asp-Phe-OMe have been characterised[214] and the influence of Thr residues in each position of an alanyl tetrapeptide has been assessed[215]. Threonine residues in 1 or 3 seem to have some influence on β-turn formation in acid solution. A study[216] of trans-Ac-Asn-Pro-TyrNHMe and trans-Tyr-Pro-Asn-NHMe using multinuclear n.m.r. spectroscopy as models for sequences in ribonuclease A have shown a predominance of the trans conformation at the X-Pro bonds, although in the protein they appear as cis X-Pro bonds. Enrichment of Tyr with ^{15}N made it possible to distinguish the resonances of the side-chain β-protons of Tyr. A conformational analysis of the Pro ring in EtCOAla-Pro-NHEt has been carried out[217], and the cis/trans isomerism in five classes of proline peptides has been made using solid-phase n.m.r. techniques[218]. ^{14}N N.m.r. investigations[219] for a single crystal of N-acetyl-D,L-valine show close agreement with X-ray diffraction results and the

effects of ^{18}O-isotopes on the ^{13}C chemical shifts of peptide carbonyl carbons have been recorded[220].

7.2 X-Ray and Related Techniques. - A crystal structure of pepstatin analogue (66) showed[221] that the first three residues adopt an extended conformation and the chain is folded back at the Sta and Ala residues to form a 12-membered ring. Phenyloxyacetyl-Leu-Val-PheOMe, an analogue of angiotensinogen(10-13), has been shown[222] to adopt a pleated sheet conformation in the solid state, while seven TRH analogues have been found[223] to possess conformational similarity with all peptide bonds trans. In a series of papers[224] on solid-state conformation of linear peptides, crystal data have been reported for DL-Ala-DL-Nva, which has similarities with D-Ala-L-Met, for L-Pro-Gly-Gly, for L-Val-Gly-Gly and for t-Boc-Gly-L-Phe. The implications[225] for the use of 1-amino cyclohexane carboxylic acid (Acc^6) in conformational design have been rationalised from X-ray data on $Boc(Acc^6)_3OMe$ (type III β-turn at $Acc^6(1)$-$Acc^6(2)$), and Boc-Pro-Acc^6-AlaOMe (type II β-turn at Pro-Acc^6). The Acc^6 residue seems to be able to occupy either position of a type III β-turn and the i+2 position of type II β-turns. Monochloroacetylated glycines, diethylated and dipropylated at the α-carbon, have been studied[226] by X-ray diffraction and the conformation about the amide bond was shown to be trans.

A crystal structure of cyclo$(L\text{-Pro-Gly})_3$ showed[227] that there were five trans and one cis peptide unit present, and confirms that the presence of the alternating prolyl residues prevents the formation of β-turns. When a 2:1 complex of the cyclopeptide with Ca^{2+} was studied it was shown that the Ca^{2+} ion is sandwiched between the two peptide molecules. All the peptide bonds have been shown[228] to be trans in cyclo-(Gly-L-Pro-D-Phe-Gly-L-Ala) and the water structure in [Phe^4, Val^6]-antanamide $12H_2O$ has been more clearly defined[229]. A crystal structure of cyclo-(D-Ala-Gly-Pro-Phe)$_2$ confirms[230] an all-trans peptide backbone with type I β-turns at Pro-Phe which makes it slightly different from the previously published cyclo-(D-Ala-Gly-L-Pro-D-Phe)$_2$ structure.

Some indication of the His-imidazole interaction modes as a function of their electrical state has come[231] from the crystal structures of tBuCO-Pro-X-NHMe (where X=His, His(τMe), His(πMe)

and His$^{+}PF_6^{-}$). The first three derivatives accommodated a βI turn but the latter protonated species showed a different conformation in which the tBuCO exhibited a strong intramolecular H-bond with the His^{+}-$\underline{N}^{\pi}$H. Studies[232] on Boc-Pro-Val-Gly-NH_2 show proof for the reasoning that valine residues are not usually involved in a α-helix or a β-turn, since in the report the data gave ($\varphi\psi$) angles close to the antiparallel β-sheet values. The crystal structure of $\underline{N}$-pivaloyl-D-Phe-L-Pro-$\underline{N}$-ethylamide shows an all-transoid type II'β conformation[233].

7.3 Infra-Red and Circular Dichroism Studies. - Many papers now appear with these techniques complementing information from other sources, so this section highlights only those papers where the technique remains the mainstream source of data. Infra-red studies[234] have proved useful in monitoring the equilibria between self-associated and unassociated species of the $\underline{N}^{\alpha}$-protected $\underline{C}$-terminal sequences of substance P. It was found that main chain length, solvent and peptide concentration all played a role in self-association and an Aib residue at position 9 drastically reduced self-association. Both infra-red and circular dichroism techniques have contributed significantly to the Schwyzer model for the membrane structure of many peptides. In the work[235] on substance P and segments it has been confirmed that the $\underline{C}$-terminal sequence (residue 3 to 4 onwards) can adopt α-helical conformation in hydrophobic environments and on lipid membranes. The $\underline{N}$-terminal segment (1-4) had a poly-(Pro) like conformation in aqueous and hydrophobic surroundings. C.D. studies[236] combined with synthesis and biological activity measurements have confirmed the importance of the $\underline{N}$-terminal portion of gastrin for optimisation of biological activity. In [des Trp^{1}, Asp$^{3-7}$]Leu-human minigastrin, substitution of the $(Asp)_5$ instead of $(Glu)_5$ as in the natural form caused a dramatic conformational change with reduction in ordered structure, and in biological activity. Some rationalisation of the Ca-dependence of gastrin activity has been made[237] as a result of c.d. measurements in trifluoroethanol on [pGlu^{10},Nle^{15}]-human gastrin HG(10-17) and [pGlu^{9},Nle^{15}]-HG(9-17). The former binds one Ca^{2+} per molecule and the latter has a second binding site as well. The change in chiroptical properties on Ca binding appears to be due to chain unfolding. C.D. studies[238] on

a gastrin analogue pGlu-$(Glu)_4$-Ala-Tyr-Gly-Trp-Nle-Asp-PheNH_2 showed evidence of a β-bend in 12% H_2O/trifluoroethanol(TFE) while in 2% H_2O/TFE evidence for the presence of a β-structure was obtained. Changes in biological activity of salmon calcitonin have been correlated[239] with conformational changes monitored using c.d. techniques in the presence and absence of lipids. Increase in biological activity of some analogues has been ascribed to increased flexibility in the molecule due to substitution of less bulky side-chains. Circular dichroism has also been used[240] to monitor the extent that peptide fragments of salmon calcitonin fold into structures of higher helical content in the presence of lipids. C.D. spectral analysis[241] of gramicidin S analogues has been used to rationalise the conformation of some of the biologically inactive analogues. Dermorphin, [L-Ala^2]-demorphin and N-terminal fragments in solution have been analysed by c.d. techniques, and a folded conformation with a high β-turn potential because of the presence of D-Ala has been proposed[242]. Interaction of Leu-enkephalin with phosphatidylserine has been studied[243] by many techniques, including circular dichroism. Data support the existence of binding between the two species with both tyrosine and phenylalanyl residues being involved. The signal sequence of *E. coli* alkaline phosphatase has been shown[244] by c.d. measurements to have α-helix and β-structure in hydrophobic environments.

C.D. Measurements and n.m.r. studies have been brought to bear on the effect of conformation on the efficiency of the His---Ser---Asp triad in the active centre of proteases. Linear analogues show[245] quite drastic conformational changes at around pH7 but cyclic analogues maintain an ordered conformation even at basic pH. FT-Infra-red spectroscopy was among a number of techniques used to establish[246] that *cyclo*-[Gly-L-Lys(Z)-Sar-L-Pro] has a *trans*-*cis*-*trans*-*trans* peptide backbone with no intramolecular H-bonds. Raman spectroscopic data[247] reveal that amide III rather than amide II frequencies are more diagnostic for β-turn structures in model cyclic peptides and have contributed[248] to establishing the conformation of methoxycarbonyl-L-Phe-L-Ala-ethyl dithioester. Conformational behaviour of monodisperse ampiphilic oligopeptides $(L\text{-Thr-L-Val})_n$ and $(L\text{-Ser-L-Leu})_n$ for

n=1-4, and models containing Aib-residues have been investigated by C.D. spectroscopy in solution[249].

7.4 Computational Methods. - Again these are techniques that researchers are using increasingly to augment experimental data, so the emphasis will be on reports where graphics, calculations and Q.S.A.R. are the main themes. Use of an adaptive, importance-sampling Monte Carlo algorithm (SMAPPS - Statistical Mechanical Algorithm for Predicting Protein Structure) has been reported[250] for determining the average conformation of Met-enkephalin, and good correlation with experimental data has been achieved when Monte Carlo techniques were used[251] on Leu-enkephalin. Energy minimisation calculations used for comparing phenazocine to the enkephalins revealed[252] the best fit between the two opiate groups came when the backbone conformer of the enkephalin was of the form previously suggested by Gorin and Marshall in 1977. Solvent-dependent conformational statistics applied to enkephalin and angiotensin II showed[253] good correlation with experimental data.

In the absence of experimental data, the 18-23 loop region of bovine prothrombin has been studied[254] by an energy minimisation algorithm, and reveals that the binding of Ca^{2+} to the 18-23 cyclic peptide may alter the equilibrium between cis and trans structures. A systematic study[255] using a semiempirical method on brain peptides has continued with the description of the structure of β-MSH in terms of eight low-energy conformations. Electrostatic and related interactions which occur in aqueous media have been taken account of[256] in a theoretical calculation of γ-melanotropin fragment His-Phe-Arg-Trp-Asp-Arg-Phe-Gly. The solution conformations of TRH(Glp-His-ProNH_2) have been predicted[257] computationally using a method which represents solution effects as a dielectric continuum surrounding the molecule. It is suggested that electrostatic or steric interaction between the imidazole ring may 'switch on' the amide group conformation of the Pro residue as implicated in potency. Secretin tetrapeptide fragments have undergone[258] conformational energy calculations, as well as C-terminal tachykinin heptapeptides[259]. A useful set of empirical rules have been put forward[260] to predict the conformation of cyclic tetrapeptides and tetradepsipeptides. Conformation allowing a γ-turn is

preferred and an ester bond always adopts a trans form. An ab initio study of intermolecular H-bonding between small peptide fragments has been investigated[261] and SIMCA, a pattern recognition technique, has been applied to structure-taste studies[262] on L-Asp dipeptides. The peptide L-AspNHCH$_2$OH on the model is predicted to have a sweet taste.

Estimation of preferred conformation, orientation and accumulation of ACTH(1-24) at an aqueous-hydrophobic interface produced[263] a model that agreed with experimental observations with lipid membranes. As in opioid peptides the amphiphilic moment is an important new parameter for determining quantitative structure-activity relationships (Q.S.A.R.) in receptor selection and biological potency. When similar considerations were applied[264] to substance P it was deduced that this neuropeptide would be expected to insert a C-terminal message into lipid membranes. The prediction of bradykinin potentiating potency of pentapeptides has come[265] from Q.S.A.R. considerations. The structure descriptor matrix based on the 20 coded amino acids described 97% of the variations in observed activity of the peptides.

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4
Cyclic, Modified, and Conjugated Peptides

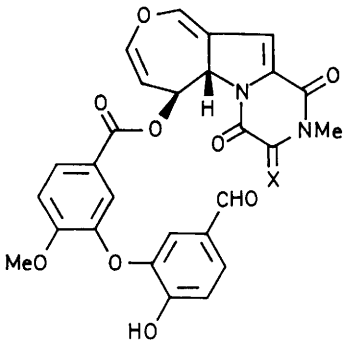
BY P. M. HARDY

1 Introduction

The number of subdivisions in this chapter has been increased in the present volume. These changes partly reflect attempts to present a growing number of references in the most useful form, and to a certain extent shifts in emphasis within the field covered. It is perhaps worth reiterating that this chapter does not now include coverage of synthetic or conformational work on peptides composed of the amino acids which occur in proteins simply because they contain D-residues, an omission based on practical rather than ideological grounds. Peptides which fall into more than one category are only mentioned once, and their classification is fairly arbitrary. Again, this has proved necessary to keep the chapter within its allotted bounds.

2 Cyclic Peptides

2.1 Naturally Occurring Dioxopiperazines (Cyclic Dipeptides)- Full details of the structure determination of the macrocyclic epidithiodioxopiperazine emestrin (ref. 1 in Vol.18), isolated from a thermotolerant fungus growing on cumin in Nepal, have been published.[1] Dethiosecoemestrin (1a), a new trioxopiperazine metabolite whose C-skeleton is identical to that of emestrin, has been isolated from the same source, Emericella striata. It is easily degraded to violaceic acid, and is active against E. coli.[2] Aurantioemestrin (1b) and silvathione (2) have been obtained from Emericella striata and Aspergillus silvaticus respectively. They are possible biogenetic intermediates in the formation of trioxopiperazines from epidithiodioxopiperazines.[3] Gliotoxin G (3a), the tetrasulphide analogue of gliotoxin (3b), has now been identified in Aspergillus fumigatus. The o.r.d. curve of gliotoxin G is very similar to that of gliotoxin except for an enhancement of 15-30% , suggesting the same absolute configuration for the sulphur bridge.[4] Gliotoxin G, which has immunosuppressive activity, has been synthesised from gliotoxin by treatment with elemental sulphur in carbon disulphide containing a catalytic



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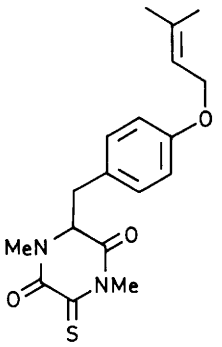
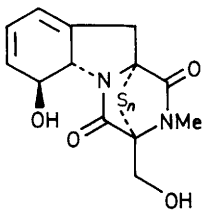
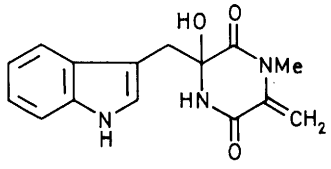
b; X = S



(2)



(3) a; *n* = 4
b; *n* = 2



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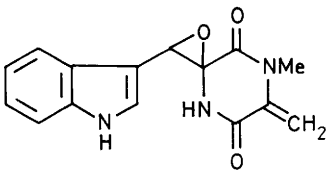

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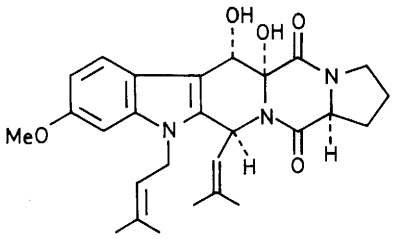

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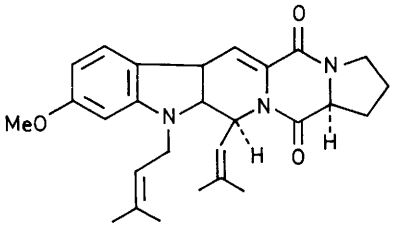


(7)

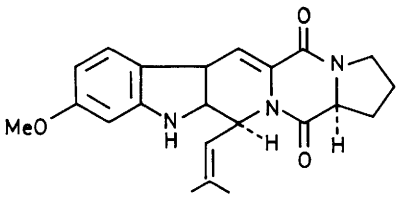
amount of thiolate.[5]

The reaction of the tryptophan derivative (4) with DDQ yields (5); this epoxide may serve as a model compound to investigate the biosynthesis of the sporidesmins.[6] Fumitremorgin B (6), a potent tremorgenic mycotoxin produced by Aspergillus fumigatus, has been synthesised by selective oxidation of the dehydro-β-carboline (7) by successive treatments with bromine in aqueous THF (which gives the wrong stereoisomer), DDQ, and sodium borohydride.[7] In an independent synthesis fumitremorgin B has been obtained from (8) by an osmium tetroxide dihydroxylation followed by N-prenylation with dimethylallyl chloride.[8]

The antifungal antibiotic piperazinomycin (9), first reported in 1982, has been made from the symmetrical dioxopiperazine (10). In the key step the strained 14-membered ring compound (11) was generated by a thallium trinitrate oxidation in 19% yield.[9] An effective intramolecular S_N2 cyclisation and Michael cyclisation have been developed for constructing the tricyclic framework of the natural mycotoxins brevianamides A and B. For example, treatment of (12) with sodium hydride furnished the tricyclic olefins (13a) and (13b) in 60% yield in a 10:1 ratio respectively.[10]

A simple route to the bicyclomycin nucleus has been described using the β-vinyl sulphone (14) as a new vinylic cation synthon. Michael addition of the anion of (15) to (14) gave the adduct (16) regioselectively. After deprotection of the hydroxyl with $Bu_4N^+F^-$, cyclisation of (16) to give the bicyclomycin nucleus (17) was achieved by a DDQ oxidation.[11] A mechanism of action for bicylomycin has been proposed. The dioxopiperazine ring is held in a boat conformation, and hence is a structural analogue of the Dap-Dap bond (Dap = diaminopimelyl) and inactivates the amidase responsible for the breakage of this bond. The Dap-Dap cleavage is required in the normal remodelling of peptidoglycan.[12]

2.2 Other Dioxopiperazines - In the presence of bases 1,4-diacetyl-2,5-piperazine-diones condense with salicylaldehyde derivatives to give mixtures of two kinds of products (Scheme 1). Irradiation with a high-pressure mercury lamp converts (18) to (19).[13] Further applications of the bislactim ether method of amino acid synthesis have been reported. The high diastereofacial selectivity of the Michael addition in the synthesis by this method of glutamic acid using cyclo(Val-Gly) and methyl 2-alkenoates is attributed to charge transfer or HOMO-LUMO interactions between the π-systems



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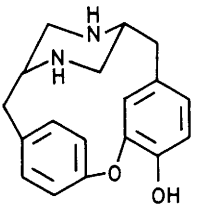

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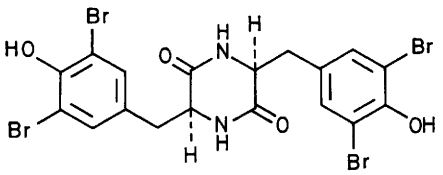

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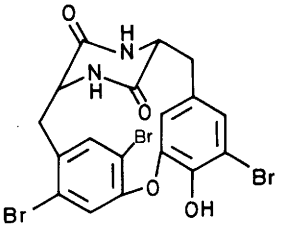

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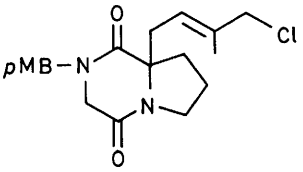
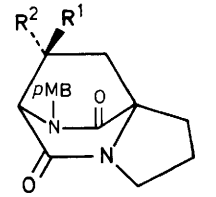
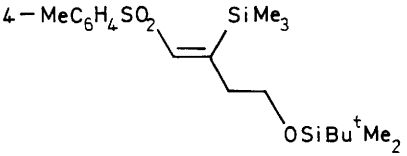


(12) *p*MB = *p*-methoxybenzyl

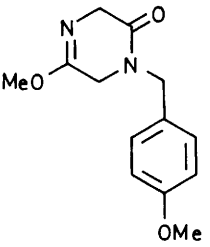


(13) a; R^1 = H, R^2 = 

b; R^1 =  , R^2 = H



(14)



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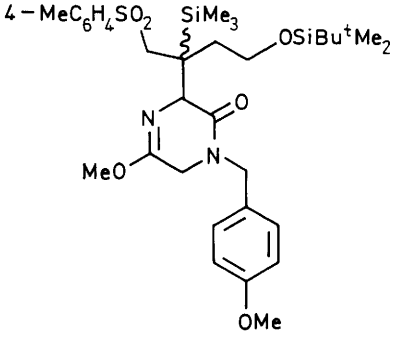
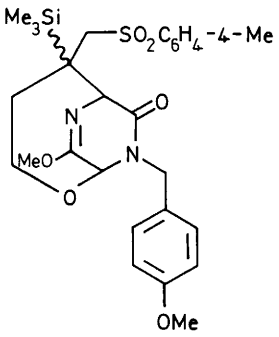


(16)



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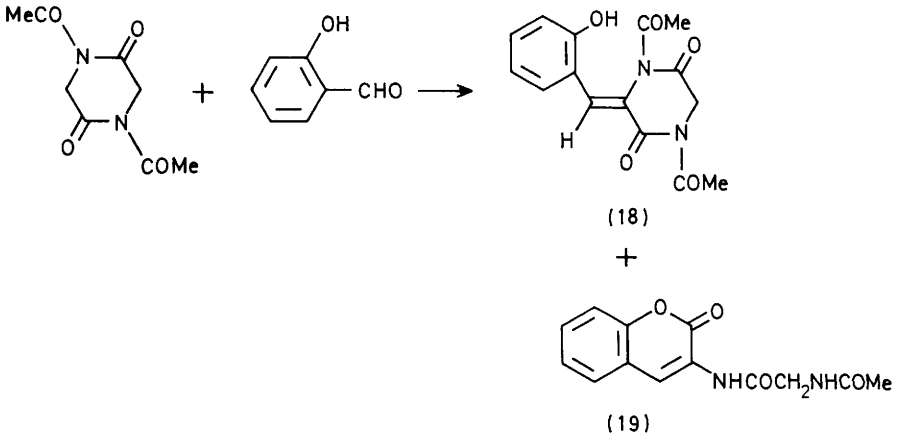
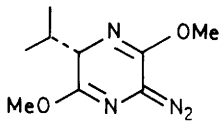


(18)

+

(19)

Scheme 1



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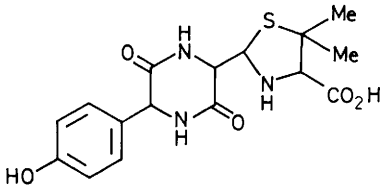

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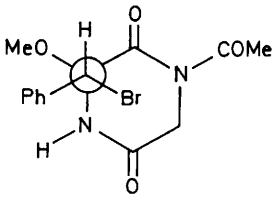


(22)

in the transition state.[14] Alkylation of lithiated cyclo(Val-Ala) also gives high asymmetric induction,[15] and 1-amino-1-cyclopropanecarboxylic acids have been prepared by the addition of olefins to the carbene derived from the diazo-compound (20).[16]

Using cyclo(Phe-His) as catalyst, optically active cyanohydrins have been obtained from substituted benzaldehydes with optical yields of 33-82%. Nonpolar solvents are advantageous; no asymmetric synthesis was observed in MeOH or DMSO.[17] The rate of cyclo(D-Val-Pro) formation from Fmoc-D-Val-Pro-$OCH_2C_6H_4OCH_2$-polystyrene following N-deprotection has been studied. Of the reagents tested, 50% piperidine -DMF proved by far the best catalyst for dioxopiperazine formation. However, restricting exposure to this mixture to 5 minutes gives quantitative deprotection with less than 4% cyclic dipeptide formation except in the most sensitive cases.[18]

Treatment of amoxocillin with glucose in solution at pH 9.2 liberates the (2R)- and (2S)-piperazine-2,5-diones (21). The former is readily epimerised to the latter in acidic solution, giving a 2:1 mixture at equilibrium.[19] In the self-condensation of neat DL-amino acid alkyl esters LL- or DD-dioxopiperazine (i.e. cis) is formed preferentially in the early stages of the reaction, but the cis:trans ratio gradually decreases as the reaction proceeds.[20] A study has been made of the racemisation of dioxopiperazines at 120 °C in aqueous phosphate buffered solutions at pH 8. The initial rate was very fast, but soon slowed down; this is attributed to hydrolysis.[21] The reaction of 3-benzylidenepiperazine-2,5-diones with N-bromosuccinimide in MeOH gives rise to 3-(α -bromobenzyl)-3-methoxypiperazine-2,5-diones. The threo-isomer is the major product, and its crystal structure shows the side-chain in the E_N conformation (22), the ring being almost planar. This conformer predominates also in $CDCl_3$ solution, but in $(CD_3)_2SO$ the ring adopts a boat conformation.[22]

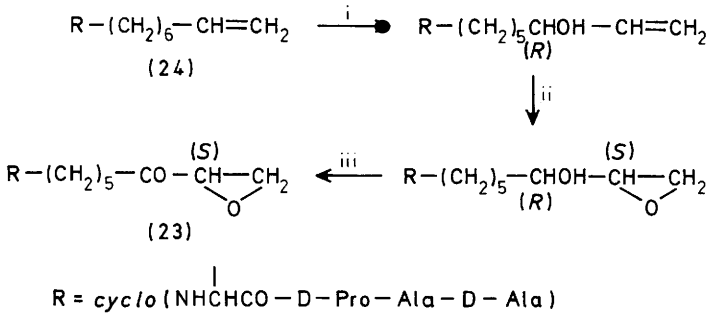
X-Ray examination of cyclo(Phe_2) shows a flattened boat conformation, one of the side-chains facing the ring and the other in an extended conformation.[23] ^1H N.m.r. data obtained from cyclo(Phe_2), cyclo(1-naphthylalanyl$_2$), and cyclo(2-naphthylalanyl$_2$) suggests all three rings are planar. In the latter two compounds one naphthyl ring is folded onto the heterocyclic ring.[24] However, for cyclo(D-9-anthranylalanine$_2$), spectroscopic studies in solution do not distinguish between folded-unfolded and unfolded-

unfolded side-chain arrangements, although theoretical calculations in this case favour the latter.[25] A ^{1}H n.m.r. study of dimeric cyclo-(hemicystinyl-Gly) shows a buckled dioxopiperazine ring with a folded side-chain. A disulphide sulphur atom is the primary co-ordination site for transition metal ions.[26]

Conformational energy calculations on cyclo (Gly-Phe) carried out by the semi-empirical MO CNDO/2 method corroborate earlier findings that the conformation with the aromatic side-chain folded over the heterocyclic ring is favoured.[27] Similar calculations on cyclo(Gly-Ala), cyclo(Gly-Val), cyclo(Ala-Ala), and cyclo-(Ala-D-Ala) show non-planar cis peptide bonds and axial side-chain orientations, which agrees well with experimental results. [^{3}H]-Cyclo(His-Pro) binds with high affinity to a single class of sites in rat liver plasma membranes without significant tracer degradation during equilibration for 60 minutes at 0 °C. Binding is increased by the addition of K^{+}, but not by the addition of Ca^{++}.[29]

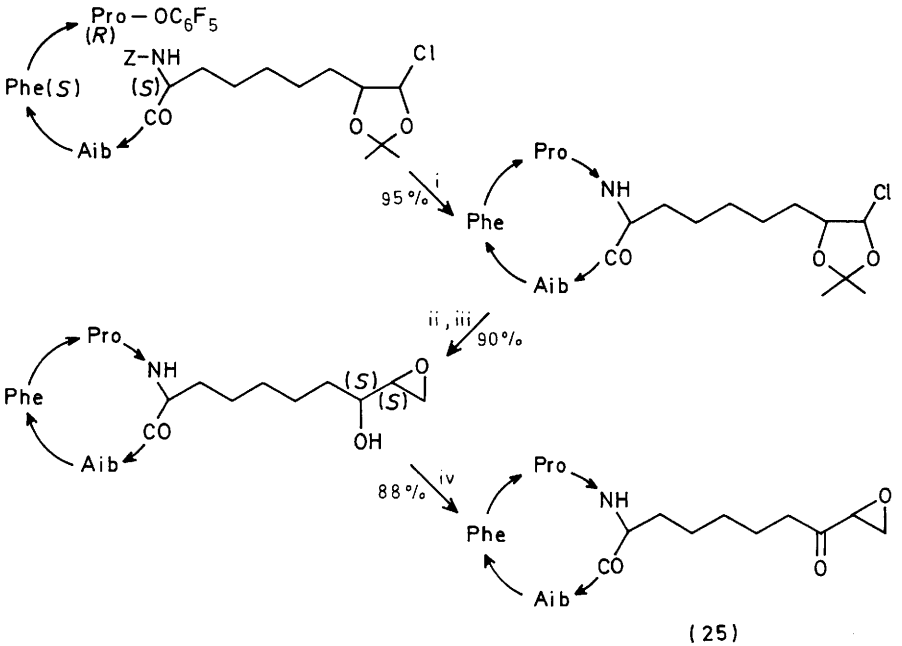
2.3 Cyclic Tetrapeptides - The sequence of the cytostatic cyclic tetrapeptide WF-3161 from Petriella guttulata has been established as cyclo(Leu-Pip-Aoe-D-Phe), where Pip = pipecolic acid and Aoe = 2-amino-8-oxo-9,10-epoxydecanoic acid. In $CDCl_3$ solution WF-3161 adopts a conformation with a possible γ-turn between LeuNH and the Aoe C=O and a cis amide bond between Leu and Pip.[30] From Alternaria citri has been isolated the stereochemically novel dihydrotentoxin cyclo(Leu-N-Me-Phe-Gly-N-Me-Ala), which displays the chlorosis-inducing activity of tentoxin, although at a reduced level. Reduction of the dehydroPhe of tentoxin generates the diastereomer cyclo (Leu-N-Me-D-Phe-Gly-N-Me-Ala), which is non-toxic.[31] Tentoxin itself and [Pro^{1}]-tentoxin have been synthesised using D,L-3-phenylserine as the precursor of the Z-dehydroPhe residue. With glycine as the C-terminal residue of the linear precursor, better yields (60%) of cyclic peptide than hitherto reported were achieved using diphenylphosphoryl azide, 1-hydroxybenzotriazole, and 4-dimethyl-aminopyridine for cyclisation.[32]

HC-Toxin (23) and its 9R-epoxide epimer have been made from an alkene precursor (24) by three successive oxidation steps (Scheme 2). The epoxyketone configuration was assigned by c.d. spectroscopy.[33] [Val^{3}]-HC toxin has also been prepared; n.m.r. studies indicate a conformation identical with that of the parent



Reagents: i, Bu^{t}OOH; ii, Sharpless epoxidation; iii, *m*CPBA

Scheme 2



(25)

Reagents: i, H_2/ Pd; ii, $(CO_2H)_2$; iii, K_2CO_3; iv, DCC / DMSO / Cl_2CHCO_2H / 0 °C

Scheme 3

cyclopeptide.[34] The cyclisation of peptides by catalytic hydrogenation of ω-Z peptide pentafluorophenyl esters, developed for the peptide alkaloids, has now been applied to homodetic cyclic tetrapeptides. As cyclisation of Z-Leu-Aib-Phe-D-Pro-OC_6F_5 gave a 95% yield of cyclotetrapeptide, the method was then applied to the preparation of chlamydocin (25). The final stages of the synthesis are shown in Scheme 3. No racemisation of proline occurred, but if the final oxidation was carried out with pyridinium chlorochromate 30% inversion at C-9 took place.[35] The Sharpless chiral epoxidation reaction has also been successfully applied to the synthesis of chlamydocin and its 9R-epimer epichlamydocin. C.d. studies of these compounds provide a simple spectroscopic method for assigning the chirality of the Aoe epoxy group in other natural products.[36]

The cyclic enkephalin analogue H-Tyr-cyclo[ε-D-Lys-Gly-Phe-γ-Glu-NH_2](see ref. 40 Vol.18) is unselective towards μ- and δ-receptors, unlike related open-chain compounds, which are μ-selective.[37] Another cyclic enkephalin analogue H-Tyr-cyclo[D-N^{δ}-Orn-Gly-Phe-Leu] and a rigid narcotic alkaloid have been studied by computer graphics to investigate potential geometrical congruencies of their respective pharmacophoric elements,[38] and theoretical and spectroscopic studies of H-Tyr-cyclo[D-A_2bu or Glu-Phe-Leu] have been reported.[39] N.m.r. studies on cyclo[Gly-Lys(Z)-Sar-Pro] indicate a trans-cis-trans-trans peptide bond sequence, but FT-i.r. data established the absence of intramolecular H-bonds. Over 10^{-3}M, the peptide exists in aggregated states; a possible intermolecular pattern of H-bonds is proposed.[40]

2.4 Cyclic Pentapeptides - [$Pro(Cl)^4$]-Cyclochlorotine, cyclo-(Ser-β-Phe-Ser-Pro(Cl)-Abu), has been synthesised. This replacement of a 3-cis-4-cis- dichloroproline by a 4-cis-chloroproline residue destroys the toxicity of the peptide, although ^{1}H n.m.r. and c.d. studies indicate a similar conformation in solution for the two peptides.[41] In the preparation of cyclo(Gly-Pro ψ [CH_2S] Gly-D-Phe-Pro), cyclisation using diphenylphosphoryl azide gave only a 10% yield at Gly-Pro but 80% at Gly-D-Phe. The cyclopeptide can adopt both β- and γ - turns in $CDCl_3$, but in contrast to its all-amide counterpart it showed evidence of cis/trans-proline peptide bond isomerism on addition of DMSO.[42]

In the crystal, all the peptide bonds of cyclo(Gly-Pro-D-Phe-Gly-Ala) are trans, although one is twisted by 19° from planarity.

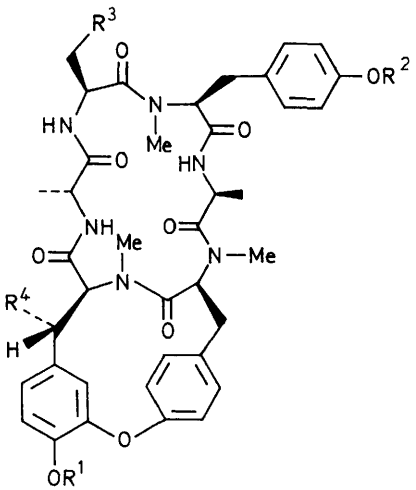
A type II β-turn encompasses the Pro-D-Phe residues, and the backbone conformation differs from that established for other cyclopentapeptides with the DLDDL chirality sequence.[43] An n.m.r. investigation of cyclo(Pro_2-Phe_2-Gly) shows that a single conformation dominates in DMSO; uniquely for a cyclopentapeptide, both Pro peptide bonds are cis. Although a β I conformation was expected for the Phe-Phe sequence, a βII' turn was in fact found.[44] Ten side-chain protected thymopoietin analogues (Table 1) have been prepared using DCC/DMAP for cyclisation, which gave better yields and purity compared with the azide method. However, racemisation or inversion was observed if the linear precursors contained respectively no C- or N-terminal D-amino acid. In solution all ten cyclopeptides appear to adopt a βII' γ-structure. The results exclude an extended conformation for the linear 32-36 fragment (Arg-Lys-Asp-Val-Tyr) as the biologically active conformation.[45]

2.5 Cyclic Hexapeptides - Two antitumour cyclic hexapeptides, RA-V and RA-VII, found in the roots of the Chinese plant Rubia cordifolia were characterised in 1983. The structures of four minor components, RA's I to IV (26) have now been elucidated. RA-V has been converted to RA-IV by successive treatment with DDQ and diazomethane.[46] The total synthesis of echinocandin D (27) has been achieved. The final cyclisation, a diphenylphosphoryl azide mediated acylation of the side-chain amino group of the ornithine, gave the cyclic hexapeptide in 50% yield as a glassy solid identical to natural material. For the more sensitive couplings in the synthesis of the linear precursor thiopyridyl esters were used.[47] Two cyclohexapeptides with further intramolecular bridges between the ε-amino groups of the two lysine residues (28) have been prepared for examination as a new type of host molecule.[48]

Five cyclic hexapeptides containing combinations of Ala, Gly, Asp, and Lys have been prepared by a variety of methods (Table 2). In all the linear precursors used glycine was C-terminal. No cyclodimers or higher polymers were detected from reaction of linear hexapeptides. The temperature dependency of peptide NH signals showed the cyclo(Gly-Xxx-$Gly)_2$ compounds to be stabilised by intramolecular H-bonds and to be resistant to temperature-induced conformational change.[47] Compared to linear and polymeric peptides with the same sequence, cyclo(Asp-εAhx-Ser-εAhx-His-εAhx) showed the greatest activity in the hydrolysis of various ester substrates. The cyclic peptide maintained an ordered

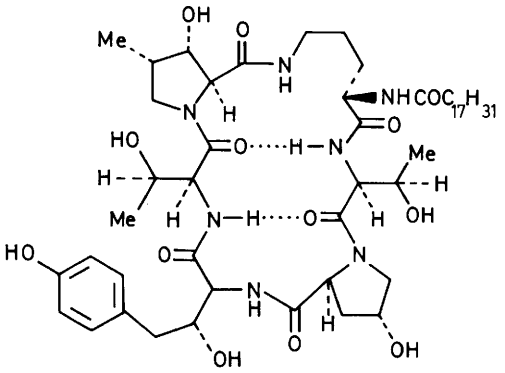
cyclo [Arg(NO_2)–Lys(Z)–Asp(OBzl)–D–Val–Tyr]
cyclo [Arg(NO_2)–Lys(Z)–Glu(OBzl)–D–Val–Tyr]
cyclo [Arg(NO_2)–Lys(Z)–Asp(OBzl)–D–Val–Trp]
cyclo [Arg(NO_2)–Lys(Z)–Glu(OBzl)–D–Val–Trp]
cyclo [D–Lys(Z)–Arg(NO_2)–Asp(OMe)–Val–Tyr]
cyclo [D–Lys(Z)–Arg(NO_2)–Glu(OBzl)–Val–Tyr]
cyclo [Arg(NO_2)–Lys(Z)–Glu(OBzl)–Val–D–Phe]
cyclo [Arg(NO_2)–Lys(Z)–Asp(OBzl)–Val–D–Tyr]
cyclo [Arg(NO_2)–D–Lys(Z)–Asp(OBzl)–D–Val–Tyr]
cyclo [Arg(NO_2)–D–Lys(Z)–Glu(OBzl)–D–Val–Tyr]

Table 1 Thymopoietin analogues



(26)

	R^1	R^2	R^3	R^4
RA – I	H	Me	OH	H
II	Me	H	H	H
III	Me	Me	OH	H
IV	Me	Me	H	OH
V	H	Me	H	H
VII	Me	Me	H	H



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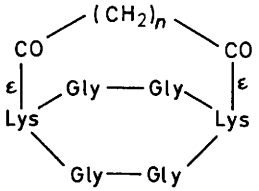


(28) $n = 4$ or 8

		Method of Cyclisation (1.1 mM conc.)			
		N_3	ONp	ONSu	DEPC
cyclo	$[Ala-Gly-Gly]_2$	57	42		
cyclo	$[Gly-Ala-Gly]_2$	50	63		
cyclo	$[Lys(Z)-Gly-Gly]_2$	64	55	57	56
cyclo	$[Gly-Lys(Z)-Gly]_2$		35	29	25
cyclo	$[Gly-Asp(OBzl)-Gly]_2$		51*		

* by cyclodimerisation ; final conc. 2.5 mM

DEPC = diethyl phosphorocyanidate

Table 2 Percentage yields on cyclisation

conformation to some extent even in the basic pH region.[50]

In the crystal, the backbone of cyclo(Pro-Gly)$_3$ is asymmetric and made up of one cis and four trans peptide units. There is a hydrogen bonded water bridge linking the carbonyl oxygens O1 and O4. The 2:1 sandwich complex that this cyclohexapeptide forms with Ca(II) has also been subjected to X-ray analysis.[51] The crystal structure of cyclo(Phe-Pro-D-Ala)$_2$ crystallised from Me_2SO/H_2O was described in 1984. A second crystalline form obtained from H_2O/MeOH/NaSCN has now been reported as having a similar conformation.[52]

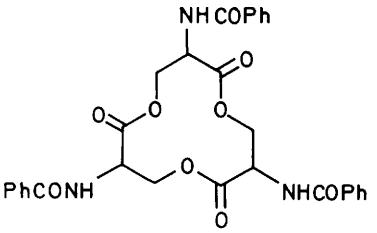
2.6 Higher Cyclic Peptides - A novel toxic cyclopeptide has been isolated from Amanita suballiacea. This compound, alloviroidin, is identical to viroidin in both mass and affinity for actin. It differs only in that it contains 2S,4S- instead of 2S,4R-4,5-dihydroxyleucine.[53] Conditions have been established for methylating α-amanitin with diazomethane which give solely 6'-O-methyl-α-amanitin. If the optimal stoichiometry is exceeded, 1'-N,6'-O-dimethyl-α-amanitin is also formed.[54]

Four cyclic octapeptides (29) containing glycine and either lysine or aspartic acid have been prepared, glycine being C-terminal in the linear precurors. These are an extension of the series of compounds described in the section on cyclohexapeptides, cyclo[Gly-Lys(Z)-Gly-Lys(Z)-Gly]$_2$ also being made.[49] Although cyclo[Gly-Lys(Z)-Sar-Pro]$_2$ efficiently transports the picrate salts of Ba(II) and Ca(II) through a chloroform 'membrane' in a U-tube, the corresponding linear or cyclic tetrapeptide and linear octapeptide sequences exhibit much less ion-transporting ability.[55] X-Ray crystallographic analysis of two crystalline forms of cyclo(D-Ala-Gly-Pro-Phe)$_2$ shows both to contain only trans peptide bonds and type I β-turns at Pro-Phe, but the second form differs from the first in that the plane of one Ala-Gly peptide bond is rotated by about 180°.[56] Three diastereoisomers of cyclo(Gly-Pro-Phe-Ala-Asn-Ala-Val-Ser) containing Phe-Ala, D-Phe-D-Ala, and D-Phe-Ala sequences have been synthesised. N.m.r. studies indicate that a single proline residue in the ring is insufficient constraint to stabilise the backbone conformations previously established for cyclo(Gly-Pro-Phe-Ala)$_2$.[57]

Comparative ^1H and ^{13}C n.m.r. studies have been carried out on the cyclic nonapeptide cyclo(Asp-β-Ala-Gly-Sar-β-Ala-Gly-His-β-Ala-Gly) and the corresponding linear compound and a polymer

cyclo [Lys(Z)–Gly_3]$_2$
cyclo [Lys(Z)–Gly]$_4$
cyclo [Asp(OBzl)–Gly_3]$_2$
cyclo [Asp(OBzl)–Gly]$_4$

(29)



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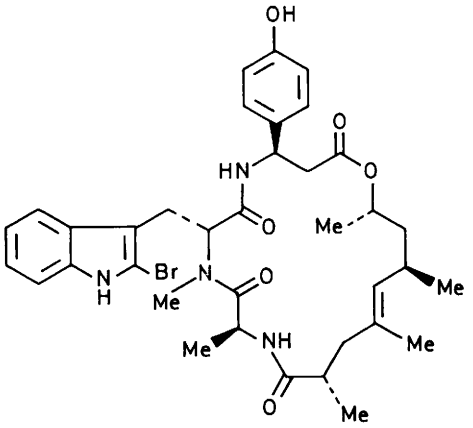

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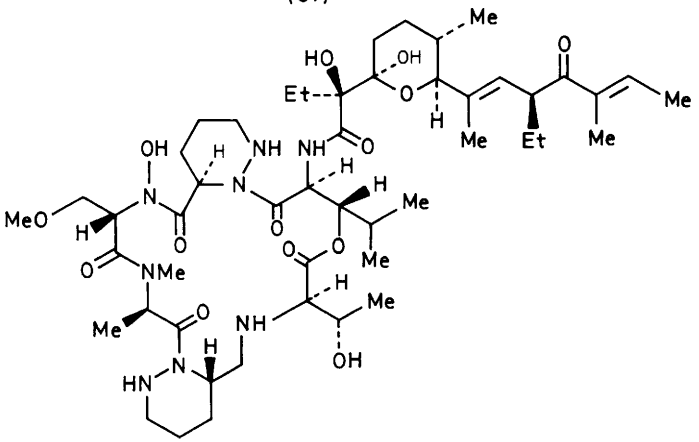


(32)

with this repeating unit.[58] Several new gramicidin S (GS) analogues have been reported. [Orn^1, Leu^2, Orn^3, Phe^4]-GS has substantial activity against Gram-negative bacteria,[59] [D-Pro$^{5,5'}$]-GS has only weak antibiotic activity,[60] while [Orn^1, Leu^2, Orn^3]-, [Orn^1, Leu^2, Orn^3, Phe^4, D-Pro^5]-,[59] [δ-Ava$^{3-4}$]-, and [δ-Ava$^{3-4,3'-4'}$]-GS (where Ava = 5-aminovaleric acid)[61] are all inactive. Two active analogues first reported in 1978, [D-Ala$^{4,4'}$]- and [Gly$^{4,4'}$]-GS, have been found to interact with a phospholipid membrane, taking the GS conformation. By contrast, the inactive [Ala$^{4,4'}$-]-GS does not interact with this membrane.[62] Measurements of the absolute distances between α-CH and NH protons in GS by the 2D n.O.e. technique lead to values in good agreement with previously published figures; deviations do not exceed 0.2 Å.[63]

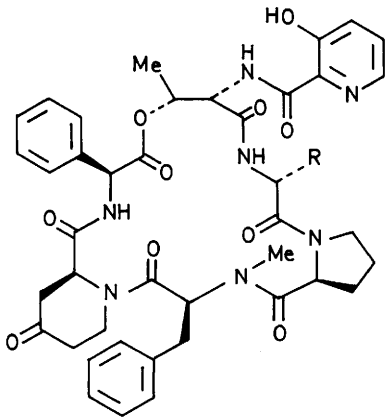
X-Ray work indicates that crystals of [Phe^4,Val^6]-antamanide grown from dioxan-H_2O are almost identical to those grown from acetone-H_2O. However, the disposition of water molecules is different, and the crystals from dioxan-H_2O may contain the first observation of a bifurcated bond between two water molecules.[64] Four analogues of cyclosporin A(CA) specifically modified in the 1-position have been synthesised. [3-OH-MeLeu^1]-CA had 1% of the biological activity of CA, and [MeThr^1]-CA 0.1%; [MeAbu^1]- and [MeAbu^1,Sar^{10}]-CA were even less active.[65] An efficient synthesis of the cyclosporin A (8-11)-tetrapeptide D-Ala-MeLeu$_2$-MeVal has been developed using bis-(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl). Racemisation of all amino acids was less than 1%.[66]

2.7 Cyclodepsipeptides - A structural analogue (30) of enterobactin has been synthesised. It lacks the two hydroxyl groups on each aromatic ring that are found in enterobactin itself but which hamper spectroscopic analyses and render it sensitive to oxygen. Like uncomplexed enterobactin, in nonpolar solvents it exists predominantly in a C_3-symmetric conformation with the side-chains in a axial right-handed orientation.[67] Four analogues of the cyclotetradepsipeptide AM-toxin I (AMT) have been prepared. [2-Amino-2,3-dehydrobutanoic acid^4]-AMT[68] and retro-enantio-AMT[69] are both inactive, while the toxicity of [L-lactic acid^2]-AMT is much weaker than that of [L-2-hydroxy-4-methylpentanoic acid^2]-AMT or of AM toxin I itself, indicating that the bulkiness of the side-chain at position 2 is important for necrotic activity.[70]

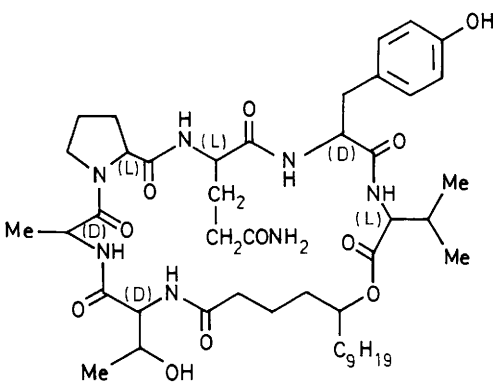
In the synthesis of cyclo[Val-Lac]$_2$ the difficult ring closure (due to the lack of N-Me groups and the all-L configuration) was effected by treating 2,4-bis(methylsulphonyl)phenoxycarbonyl-[Val-Lac]$_2$-OH with NEt_3-CH_2Cl_2-4Å molecular sieve. Elimination of disulphonylphenol occurs, and the isocyanate formed undergoes an intramolecular reaction to give the cyclic mixed anhydride, which then loses CO_2 to give the cyclotetradepsipeptide in 33% crude yield.[71] The novel metabolite jaspamide (31), of mixed peptide-polyketide biosythesis, has been obtained from a sponge. of the Jaspis sp. collected in Fiji. Jaspamide shows both insecticidal and antifungal activity. Its stereochemistry was completely defined by X-Ray crystallography. The 2-bromoabrine residue is apparently a novel amino acid.[72] Also from a soft-bodied sponge of the Jaspis sp. has been isolated jasplakinolide, which has similar biological activity to jaspamide, and apparently differs in structure only by the replacement of 2-bromoabrine by glycine (the configurations of the ring components are not known).[73] Synthetic studies have now established that in the didemnins, cyclic depsipeptides from a Caribbean tunicate, the hydroxyisovalerylpropionyl unit has the (2S,4S)-stereochemistry and the 2-methyl-4-isopropyltetronic acid has the S-configuration.[74]

The component from Streptomyces sp. X-14950 showing activity against Gram positive bacteria has been identified as azinothricin (32); the name is derived from the azino groups of the piperazic acid moieties. It shows a similar antimicrobial spectrum to the monamycins, which also contain piperazic acid. Due to the presence of N-hydroxy-O-methylserine, the 19-membered lactone ring of azinothricin can also be regarded as a cyclic monohydroxamic acid.[75] Two syntheses of virginiamycin S_1 (33a) and one of virginiamycin S_4 (33b) have been described. In one synthesis of (33a) the exocyclic 3-hydroxypicolinic acid residue was added before cyclisation, and in the other after cyclisation. Cyclisation was effected between proline and N-MePhe in very modest yield.[76]

Verlamelin(34), a new antifungal agent from Verticillium lamellicola which is particularly active against rice blast, contains 5-hydroxymyristic acid as an integral part of the ring structure. Most peptolides have the fatty acid merely attached to the peptide ring.[77] A family of new inhibitors of phospholipase A_2 from Bacillus cereus, the pliplastins (35), seem



(33) a; R = Et
b; R = Me



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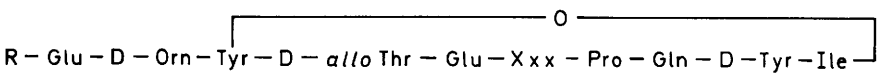


(35)

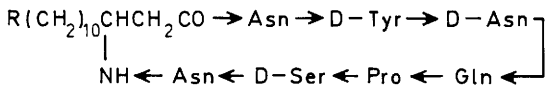
	R	Xxx
A_1	$Me(CH_2)_{12}CH(OH)CH_2CO-$	D — Ala
A_2	$EtCH(Me)(CH_2)_{10}CH(OH)CH_2CO-$	D — Ala
B_1	$Me(CH_2)_{12}CH(OH)CH_2CO-$	D — Val
B_2	$EtCH(Me)(CH_2)_{10}CH(OH)CH_2CO-$	D — Val

to be the first examples of natural peptide lactones involving an ester bond to a tyrosine hydroxyl. A ^{1}H n.m.r. study of the conformations indicates all amide bonds to be trans and two β-turns to be present.[78] The structure of mycosubtilin has been revised to (36) following a study of the peptide resulting from treatment with N-bromosuccinimide, which cleaves the C-peptidyl bond of the tyrosine residue.[79] Full details of the characterisation of neopeptins A, B, and C have appeared (see Vol. 17 p 139 figure 26).[80]

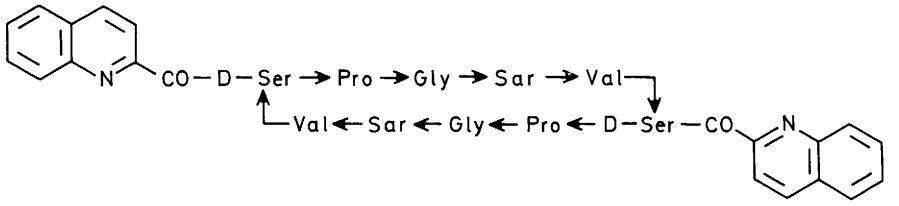
A synthetic analogue (37) of the cyclic decadepsipeptide intercalation agent luzopeptin A has been prepared which incorporates L-proline in place of tetrahydropyridazinecarboxylic acid and L-valine in place of N-methyl-β-hydroxy-L-valine. Its binding to DNA has yet to be reported.[81] Theonellapeptolide 1D (38), which inhibits development of the fertilised eggs of the sea urchin Hemicentrotus pulcherrimus, is the major component of a mixture of peptolides isolated from a marine sponge of the Theonella sp. The structure was largely determined from fragments produced by hydrolysis with 30% TFA (110 °C, 40 min).[82] Another group has independently characterised this peptide from the same sponge. In this case it has been named theonellamine B, and is described as the first peptide to be found that inhibits Na, K-ATPase.[83]

2.8 Cyclic Peptides Containing Thiazole and Oxazoline Rings - Three new minor components, prelissoclinamide 2 (39), prepatellamide-B formate (40), and preulicyclamide (41), of Lissoclinum patella have been identified. They are probably biosynthetic intermediates in the peptide pathway rather than degradation products. A detailed analysis of 2D COSY 45 n.m.r. data for all lissoclinum peptides has revealed homo-allylic coupling between the α-amino protons both through the peptide bonds and the oxazoline rings.[84] The total synthesis of patellamide B (42) has been achieved, confirming the revised structure reported last year (refs. 103 and 104). Cyclisation was carried out on a linear sequence containing preformed oxazoline rings by the pentafluorophenyl ester method (20% yield).[85]

Three total syntheses of ulithiacyclamide (43) have been reported. That of Schmidt and Weller involves a double penta-fluorophenyl ester ring closure of (44) followed by treatment with thionyl chloride to generate the oxazoline rings.[86] Of the two reported by Kato, Hamada, and Shioiri, one also involves a twofold



(36) R = $MeCH_2CH_2-$, Me_2CH-, Me_2CHCH_2, or $MeCH_2CH(Me)-$



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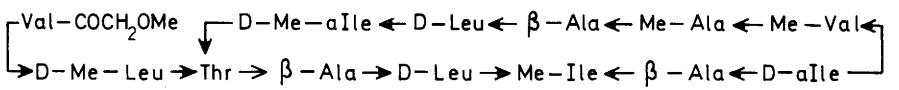

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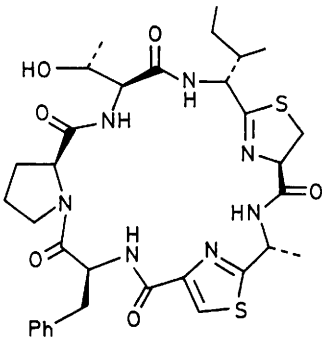

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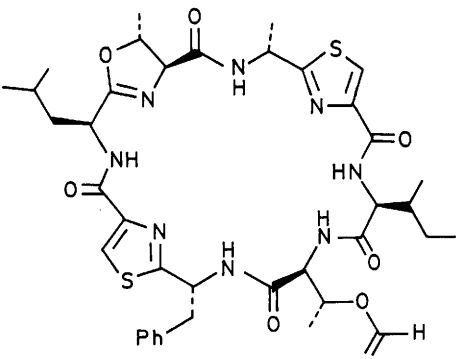

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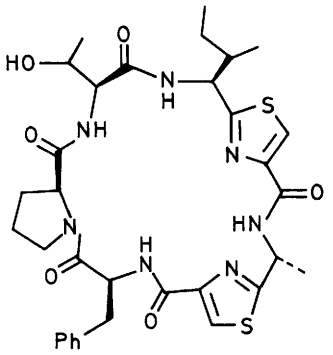

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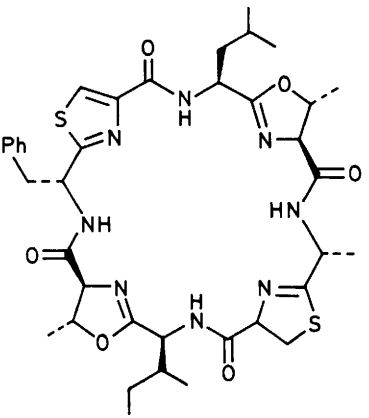

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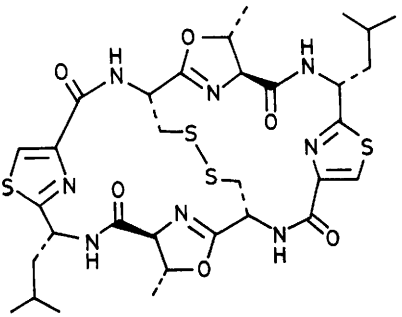

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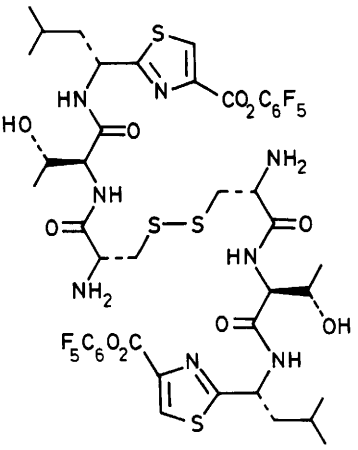
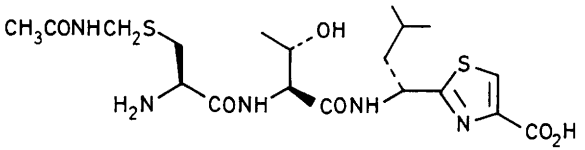


(44)

cyclisation of disulphide-bridged tripeptides, and the other the cyclodimerisation of (45); in the latter synthesis S-deprotection and generation of the disulphide bridge then preceded formation of the oxazoline rings.[87] Four groups have published papers involving dolastin 3, which has an exceptional *in vitro* antineoplastic activity, but whose structure is in some doubt as it is only available in small amounts. Comparison of synthetic cyclo-[Pro-Leu-Val-(R,S)-(Gln)Thz-(Gly)Thz)] with dolastin 3 shows that the natural peptide possesses a clearly related structure, possibly involving (R)-(Gln)Thz and/or a modified Pro-Leu-Val sequence.[88] The simplified dolastin 3 analogue cyclo[Pro-Leu-Val(Gly)Thz-(Gly)-Thz] has also been prepared. A ^{1}H n.m.r. study indicates that the LeuNH is involved in a strong intramolecular H-bond, the leucine also being in the conical shielding zone of a thiazole ring.[89] The synthesis of protected (R)- and (S)-(Gln)Thz and (Gln)Thz-(Gly)Thz has been reported,[90] and the unequivocal FAB-tandem m.s. sequencing of cyclo[Pro-D-Leu-Val-D(Gln)Thz-(Gly)Thz] and cyclo[Val-D-Leu-Pro-(Gly)Thz-D-(Gln)Thz] achieved, opening a way to elucidate the structure of dolastin 3.[91]

2.9 Cyclic Peptides Containing Other Nonprotein Ring Components - A third peptide alkaloid, discarine I (46), has been isolated from the root bark of Discaria febrifuga. It differs from discarine B only in that the N-terminal amino acid is monomethylated.[92] A cyclic peptide (47) containing a rigid spacer which forces hydrogen bridging between antiparallel peptide chains in a similar manner to a β-turn has been prepared. The γ-loop involving the valine residue is in concordance with the n.O.e. effects observed, but the conformation may be somewhat flexible.[93] Synthesis of the ring B part of the antibiotic nisin (48) has been achieved by desulphurisation of the corresponding disulphide with tris(diethylamino)phosphine.[94] Three cyclotripeptides containing β-amino acids have been made. Cyclo-tri-β-aminoisovaleryl was cyclised from the linear tripeptide using pivaloyl chloride in pyridine: for cyclo-tri-β-aminopivaloyl and cyclo-tri-β-amino butyryl o-phenylenephosphochloridite was used.[95]

Two cyclic peptides (49) have been synthesised as models of the metastable binding sites of α_2-macroglobulin and other human serum proteins. These isomeric hexapeptides are spontaneously interconverted in water.[96] Quaternary salts of the amides of N-(3-quinolylcarbonyl)amino acids have been found to cyclise under the influence of base to give 14-membered ring cyclic



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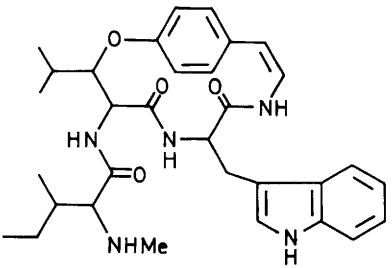

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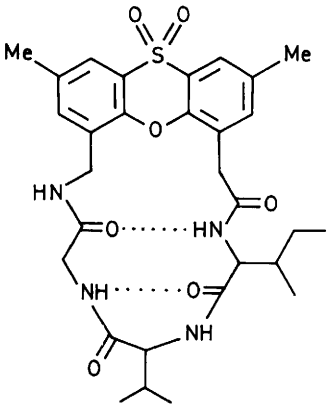

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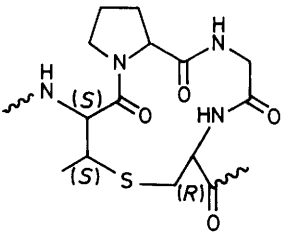


(48)

MeCO – Gly – Cys – Gly – Glu – Glp – Asn – NH_2

MeCO – Gly – Cys – Gly – Glu – Glu – Asn – NH_2

(49)

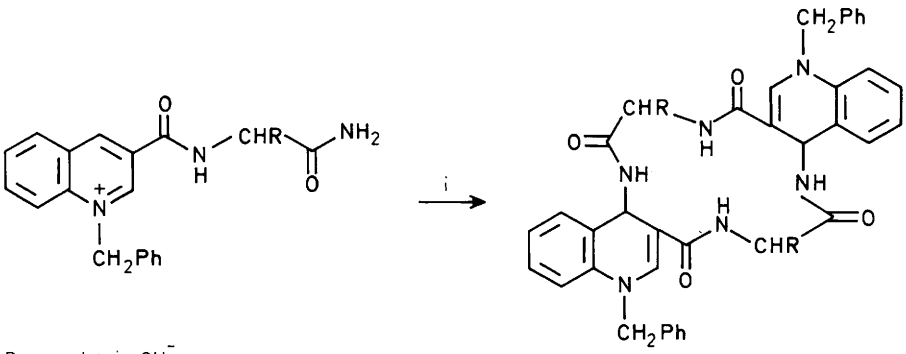
peptides (Scheme 4).[97] The trypanosomatids so far examined, including the one responsible for African sleeping sickness, possess a flavoprotein disulphide reductase which utilises as substrate the novel cyclic peptide tryptathione (50). This has now been synthesised, together with N^8- and N^1-monoglutathionyl spermidines, open chain analogues.[98]

The mycotoxin phomopsin A, the cause of a liver disease of sheep and cattle in Australia, was reported in 1983 to be a homodetic cyclic peptide. X-Ray crystallography has now shown it to in fact be (51), containing an ether bridge incorporating a meta-fused benzene ring.[99] The bush Laportea moroides, which grows in Eastern Australia, has hairs on the leaves which on cutaneous contact can cause severe reactions lasting up to 30 h. A bicyclic octapeptide moroidin (52) has now been isolated from this species and contributes at least partly towards its toxicity. The stereochemistries of the β-substituted-Leu and the Trp residues have not yet been determined; all the other amino acids are of the L-configuration. It is possible that it is the other imidazole nitrogen which is attached to the indole.[100]

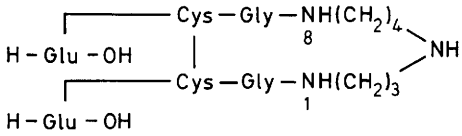
3 Modified Linear Peptides

3.1 Enzyme Inhibitors - The tetrahydro-3-isoquinoline carboxylic derivative quinapril (53a) and its dimethoxy analogue (53b) possess the *in vitro* potency and the *in vivo* efficiency of enalapril. Quinapril has proven to have a high margin of safety in animal toxocological studies and has shown efficiency in early clinical trials in essential hypertension.[101] As a result of an investigation defining the 3D relationship of the three binding sites of ACE inhibitors, cilazapril (54), a highly active *in vivo* antihypertensive, has been developed. The dominant conformation, both in solution and the solid state, is the chair-chair form.[102]

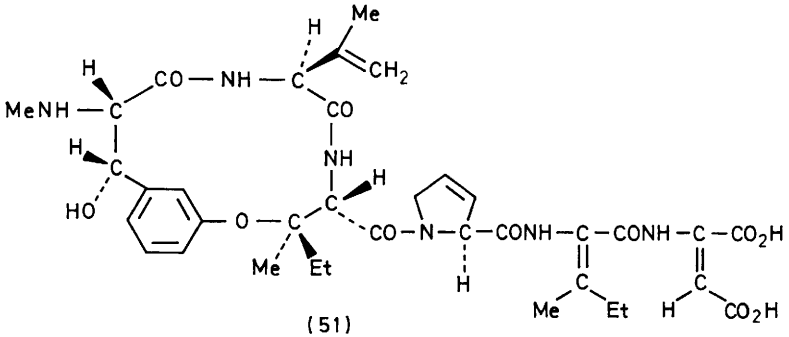
A series of enalaprilat analogues (55) in which the Ala-Pro portion is replaced by a series of monocyclic lactams has been prepared. The most potent contained eight- and nine-membered rings.[103] The X-Ray crystal structure of enalapril shows a similar Ala-Pro conformation to that observed earlier for captopril,[104] and a comparison of the crystal structures of enalapril and two mercaptoalkanoyl derivatives, (56) and (57), which are potent ACE inhibitors shows a common conformation with the polar groups on one side of the molecule and the hydrophobic groups on the opposite



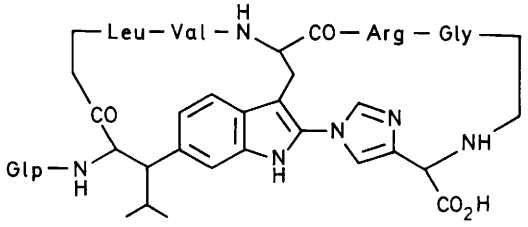
Scheme 4



(50)



(51)



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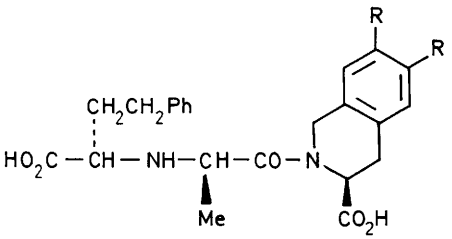
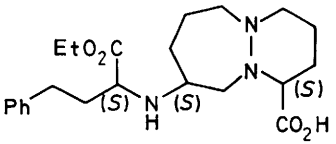


(53) a; R = H
b; R = OMe



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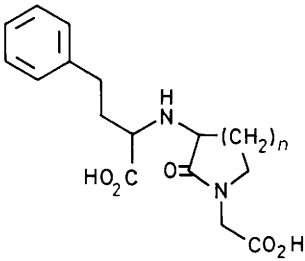
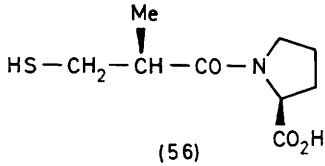


(55)



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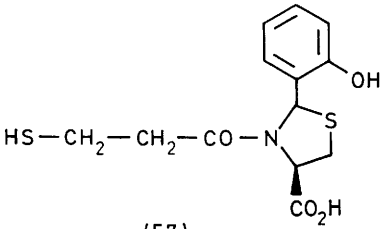

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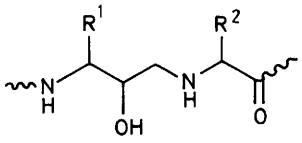
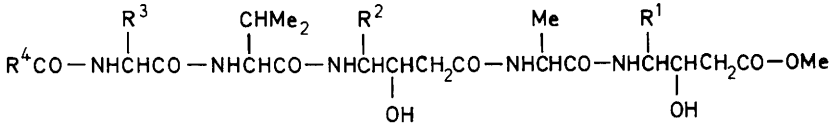


(58)



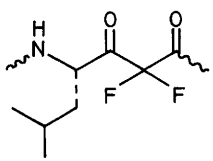
(59)	R^1	R^2	R^3	R^4
A	$-CH_2Ph$	$-CH_2CHMe_2$	$-CHMe_2$	$-C_3H_7$
B	$-CH_2Ph$	$-CH_2CHMe_2$	$-CHMe_2$	$-C_3H_7$
C	$-CH_2CHMe_2$	$-CH_2CHMe_2$	$-CHMe_2$	$-CH_2CHMe_2$
D	$-CH_2CHMe_2$	$-CH_2CHMe_2$	$-CHMe_2$	$-C_3H_7$
E	$-CH_2Ph$	$-CH_2CHMe_2$	$-CHMe_2$	$-CH_2CHMe_2$
F	$-CH_2CHMe_2$	$-CH_2CHMe_2$	$-Ph$	$-CH_2CHMe_2$
G	$-CH_2Ph$	$-CH_2Ph$	$-CHMe_2$	$-CH_2CHMe_2$

side.[105]

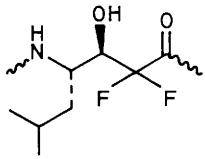
Details have been published of the chemical methodology useful in the synthesis of 'peptidyl amino alcohols' (58) found in 1985 to be novel ACE inhibitors.[106] Of a series of N-(1-carboxy-5-aminopentyl)dipeptides tested for ACE inhibitory activity, the most potent was the Ala-Ala derivative (IC_{50} 10 nmol/l). When coupled to Sepharose B, the most suitable as ligands for affinity chromatography were the Gly-Ala and Ala-Gly compounds. The enzyme is almost irreversibly bound by the Ala-Ala derivative.[107] In the course of screening for new acid protease inhibitors from actinomycetes, Streptomyces sp. WK-142 was found to produce the ahpatinins (59) which are active against renin and pepsin. The least active of the series, ahpatinin C, is identical with pepstatin A. Four of the components contain the unusual amino acid 4-amino-3-hydroxy-5-phenyl-pentanoic acid.[108]

Novel renin inhibitors containing aminostatine (Asta; 3,4-diamino-6-methylheptanoic acid) have been prepared. In each case, the (S,S)-Asta analogue is of similar potency to its (S,S)-statine congener. The peptide H-His-Pro-Phe-His-(S,S)-Asta-Val-Ile-Phe-OH, for example, has an IC_{50}, 10^{-6}M of 0.06. In the less potent diastereoisomers (S,R)-Asta confers much higher potency than (S,R)-statine. The increased binding is attributed to the additional ionic interaction of the extra amino group.[109] Peptides such as Boc-Phe-His-Xxx-Ile-2-pyridylmethylamide, where Xxx is difluorostatine (60) or difluorostatone (61), are potent inhibitors of renin. The readily hydrated fluoroketone is thought to mimic the tetrahedral intermediate formed during the enzyme-catalysed hydrolysis of a peptide bond.[110] A new class of renin inhibitors containing an aminoalcohol grouping -CHOH-CH_2-NH- in place of a peptide bond have been developed. The modified octapeptide H-His-Pro-Phe-His-Leu ψ [CHOH-CH_2-NH]Val-Ile-Phe-OMe has an activity comparable to the -CH_2NH- peptide bond analogue and statine, although it is significantly weaker than the hydroxyethylene isostere.[111] In vitro, Boc-Pro-Phe-N^{α}MeHis-Leuψ[CHOH-CH_2]Val-Ile-Amp is a potent inhibitor of human plasma renin, but is a much weaker inhibitor of other aspartic proteases such as porcine pepsin or bovine cathepsin D.[112]

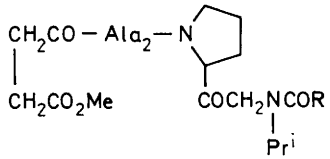
Pepstatin analogues corresponding to the general formula A-Xxx-Yyy-Sta-Ala-Sta-OH have been prepared. Boc and isovaleryl were found to be the most effective A groups, and peptides with Xxx=Phe and Yyy=His,Nleu, or Nval showed the highest inhibition

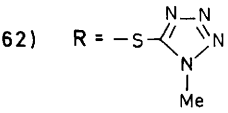


(60)

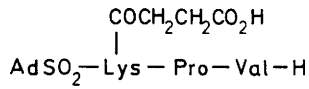


(61)

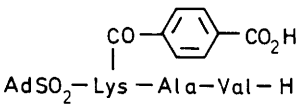




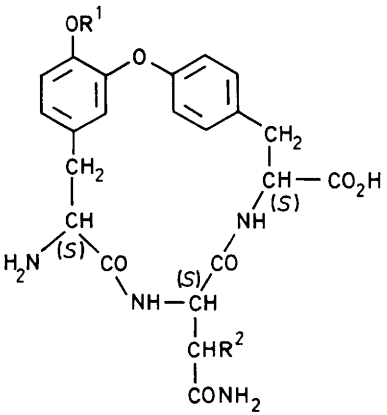
(62)



(63) Ad = 1-adamantane

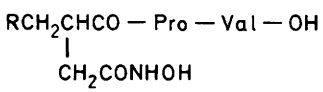


(64)



	R^1	R^2
I	Me	OH
II	H	OH
III	Me	H
IV	H	H

(65)



(66) a; R = Et
b; R = Me_2CH

of human plasma renin.[113] Carboxyl modified dipeptide derivatives of the type H-Gly-Phe-X have been examined as protease inhibitors. Where X=CN or $CONH_2$, they are competitive inhibitors of dipeptidyl aminopeptidase and if N-acetylated they also inhibit papain. Where X= trans-$CH=CHCO_2Me$ the compounds act as irreversible affinity labels.[114] It has been found that novel inhibitors can be targeted against any serine protease of known specificity by replacing the amide at the cleavage site of the protease substrate by a fluoromethyl ketone. By analogy with the corresponding aldehydes it is assumed that the fluoromethyl ketones react with the γ-OH of the active-site serine to form a stable hemiacetal. In all cases the difluoro- and trifluoro-methyl ketones are better inhibitors. Of a series of compounds, Ac-Leu-Phe-CF_3 was the best chymotrypsin inhibitor and Ac-Ala-Ala-Pro-Ala-CF_3 (and CHF_2) good elastase inhibitors.[115]

The design and synthesis of 13 novel peptidyl carbamates, of which six of general formula (62) specifically inhibit elastase without affecting trypsin or chymotrypsin, has been reported. Preliminary work on one inhibitor indicates that the inhibition is reversible and proceeds via the rapid formation of a strong enzyme-inhibitor complex, followed by slow acylation of the serine residue on the active site of the enzyme. Compound (62a) exhibits a K_i value of 4×10^{-6}M against human leucocyte elastase.[116] Two peptide aldehydes (63) and (64) earlier reported as highly specific elastase inhibitors[117] have now been examined in the treatment of emphysema. When compared to α_1-antitrypsin they showed in vitro advantages in terms of potency, specificity, and stability to cigarette smoke.[118]

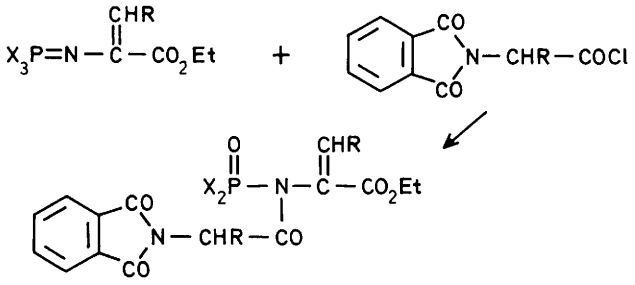
Replacement of the Phe-Phe dipeptidyl unit of the good cathepsin D substrate Boc-Phe-Leu-Ala-Phe-Phe-Val-Leu-OR with (S,S)-statine gives a compound which inhibits the enzyme with a K_i value of 1.1 nM. Thirty-five known pepstatin analogues were also evaluated as cathepsin D inhibitors. The best were about equivalent with the statine analogue above.[119] Suc-Tyr-D-Leu-D-Phe-p-nitroanilide has been found to be an effective and specific inhibitor of chymotrypsin, the p-nitroanilide moiety participating in binding with some part of the enzyme.[120] A number of peptide hydroxamic acids have been synthesised and shown to be inhibitors of human skin collagenase. The most potent, Z-Pro-Leu-Gly-NHOH, has an IC_{50} value of 4×10^{-5}M. Replacement of the hydroxamic function by an amide, carboxylate, or aldehyde gives compounds with

little or no inhibitory activity.[121]

Four new inhibitors of aminopeptidase B (65) have been isolated from the fungus Penicillium rugulosum. The erythro-β-hydroxyasparagine residue is the same stereoisomer as found in mammalian urine.[122] Two new enkephalinase B inhibitors extracted from kitosporia setae, propiotoxins A and B, have been identified as (66a) and (66b), the structures being proved by synthesis. Devalylpropiotoxin A was also synthesised, and found to have a higher K_i value for enkephalinase B than propiotoxin A itself.[123] Cyanamide is a potent aldehyde dehydrogenase inhibitor used therapeutically as an alcohol deterrent agent. In vivo acetylcyanamide is deacetylated, and on this basis several acyl derivatives of cyanamide, including Z-Gly-Leu-NHCN, Glp-Leu-NHCN, and Glp-Phe-NHCN have been synthesised as prodrugs. All raised ethanol-derived blood acetaldehyde levels in rats significantly over controls.[124]

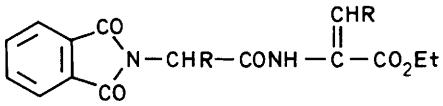
3.2 Dehydropeptides - [Z-Δ Phe^3]-, [Z-ΔPhe^5]-, and [Z-Δ $Phe^{3,5}$]-dermorphin(1-5) amide have been prepared. The compounds showed only low opioid activity on the GPI preparation, the unsaturation at position 3 being particularly detrimental to μ-activity. A similar result was observed on extending the work to dermorphin itself. A c.d. study of these analogues indicates that both in water and alcohol they adopt different conformations to the parent peptides.[125] The E-isomer of [D-Ala^2, Δ Phe^4, Leu^5]-enkephalin has been made from the Z-isomer by photoisomerisation with 310 nm radiation. It showed an extremely diminished affinity for both μ- and δ- receptors compared to its precursor.[126]

A full report (cf. ref 129 Vol. 16) has appeared on the one-pot synthesis of N-protected Δ^2-dehydrodipeptide and tripeptide esters. N-Carboxy α-dehydroamino acid anhydrides can be acylated by N-protected amino acids using DCC and pyridine, the latter being essential for reaction. Subsequent hydrolysis with water gives the dehydrodipeptide, while treatment with C-protected amino acids gives the Δ^2-dehydrotripeptide. Using this method the C-terminal peptide of the antrimycins, Boc-Leu-ΔIle-Ser-OMe, has been prepared.[127] 2-Triethoxy- and 2-triphenylphosphoranylidene-amino-2-alkenoates (67), derived from reacting alkyl (Z)-2-azido-2-alkenoates with $P(OEt)_3$ or PPh_3 respectively, yield dehydrodipeptide derivatives with N-phthaloylamino acid chlorides (Scheme 5). When X=OEt, treatment of (67) with HBr/CH_3CO_2H gives (68); where

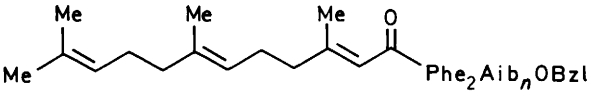


(67)

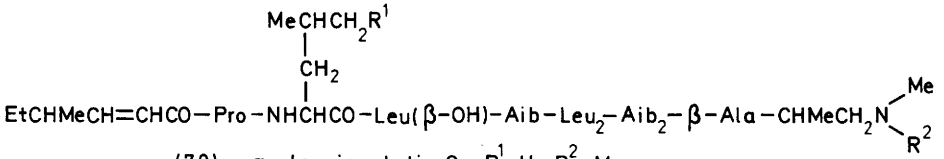
Scheme 5



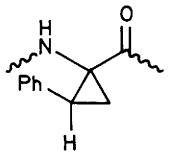
(68)



(69) $n = 2-4$



(70) a; leucinostatin C , R^1 = H , R^2 = Me
b; leucinostatin D , R^1 = H , R^2 = H



(71)

X=Ph, (68) can be obtained from (67) simply by treating with aqueous $NaHCO_3$.[128]

The coupling of N-protected dipeptides containing C-terminal (Z)- Δ Phe- or Δ -Ala with glycine esters has been examined in some detail. In general it was found that reaction took place more slowly than with the corresponding saturated residues. Side reactions were observed; the mixed anhydride method gave 9% of N-isobutyloxycarbonyl-Gly-OBu^t, and diphenylphosphorazidate (DPPA) gave 19% of N-diphenylphosphoryl-Gly-OBu^t. In the Δ -Ala case using DPPA, addition of HN_3 to the double bond also occurred; 16% of azidotripeptide was isolated. With water-soluble carbodiimide in the presence of 1-hydroxybenzotriazole (HOBt) the only peptide product observed was that in which HOBt had added to the Δ-Ala double bond.[129]

The enkephalin analogue Z-Tyr(O-tosyl)-Gly_2- Δ Phe-Leu-OMe has been prepared and its asymmetric hydrogenation examined as a potential tool for stereoselective labelling. Complete reduction was attained with (Rh dipamp COD)$^+BF_4^-$ and hydrogen at 10 bar to give a 93% diastereoisomeric excess of (S)-Phe.[130] More generally, the asymmetric reduction by chiral rhodium complexes of the series Ac-ΔPhe-Gly_n-Leu-OH or OMe has been investigated. The results are analysed in terms of degree of substance control or catalyst control of reaction.[131] Protected aspartame has been generated by the asymmetric hydrogenation of Z-Asp- Δ Phe-OMe; use of (R)-prophos as the rhodium ligand gave an L,L:L,D ratio of 9:1.[132] The hydrogenation in DMF of cyclo(Δ Tyr(Me)-Ala) and cyclo(Δ Amp-Ala), where Amp=L-2-amino-5-(p -methoxyphenyl)pentanoic acid, has been examined using Pd black as a method of synthesis of O-MeTyr and Amp. The content of LL-isomers obtained was over 94%. Acid hydrolysis of the resulting dioxopiperazines with 0.5 M HCl-dioxan (1:1), 110 °C, 96 h gave the free amino acids with neglibible dimethylation.[133]

3.3 Peptides Containing α,α -Dialkylamino Acids -Aib residues have been incorporated into peptide derivatives of farnesic acid (69) to improve solubility in organic solvents. Epoxidation of these compounds gave an excess of R-epoxide with NBS and an excess of S-epoxide with MCPBA, although asymmetric induction does not exceed 25%. These studies are related to the last stage of cholesterol biosynthesis in which squalene is regio- and enantio-selectively converted to the 2,3-epoxide.[134] The reaction of Boc-Leu_3-Aib-OH

with H-Leu$_3$-OBzl using DCC as coupling agent proceeds via the 4,4-dimethyl-5-oxazolone, which reacts faster with 1-hydroxysuccinimide than with the peptide ester. In contrast, HOBt does not react with the oxazolone but strongly catalyses its aminolysis in dichloromethane to form peptide bonds in nearly quantitative yield.[135]

The 4-chlorobenzoyl derivatives of some di- to penta-peptides rich in Aib residues have been found to be highly crystalline materials which can be detected and integrated in chromatographic effluents at ca. the 10 ng level. They may be prepared by reaction of peptide esters with 4-chlorobenzoyl azide, a relatively stable crystalline solid.[136] From the reaction mixture of Z-Cl and H-Aib-OH three crystalline compounds have been isolated and characterised by X-Ray analysis. Two are polymorphic forms of Z-Aib-OH; the third is Z-Aib$_2$-OH. The crystal structure of (Z-Aib)$_2$O has also been determined.[137]

Boc-Asp-Pro-Aib-His or Lys-NHMe have been prepared to investigate intramolecular electrostatic interactions between ionisable side-chains. N.m.r. evidence supports intramolecular salt bridges and diminished backbone flexibility, and suggest that proximity effects confer greater stability to intramolecular ion-pair interactions vis à vis their intermolecular counterparts.[138]

The electron diffraction pattern of an oriented film of poly(Aib) in the 3_{10}-helical conformation indicates the presence of well-defined intermolecular channels, the first time this has been observed in a synthetic polypeptide.[139] Peptides containing Aib at other than C-terminal positions give molecular ions of low abundance on EI-m.s. but abundant ions corresponding to M^+-H_2O. This dehydration reaction does not occur with CI-m.s., or in permethylated derivatives.[140] Using a new modification of the liquid chromatography-FAB m.s. direct coupling interface, leucinostatins C and D (70; stereochemistry not indicated) have been assigned structures for the first time. Leucinostatins E and F were found to correspond respectively with leucinostatins A and C with an extra oxygen atom in each case.[141]

The conformational preferences of homo-oligomers of Aib have been reviewed, the 3_{10}-helix being confirmed as the favoured structure adopted.[142] Further conformation work on Aib-rich peptides is listed in Table 3.

Table 3 Conformational studies of Aib peptides

Peptide	Techniques used	Ref.
Boc-Aib-Val-Aib_2-Val_3-Aib-Val-Aib-OMe	^{1}H n.m.r.	143
Boc-Aib-Leu-Aib_2-Leu_3-Aib-Leu-Aib-OMe	^{1}H n.m.r.	144
TFA.H-Pro-Glu-[Ala-Aib-Glu-Aib]$_4$-Gly-OH	^{1}H n.m.r.	144
HCl.H-Pro-Ala-Aib[Glu-Ala_2-Aib]$_2$-Glu-Ala--Aib-Gly-PEGM*	^{1}H n.m.r.	144
TFA.H-Ala-Aib-[Glu_2-Ala-Aib]$_3$-PEGM	^{1}H n.m.r.	144
Ac-Phe-Aib_3-D-Iva-Gly-Leu-Aib_2-Hyp-Gln-D--D-Iva-Hyp-Aib-Pro-Phol	^{1}H n.m.r.	145
Boc-Met-Aib-Phe-OMe	X-Ray, ^{1}H n.m.r.	146
Z-Aib_3-OH	X-Ray	147
H-Pro-Leu-Aib_2-Glu-Valol	X-Ray	148
Z-Aib_3-Ala_2-Aib-OBu^t	X-Ray	149
4-BrC_6H_4CO-Aib_8OBu^t	X-Ray	150
4-BrC_6H_4CO-Aib_3-Val-Gly-Leu-Aib_2-OMe	X-Ray	150
Boc-Trp-Ile-Ala-Aib-Ile-Val-Aib-Leu-Aib-Pro-OMe·$2H_2O$	X-Ray, ^{1}H n.m.r.	151

* PEGM = polyethylene glycol monomethyl ether

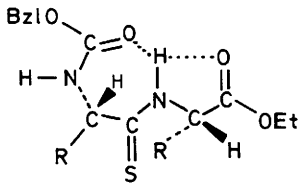
Four diastereoisomeric [D-Ala^2, ∇-Phe^4, Leu^5]-enkephalins (∇-Phe = cyclopropylphenylalanine) have been synthesised. Only the compounds containing Z-∇Phe (71) showed strong binding affinity. No correlation between c.d. spectra and bioactivity could be made.[152] Three peptide analogues containing dibenzylglycine (Dbg) have been prepared. Although [Glp^6, Dbg^7]-substance P (6-11) possessed no activity, [Dbg^4, Leu^5]-enkephalinamide was 8.4 times as potent as its parent in isolated tissue assays for opioid activity, and was highly (∿300 fold) δ-selective. [N,N-diallyl-Tyr^1,Dbg^4,Leu^5]-enkephalinamide is a moderately potent opioid antagonist, but shows little μ or δ selectivity.[153]

An X-Ray study of Boc-$(Acc^n)_2$-NHMe (Acc^n=1-aminocycloalkanecarboxylic acid with n atoms in the cycloalkane ring) shows a type III β-turn with a (4→1)H-bond. The φ,ψ values for both Acc residues are close to those expected for the ideal 3_{10}-helical

conformation. ^{1}H N.m.r. also shows a β-turn in solution,[151] as it does too for Boc-$(Acc^{6})_{3}$-OMe and Boc-Pro-Acc^{6}-Ala-OMe. The crystal structures of the latter compounds show respectively type III and type II β-turns, establishing that Acc^{6} residues can occupy either position in a type III turn and the i+2 position of a type II β-turn.[155]

3.4 Amide-Bond Analogues. - The thionation of Boc-Ala-Aib-Ala-OMe with Lawesson's reagent gives mainly Boc-Ala-Aib ψ[CSNH]-Ala-OMe, with a small amount of Boc-Ala ψ[CSNH]-Aibψ[CSNH]-Ala-OMe. Ac-Ala-Aib-Ala-OMe, however, gives Ac-ψ[CSNH]-Ala-Aib-Ala-OMe. Boc-Alaψ[CSNH]-Aib-OH has been converted to the thiazlactone with pivaloyl chloride, and on treatment with H-Ala-OMe gives Boc-Alaψ[CSNH]-Aib-Ala-OMe. This seems to be the first thiopeptide C-terminal prolongation recorded, albeit a special case.[156] The solution conformations of a series of N-Z-endothiodipeptide esters have been examined, and there appear to be two main conformers present. One (72) predominates in nonpolar solvents and the other (73) in H-bond accepting solvents such as DMSO. The former has a bifurcated intramolecular H-bond, and the latter is more extended and flexible in the N-terminal region.[157] The thiopeptide N-(furacroyl)-Pheψ[CSNH]-Gly-Pro-OH is readily hydrolysed by ACE, but replacement of the Gly by Ala makes the peptide resistant to this enzymic cleavage. It is thought that the larger size of the sulphur atom prevents coplanarity of the Phe CO oxygen and the alanine methyl group.[158] N.O.e. measurements have been used to show that the thionopeptide bond in Z-Gly ψ[CSNH]-Gly-OBzl has the trans configuration. 3-Benzylpiperazine-2,5-dithione was synthesised as a reference cis compound.[159]

Thioamide, amidoxime (-C(=NOH)NH-), cyanoamidine (-C(=NCN)NH-), and amidrazone (-C(=NNH_2)NH-) analogues have been prepared of the chemotactic peptide OCH-Met-Leu-Phe-OMe/OBu^{t}. The thioamides were converted to amidoximes by NH_2OH-$Hg(OAc)_2$ treatment, to the cyanoamidines by NH_2CN-$Hg(OAc)_2$ and to the amidrazones by N_2H_4-$Hg(OAc)_2$. Mixtures of geometrical isomers were obtained, the Z-form predominating. Where the amidrazones or amidoximes were at the Leu-Phe peptide bond of the methyl ester, spontaneous cyclisation occurred to give (74a) and (74b) respectively. In tests for the ability to induce the release of lysozyme from human neutrophils, the thioamides and the cyanoamidines were inactive, the amidoximes had significant activity, and the cyclic amidrazones and amidoximes strong activity.[160]



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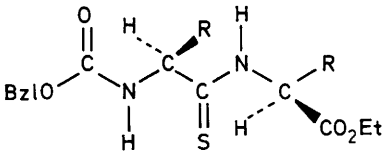

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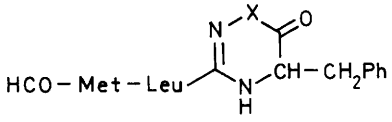
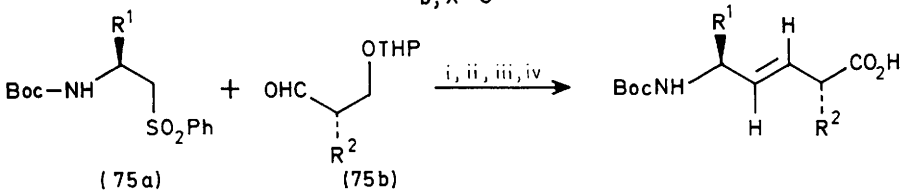


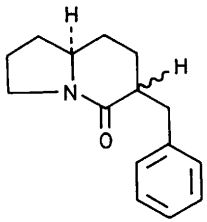
(74) a; X = NH
b; X = O



(75a) (75b)

Reagents: i, 2.0 eq CH_3Li-Et_2O, THF, -78 °C + (75a) $CH_3OAlBu^i_2$ + (75b), then mix; ii, 5% Na(Hg), Na_2HPO_4, MeOH, 0 °C, 4h; iii, $PyrH^+OTs^-$, MeOH, 25 °C, 12h; iv, CrO_3, H_2SO_4, H_2O, MeCOMe, 0 °C, 0.5h.

Scheme 6



(76)

The substance P(SP) analogue [Glp^6(RS)-Phe$^8\psi$($COCH_2$)Gly^9]-SP_{8-11} is a potent inhibitor of SP degrading activity in rat diencephalon membranes and is also a full agonist with a potency of 70% of the parent hexapeptide. Replacement of Glp by a benzoyl group gives only a weak enzyme inhibitor.[161] Ketomethylene-containing analogues of the snake venom pentapeptide Glp-Lys-Trp-Ala-Pro-OH, which lowers blood pressure in various models of hypertension, have been shown to be potent ACE inhibitors. Although the most potent compounds, PhCO-Pheψ[$COCH_2$]-Ala-Pro-OH and cyclobutanecarbonyl-Lys-Pheψ[$COCH_2$]-Gly-Pro-OH, are five times more potent than captopril, they are relatively weak inhibitors of [^{3}H]-captopril binding to membrane-bound ACE. It therefore seems that they bind to an enzyme site distinct from the captopril binding site.[162]

The peptide bonds in H-Pro-Leu-Gly-NH_2 have been replaced by iminomethylene groups. The analogue, which adopts a conformation in solution similar to the postulated β-turn in the natural hormone, H-Pro-Leuψ[CH_2NH]-Gly-NH_2, has an activity approximately equal to its parent compound.[163] The enkephalin analogues H-Tyr-cyclo-(ε-D-Lys-Gly-Pheψ[CH_2S]-L or D-Leu) have high activity in isolated tissue assays, but in contrast to the parent peptide show no selectivity for μ-receptors. The corresponding linear pseudopeptide analogues are both less potent and less δ-selective than their parents.[164] The angiotensinogen analogue H-Pro-His-Pro-Phe-His-Leuψ[CH_2NH]-Val-Ile-His-Lys-OH has been submitted to Edman degradation. Multiple cleavages were observed in the first cycle due to phenylthiocarbamylation of the internal secondary amine as well as spontaneous alkaline cyclisation and subsequent recoupling with the Edman reagent. A similar heterogeneity results from Edman degradations of N-terminally alkylated proteins.[165]

New methods for preparing methyleneimino and methyleneoxy isosteres of peptides have been described, and these and other peptide bond replacements have been incorporated in an extensive series of compounds which have been tested for their ability to reverse electroconvulsive shock-induced amnesia in rats. The most active materials were Z-Pro-ψ[CH_2NH]Leuψ[CH_2S]Gly-OH, Z-Pro(4-CO)-Leuψ[CH_2S]-Gly-OH, and Z-Pro-Leuψ[CH_2SO_2]-Gly-OH; none showed significant activity in enhancing memory. Other compounds prepared and tested are listed in Table 4.[166]

Table 4 Peptides Containing Amide Bond Analogues

Z-Pro-ψ[CH_2O]Gly-OH	Boc-Pro-ψ[CH_2NH]D-Lys-(Z)-Gly-NH_2
Z-Pro-ψ[CH_2O]Leu-Gly-OH	H-Proψ[CH_2NH]D-Lys-(Z)-Gly-NH_2
Z-Pro-ψ[CH_2O]Gly-Gly-OH	Z-Proψ[CH_2NH]Leuψ[CH_2S]Gly-NH_2
Z-Proψ[CH_2O]Gly-Gly-NH_2	Z-Pro-Leuψ[CH_2S]Gly-OH
Z-Proψ[CH_2O]Leu-Gly-NH_2	Z-Glp-Leuψ[CH_2S]Gly-OH
Z-Pro-Leuψ[CH_2O]Gly-OH	Z-Pro-Leuψ[CH_2S]Gly-NH_2
Z-Pro-Leuψ[CH_2O]Gly-NH_2	Z-Proψ[CH_2S]Lys(Z)-OH
Z-Pro-Pheψ[CH_2O]Gly-NH_2	Bz(4-OMe)-Pro-Leuψ[CH_2S]Gly-OH
Z-Pro-Leuψ[CH=CH]Gly-NH_2	Z-Proψ[CH_2S]Leu-NHC_3H_7
Glp-Leuψ[CH=CH]Gly-NH_2	Z-Proψ[CH_2S]Leu-NHCH_2CH_2SEt
Z-Proψ[CH_2NH]Leu-Gly-NH_2	
Z-Pro-Leuψ[CH_2NH]Gly-NH_2	
Glp-Leuψ[CH_2NH]-Gly-OH	
Z-Pro-ψ[CH_2NH]Leuψ[CH_2NH]Gly-OH	
Tos-Proψ[CH_2NH]Leuψ[CH_2NH]Gly-OH	
Tos-Proψ[CH_2NH]Leu-Gly-OH	
Tos-Pro-Leuψ[CH_2NH]Gly-OH	
Tos-Proψ[CH_2NH]D-Leu-Gly-NH_2	

Six protected dipeptides containing methylene-oxy units have also been prepared (Table 5),[167] and a general synthesis of the

Table 5 Dipeptide Isosteres

Pht-Glyψ[CH_2O]Gly-OBu^t
Z-Tyr(Bu^t)ψ[CH_2O]Gly-OBu^t
Z-Tyr(Bzl)ψ[CH_2O]Gly-OBu^t
Boc-Pheψ[CH_2O]Gly-OEt
Boc-<u>trans</u>Hyp(Bzl)ψ[CH_2O]Gly-OEt
Boc-<u>trans</u>Hyp)Bzl)ψ[CH_2O]Ala-OEt

hydroxymethylene isostere has been described which is complementary to existing methods and allows for wide variation in the two 'amino acid' side chains involved.[168]

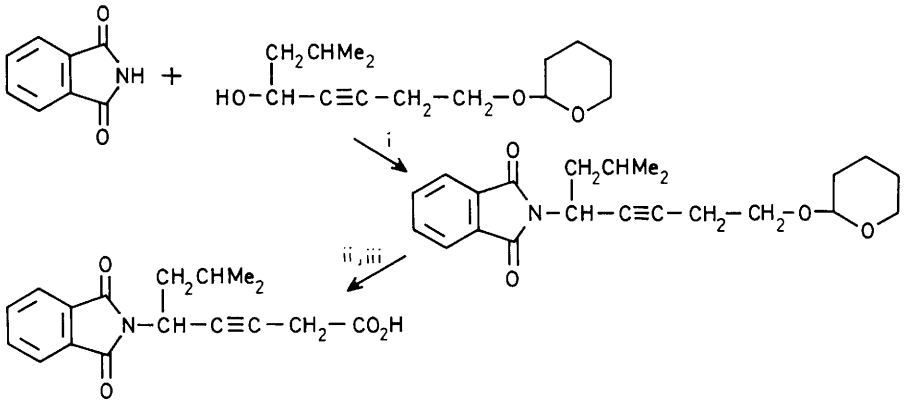
A general synthetic route to <u>trans</u>-alkene isosteres of protected dipeptides has also been developed and tested in the

preparation of analogues of, e.g., Tyr-Ala-Phe-Phe and Leu-Leu. It involves a stereocontrolled sulphone-aldehyde coupling (Scheme 6) in which the aldehyde (75a) is precomplexed with diisobutylaluminium methoxide and the sulphone (75b) preconverted to the dilithium dianion.[169] In order to determine the configuration of the diastereoisomers of H-Pro ψ [CH=CHE]L,D-Phe-OH a method has been developed in which the double bond is catalytically reduced and the product cyclised to the lactam (76). The ^{1}H n.m.r. spectra of the separated isomers allows the assignment of S,S, or R,S- configuration.[170]

A racemic dipeptide analogue of Leu-Gly has been prepared in which the peptide bond is replaced by an ethyne unit. Key steps in the synthesis involve introduction of the amino function by a Mitsonobu reaction, and a Jones oxidation to generate the carboxyl (Scheme 7).[171] Analogues of enkephalin of the type H-Tyr-Xxx-Gly-Phe-Leu-OH, where Xxx = N-hydroxy-Gly, Ala, or β-Ala, have been made. They are resistant to aminopeptidase M, and a qualitative analgesia test shows the N-OH-Ala analogue to have a comparable potency to [Leu^{5}]-enkephalin but a longer duration of action.[172]

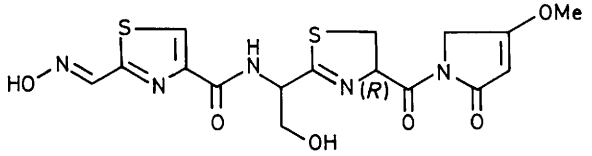
3.5 γ-Glutamyl Peptides - The major component of the defensive secretion of the Colorado beetle, a notorious pest of potato plants, is a toxic dipeptide now identified as γ-glutamyl-L-2-amino-3-(Z)-5-hexadienoic acid. It is toxic to ants at a concentration of 10^{-2} molar. Catalytic reduction of the natural material gives γ-glutamylnorleucine.[173] Three new γ-glutamyl peptides have been isolated from the seeds of Vigna radiata (mung beans) γ-Glu-SMeCys-β-Ala-OH (S-methylhomoglutathione), γ-Glu-N$^{\delta}$Ac-Orn-OH, and γ-Glu-γ-Glu-SMeCys-OH. The structures were confirmed by comparison with authentic speciments.[174] The novel dipeptide γ-Glu-β-D-aminophenylpropanoic acid from the water fern Azolla caroliniana is not detectable or present only in trace amounts in other Azolla species and is therefore not a common constituent of the genus.[175] The administration of certain γ-glutamyl amino acids appears to be a specific and non-toxic procedure for in vivo inhibition of glutamyl transpeptidase that may be useful in experimental work on glutathione metabolism and function and also for treatment of toxicity produced by heavy metals and nephrotoxic drugs.[176]

3.6 Peptides Containing Modified Protein Constituents - Four analogues, (77) to (80), of the antibiotic althiomycin have been synthesised. The absence of the hydroxymethyl group does not



Reagents : i, PPh_3, $EtO_2CN{=}NCO_2Et$; ii, HCl-MeOH; iii, CrO_3-H_2SO_4-MeCOMe

Scheme 7



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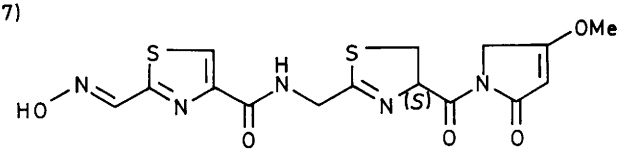

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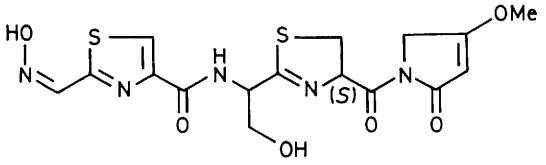

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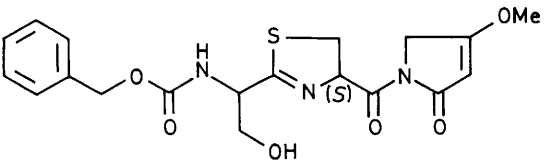


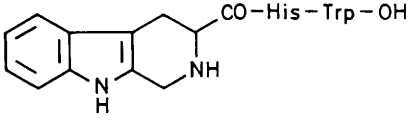
(80)

affect antibacterial activity, but inversion of configuration at C-4 in the central heterocyclic ring or the aldoxime decreases biological activity, as does replacement of the N-terminal thiazole by a Z-group.[177] A new α-mating pheromone has been isolated from Saccharomyces kluveri, α^{skI} β-Car-His-Trp-Leu-Ser-Phe-Ser-Lys-Gly-Glu-Pro-Met(O)-Tyr-OH, which contains an N-terminal 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (βCar) group. Treatment of H-Trp-His-Trp-OH with CH_2O - H_2SO_4 gives a compound (81) identical with the N-terminal tripeptide of α^{skl} obtained by thermolysin digestion.[178]

Two models of collagen, Ac-Ala-Gly-Pro-Ala-Glc-Pro-NHMe and Ac-Ala-Glc-Pro-Ala-Gly-Pro-NHMe (where Glc = glycolic acid), have been prepared. A ^1H n.m.r. study indicates that while in DMSO-d_6 the conformations are random in TFE the glycine amide protons are less solvent exposed than the other amide protons.[179] The end-group modified retro-inverso analogue of glutathione (82a) is a substrate for glutathione-S-transferase, glutathioneperoxidase, and glyoxylase I with K_c and K_c/K_m values 10^2-10^3 fold lower than the natural peptide. The decarboxylated peptide (82b) is also a substrate mimic for these three enzymes.[180] The two analogues [D-Thr^2,Δ^3Pro^5]- and [D-Thr^2,Thz^5]-enkephalinamide have been synthesised by solution methods (a solid-phase synthesis of the latter was earlier reported). Both are potent analgesic agents, the latter showing high μ-receptor affinity but little δ-receptor affinity.[181]

Bz-Phe-Pro-p-guanido-Phe-p-nitroanilide has been prepared and its rates of hydrolysis by bovine trypsin and thrombin compared with Bz-Phe-Pro-Arg-p-nitroanilide. The results suggest that the subsite of trypsin is very different from that of thrombin.[182] Three substance P analogues containing p-fluorophenylalanine in positions 7 and or 8 have been made by solid-phase methods; their effects in GPI assays approximate that of the parent peptide.[183] Substitution of D-p-bromo-Phe in positions 1 or 2 of an LH-RH inhibitory peptide results in improved activity, but similar substitutions with D-p-fluoroPhe, D-homoPhe, and pentamethyl-D-Phe give less potent compounds.[184]

3.7 Peptides Containing β- and Higher Amino Acids. - The tetrapeptide Boc-β-Ala-Trp-Leu-Asp-Phenethyl ester has proved the most potent inhibitor of gastrin-induced acid secretion of a series of analogues in which the Phe of the gastrin C-terminal tetrapeptide



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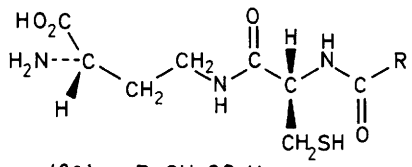
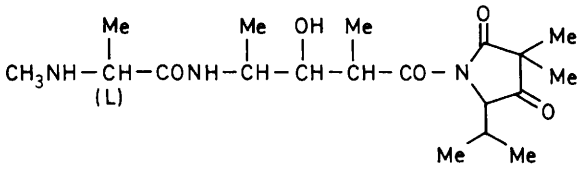


(82) a ; R = CH_2CO_2H
b ; R = CH_3



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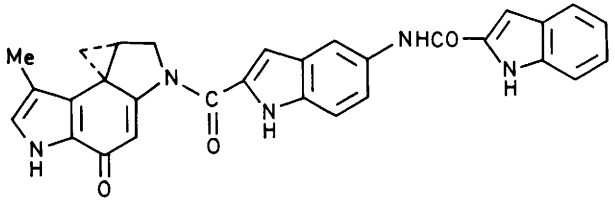

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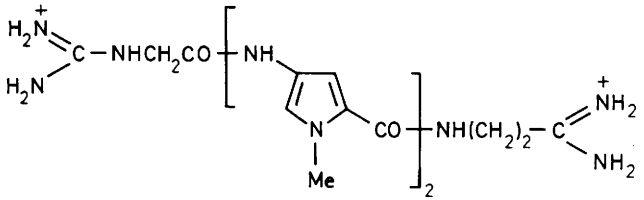

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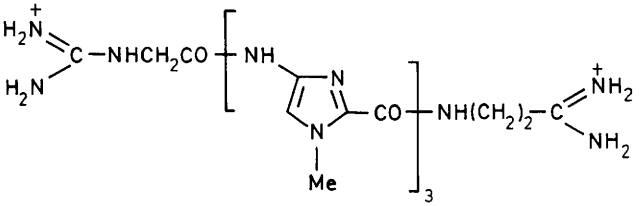


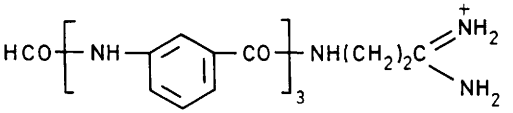
(86)

has been replaced by a phenethyl group.[185] In the series Boc-(β -Ala)$_n$-OBzl, where n=3 to 8, i.r. indicates that in the solid state the β -sheet structure commences to occur when n = 5. Insolubility in polar solvents begins at the heptapeptide level, attributable to β -sheet aggregation.[187] The unusual peptide janolusimide (83) has been isolated from the Mediterranean nudibranch Janolus cristatus. It is neurotoxic to mice, seemingly affecting the acetylcholine receptors. The stereochemistry of the central 4-amino-3-hydroxy-2-methylvaleric acid residue is not yet known.[188]

A highly potent analogue (84) of the antitumour antibiotic CC-1065 (see SPR Vol. 16 p 377 diagram 65) has been synthesised. Like CC-1065, it binds to double-stranded B-DNA, and is equipotent with its parent, but it does not cause delayed death in mice. Its enantiomer is biologically inactive.[189] The cyclopropapyrroloindole unit of CC-1065 has been made by a route which involves construction of both pyrrole rings by thermolysis of vinyl azides,[190] while further adaptation of a strategy for the synthesis of 3,3´-bipyrroles has allowed the synthesis of all three such fragments of CC-1065.[191]

Analogues of the antiviral antibiotic netropsin (85) have been prepared in which each of the pyrrole units is successively replaced by one or more imidazole moieties. The higher homologue of this series (86) has also been synthesised. These analogues bind to duplex DNA but not to singly stranded DNA, and as the number of imidazole residues increases, there is a progressively decreasing preference for AT-rich sites in minor groove binding.[192] An analogue of distamycin (87) has been made in which the N-methylpyrrole rings are replaced by 1,3-disubstituted benzene rings, increasing the curvature of the molecule. The binding to DNA seems to be A-T specific, though this is less pronounced than with distamycin.[193] Bis(distamycin)fumaramide has also been reported; it binds nine contiguous A-T pairs in the minor groove of double helical DNA.[194] Theoretical calculations on distamycin and netropsin accurately predict the absorption spectra, and also the signs of the c.d. bands except the negative 275 nm band.[195]

3.8 Peptides Containing Other Unusual Amino Acids. - The synthesis of (-)- Detoxinin D_1 (88), a depsipeptide from Streptomyces caespitosus, has been reported. The lactone (89) was a key intermediate; ring opening without β -elimination of the 7-oxy sub-



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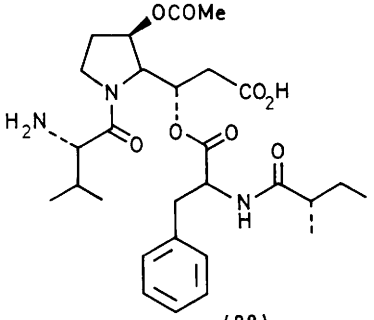

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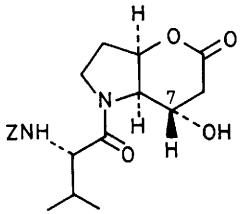

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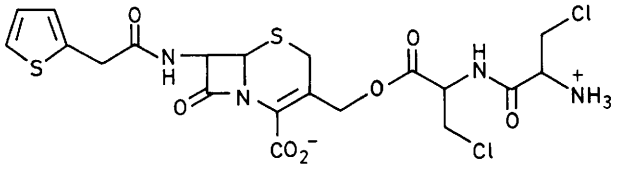
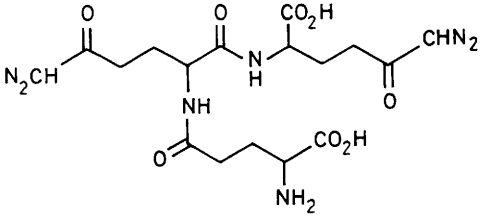
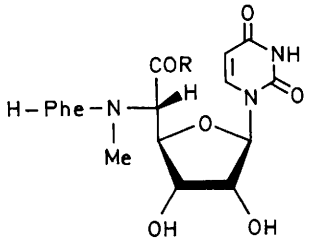


(90)



(91)

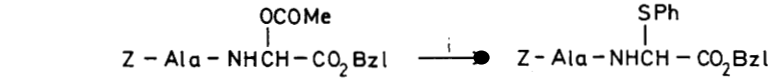


(92) R = Ala–OH or NH_2

stituent proved possible.[196] A dipeptidyl ester of cephalosporin (90) has been prepared to examine the possibility that cephalosporin may be used to 'carry' a second bactericidal compound which would be released by the action of β-lactamase.[197] A large number of structurally diverse di- and tri-peptides also containing the alanine racemase inactivator β-chloro-L-alanine have been synthesised and their antibacterial properties evaluated in vitro. None improves dramatically on the antibiotic efficiency of the previously described H-β-Cl-L-Ala-βCl-L-Ala-OH.[198]

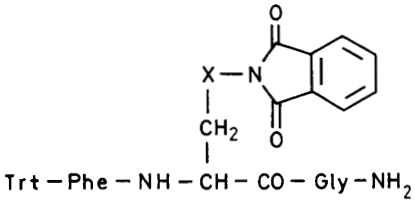
A total synthesis of the Streptomyces ambofaciens anticancer constituent (-)-azotomycin (91) has been accomplished in nine steps, the most challenging reaction being the conversion of two carboxyl groups to diazoketones. This synthesis was undertaken to circumvent problems arising in fermentation production.[199] N-Methylated di- and tri-peptide polyoxin analogues (92) have been prepared using the Ugi four-component condensation reaction. The products exhibited modest inhibition of chitin synthase activity.[200] Two dipeptides and a tripeptide, H-Ala-Tpg-Ala-OH, containing 2-thiophenylglycine have been constructed for the investigation of microbial peptide transport. The novel side-chain was introduced as shown in Scheme 8.[201]

The fluorescent enkephalin analogue [Pya^1,Leu^5]-enkephalin, where Pya = 1-pyrenylalanine, is δ-preferential with a fairly good affinity. Its methyl ester binds 24-fold more strongly to μ- than to δ-receptors.[202] N,O-Ditritylhomoserine has been made and used for the synthesis of some homoserine-containing peptides, e.g. Trt-Phe-Hse-Gly-NH_2. Condensation of this tripeptide with PPh_3 and diethyl azodicarboxylate gives a compound (93a) containing a 1,4-diaminobutyryl residue; a similar condensation with N-hydroxyphthalimide gives the canalyl tripeptide (93b). Selective removal of the phthaloyl group can be achieved with acetic acid-hydrazine hydrate.[203] Boc-[Aha^{11}]-substance P_{5-11}(Aha = L-2-amino-4-hexynoic acid) has been prepared and catalytically reduced to the corresponding Nle^{11} compound with a specific radioactivity of 96 Ci/mmol.[204] Six substance P analogues in which the glutamine residues at position 5 and/or 6 are replaced by homoglutamine (Hgn) have been synthesised by the solid-phase method. The Boc_2-Hgn-OH used was derived from an ester of Boc_2-Lys-OH by ruthenium tetroxide oxidation. The products showed enhanced activity on GPI tests, [Hgn]^5-substance P_{4-11} being the most potent. The same compounds have also been made with D-Hgn. All had reduced activity,

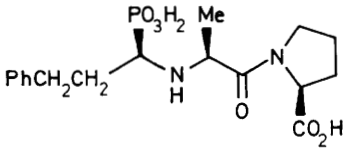


Reagent : i, PhSH, DMF, NEt_3, 20 °C

Scheme 8



(93) a; X = $-CH_2-$
b; X = $-CH_2O-$



(94)

*p*Aad – Leu – Pro – NH_2
*p*Aad – Nva – Pro – NH_2
Glp – Leu – Pip – NH_2
Glp – Leu – Tca – NH_2
Glp – Nva – Tca – NH_2
*p*Aad – Nva – Tca – NH_2

(95) *p*Aad = L – pyro – 2 – aminoadipic acid
Pip = L – pipecolic acid
Tca = L – thiazolidine – 4 – carboxylic acid

H – Pro – Leu – Aze – NH_2
H – Pro – Leu – Tca – NH_2
H – Pro – Leu – Pip – NH_2

(96) Aze = azetidine – 2 – carboxylic acid

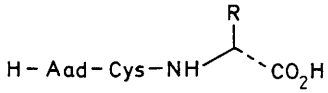
but [D-Hgn^6]-$SP_{4-11}$was an SP antagonist.[205]

The dipeptide Ac-Tyr-2,4-MePro-NHMe, where 2,4-MePro = 2-carboxy-2,4-methanopyrrolidine, adopts in aqueous solution >95% a trans peptide bond conformation whereas Ac-Tyr-Pro-NHMe exists as a mixture of the cis and trans forms.[206] Although L-2-amino-pimelic acid (Apa) is itself devoid of antibacterial activity, dipeptides containing Apa and either Ala or Pro have been prepared and found to have activity against a range of Gram-negative organisms. These peptides inhibit diaminopimelic acid production in intact resting cells; the most potent of those tested was H-Apa-Ala-OH.[207]

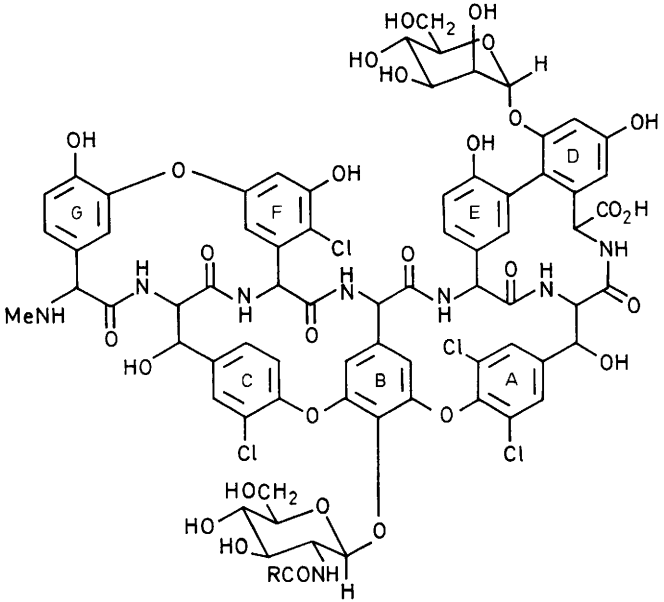
Another series of dipeptides containing N-terminal Ala or Leu and a wide range of racemic P-terminal 1-aminoalkane-phosphonates have been examined for antibacterial activity. Those containing 4-amino-4-phosphonobutyric acid and 1-amino-1-methylethanephosphonic acid were comparable in antibiotic power to alafosfalin.[208] A method for the large-scale synthesis of alafosfalin, Ala-Ala(P), has been developed, and a number of new analogues explored. Of these, Nva-Ala(P) is more potent, and Sar-Nva_2-Ala(P) shows a broader antibacterial spectrum in vivo as well as in vitro.[209] The phosphonic acid analogue (94) of the potent ACE inhibitor MK-422 is also a good inhibitor, but shows a loss in binding activity. It has been tentatively assigned the R,S,S-configuration.[210]

Two groups have explored modifications of the potent thyrotropin-releasing hormone (TRH) analogue H-Pro-Leu-Gly-NH_2 in which the terminal residues are replaced by a range of heterocyclic residues. Of the series in (95), all the compounds have a stronger anticataleptic effect than TRH with negligible or no hormonal activity, pAad-Leu or Nva-Pro-NH_2 being the most active.[211] Both H-Pro-Leu-thiazolidine-2-carboxamide and H-Pro-Leu-$\Delta^{3,4}$-Pro-NH_2 show a 2-3 fold enhancement of the binding of the dopamine agonist 2-amino-6,7-dihydroxynaphthalene to dopamine receptors in bovine brain tissue. Other compounds made which enhance binding to a lesser extent are shown in (96).[212] Glp-Leu-Gly-NH_2, Pip-Leu-Gly-NH_2, Aze-Leu-Gly-NH_2, and H-$\Delta^{3,4}$-Pro-Leu-Gly-NH_2 show activity comparable to H-Pro-Leu-Gly-NH_2, but Thz-Leu-Gly-NH_2 and H-D-$\Delta^{3,4}$--Pro-Leu-Gly-NH_2 are inactive.[213]

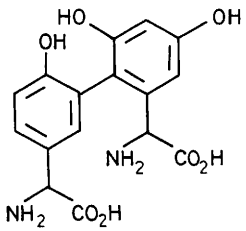
Four saturated peptide substrates for isopenicillin N synthase have been prepared, two containing allene side-chains (97 a and b)[214] and two alkenes (97 c and d).[215]



(97) a ; R = $-CH_2-CH=C=CH_2$
b ; R = $-(CH_2)_2-CH=C=CH_2$
c ; R = $CH=CH_2$
d ; R = $-C(Me)=CH_2$



(98) Kibdelin A, R = $-(CH_2)_8Me$
B, R = $-(CH_2)_7CHMe_2$
C_1, R = $-(CH_2)_8CHMe_2$
C_2, R = $-(CH_2)_{10}Me$
D, R = $-(CH_2)_2CH=CH(CH_2)_4Me$



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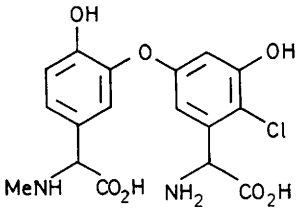


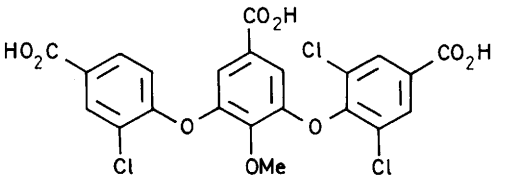
(100)

4 Conjugate Peptides

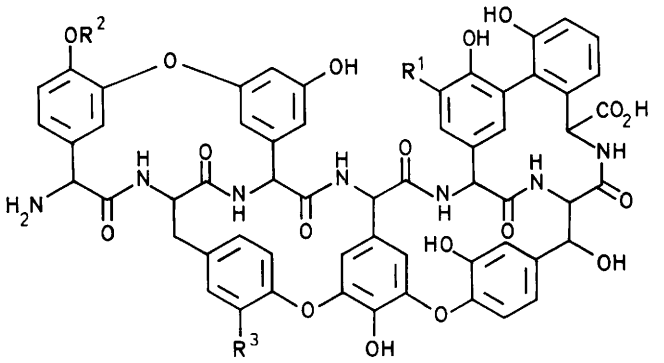
4.1 Glycopeptide Antibiotics - A new glycopeptide antibiotic complex has been isolated from Kibdelosporangium aridum by affinity chromatography on a D-Ala-D-Ala agarose column. The major components were then resolved by hplc. These compounds, the kibdelins (98), have the same mannosyl aglycon as the aridicins, and differ from the aridicins only in the oxidation level at the C-6 position of the amino sugar. They thus join the teicoplanins as the second example of glycopeptide antibiotics containing N-acyl-β-glucosamine moieties β-linked through the phenolic oxygen in ring B to the parent tetrapeptide core of the aglycon.[216] Three fragments have been obtained from the aridicin A aglycon which account for 54 of the 59 C atoms. Compounds (99) and (100) were obtained on acid hydrolysis, and (101) after oxidation and basic hydrolysis of the permethylated aglycon. These fragments are in agreement with the aglycon depicted in (98) but do not distinguish whether it is ring A or ring C in (98) which is dichlorinated.[217]

A new glycopeptide antibiotic complex A41030 has been obtained from the soil micro-organism Streptomyces virginiae, being active against Gram-positive bacteria. All components (102) contain a chlorinated ristocetin-like peptide core, and four of them are unique amongst antibiotics of this type in containing no attached sugar.[218] A strain of Streptomyces toyocaensis from a sample of sandy soil collected at low tide in Washington U.S.A. has a ristocetin-like peptide core, but is an aglycon and is unique among reported glycopeptides in that it bears a sulphate ester on ring G.[219]

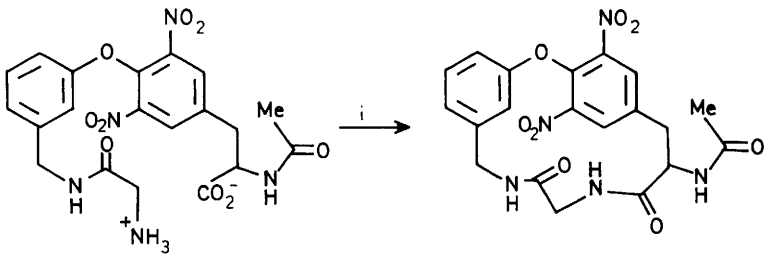
The sites of iodination of vancomycin and ristocetin A have been determined by n.m.r. and degradation. The former is substituted at the para-position of the resorcinol ring of actinoidinic acid, and the latter predominantly on residue 3 at the ortho-position distal to the diphenyl ether linkage. However, if the sugar attached to the aromatic ring of residue 7 of ristocetin A is removed, iodination occurs in the same position as in vancomycin.[220] A synthetic analogue (103) of the region of vancomycin which contains the pocket for the binding to the carboxylate of D-Ala-D-Ala- containing peptides has been prepared (Scheme 9) and shows spectroscopic similarity to the natural molecule. The nitro groups will, in future work, allow the introduction of halide



(101)



(102) *factor*	R^1	R^2	R^3
A	Cl	H	Cl
B	H	H	Cl
C	Cl	Gal	Cl
D	Cl	H	H
E	H	H	H
F	Cl	Gal-Gal	Cl
G	Cl	Gal-Gal	Cl



(103)

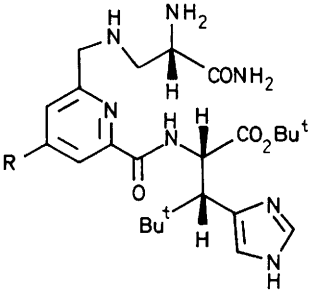
Reagent : i, diphenylphosphorazidate , DMF

Scheme 9

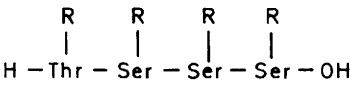
and other groups to vary the steric and electronic properties of the pocket.[221] The interaction between ristocetin pseudo-aglycon and Ac_2-Lys-D-Ala-D-Ala-OH has been examined by n.m.r. and molecular modelling. It was concluded that no one structure could fit all of the n.m.r. data. These were best explained by considering an average structure.[222]

A synthetic model binding compound PYML-4 (104) for the metal binding site of bleomycin showed dioxygen activation 71% of that of bleomycin.[223] Addition to PYML-4 of a methoxy group at R^1 gave PYML-6, a compound showing oxygen activation virtually equivalent to that of bleomycin itself.[224] Incorporation of the 4-methoxypyridine ring and the C-Bu^t group of PYML-6 into bleomycin gave an analogue with potent DNA-cleaving activity.[225] The irradiation with light of Cu(II)bleomycin and its bithiazole model compound methyl 2'-methyl-2,4'-bithiazole-4-carboxylate has been shown to induce isomerisation of the 2,4'-bithiazole ring system to a 4,4'-bithiazole. The phototransformed bleomycin has a nucleotide sequence cleavage mode almost identical with that of the original bleomycin.[226]

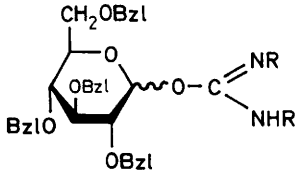
4.2 Other Glycopeptides - The synthesis of the tetrapeptide sequence 12-15 of asialoglycophorin A with four disaccharide side-chains attached (105) has been achieved. This synthesis involved the coupling of Fmoc-Ser or Thr benzyl esters with the appropriate acetylated disaccharides as the first step.[227] The reaction of 3,4,6-tri-O-acetyl-2-azido-2-deoxy- β -D-galactopyranosyl chloride with Z-Thr-OMe, Z-Thr-Ala-OMe, and Z-Thr-Pro-OMe gives yields of O-glycosyl derivatives of 42-49%, but no O-glycopeptides were obtained from Z-Ala-Thr-OMe or Z-Ala-Thr-Ala-OMe. This is ascribed to steric interactions between the benzyl of the Z-group and the AcO-4 of the sugar.[228] The O-glycosyl pseudourea (106) formed by treatment of tetra-O-benzyl-D-glucopyranose with carbodiimides and CuCl at 80 °C affords the corresponding D-glycopyranosyl ethers (107) on fusion with peptide derivatives at 80 °C. Stereoselectivity is moderate to fairly good.[229] It has been found that a high sugar content in a glycopeptide can result in a reduction in intensity, or suppression, of its FAB-m.s. In principle, the problem can be solved either by use of a more hydrophilic matrix or by preparation of a derivative of more suitable hydrophobicity. The former is more desirable, but the latter easier to achieve in practice.[230]



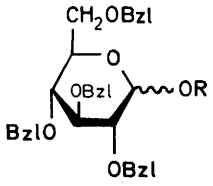
(104) R = H



(105) R = β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-(1→O)-



(106) R = C_6H_{11} or 4-MeC_6H_4



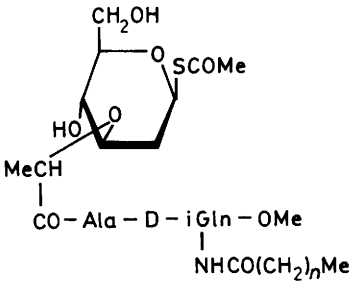
(107) R = Z-Gly_2-Phe- or Boc-Tyr(Bzl)-Gly_2-Phe-Leu-

A number of lipophilic derivatives of N-acetyl-1-thiomuramyl--Ala-D-isoGln have been synthesised. Three compounds (108) showed efficient protective activity in mice and guinea pigs. When acyl groups of a similar size were introduced also on to the sulphur, the activity was almost completely lost.[231] The 6-(octadecyl hydrogen phosphate) and the 6-(2-docosyltetracosyl hydrogen phosphate) derivatives of N-Ac-muramyl-Ala-D-iGln have been prepared. The latter more lipophilic compound showed high activity in a tumour regression test.[232] Four analogues of the immunoadjuvant muramyl dipeptide in which the D-lactic acid has been replaced by D- or L-Ala have been prepared, but no biological activities given.[233]

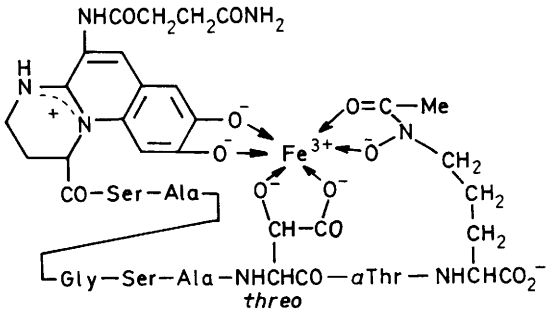
Following an effective synthesis of 1,6-anhydro-β-muramic acid, β-D-GlcNAc(1→4)-1,6-anhydro-β-MurNAc-Ala-D-iGln-OH has been prepared,[234] and of a series of acylated, amidated, and esterified derivatives of N-acetylglucosaminyl-β(1→4)-N-acetylmuramyl tri- and tetrapeptides examined for their effect in reducing pyrogenicity in the rabbit, the L- and D-Ala-D-iGln-L-stearoyl-D-meso-2,6--diaminopimelic acid (D)-amide have proved worthy of further investigation.[235]

4.3 Non-Carbohydrate Conjugate Peptides - The structure of the yellow-green fluorescent siderophore pseudobactin A214 (109), produced from bean-deleterious Pseudomonas A214 under iron-limiting conditions, has been determined. This metabolite, which may contain some D-residues, is very similar in structure to the siderophores of plant growth promoting and plant-deleterious Pseudomonas B10 and 7SR1 respectively.[236] Bivalent ligands containing the oxymorphamine (110a) or naltrexamine (110b) pharmacophores connected to spacers of varying lengths have been prepared and evaluated for their selectivity at opioid receptors. Peak agonist activity in the oxymorphine compounds was observed with four glycyl units. The naltrexamine ligand that most effectively antagonised the μ-receptor against morphine in the GPI also contained the four glycine residues.[237]

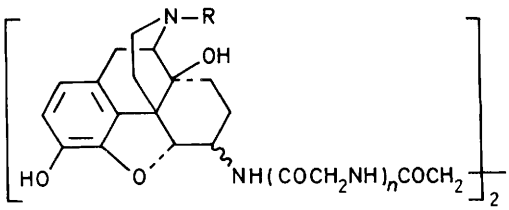
A series of unsaturated derivatives of a tripeptide (111) have been evaluated as substrates for isopenicillin N synthetase to establish if the production of hydroxylated products is a general phenomenon with this enzyme. In two cases (111a and b) the alkene is apparently too remote for hydroxylation to occur, but where the carbon chain is shorter (111c and d), hydroxylation concomitant



(108) n = 8, 12 or 16

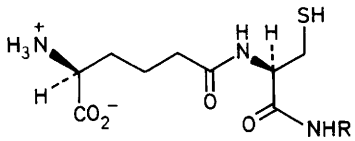


(109)



(110) a; R = Me, C-6α

b; R = $-CH_2-CH(CH_2)_2$, C-6β



(111) a; R = $-CH(CO_2H)CH_2CH_2CH{=}CH_2$

b; R = $-(CH_2)_3CH{=}CH_2$

c; R = $-CH{=}CH_2$

d; R = $-CMe{=}CH_2$

e; R = $-C(CO_2H){=}CMe_2$

with ring formation is a major pathway. The αβ -alkene (111e) is not converted into bicyclic product.[238] $\underline{N}^{\varepsilon}$-Retinylidene derivatives of Boc-Lys-Phe-OMe, Boc-Lys-Phe_2-OMe, and Boc-Lys-Phe or Gly-Tyr-OMe have been prepared in order to investigate the effect of the surrounding microenvironment on the absorption maximum of the chromophore of bacteriorhodopsin. In the case of the tyrosine-containing peptides, but not in the other derivatives, there was a shift to the red confirming an interaction between Tyr and the retinylidene moiety in low dielectric media.[239]

Treatment of polyglutamic acid (Mv 200,000) with p-aminoazobenzene and DCC-HOBt has given a conjugate with an 85% azobenzene content. Addition of 15% water to a hexafluoro-2-propanol solution of the modified polymer causes precipitation. Irradiation for a few seconds of the suspension at 338 nm causes the solid to redissolve, the resulting solution exhibiting the absorption spectrum of the cis-azopolypeptide. Further irradiation at 450 nm or dark adaption will reprecipitate the polymer.[240] Photocontrol of the secondary structure of solid membranes composed of polyglutamic acid containing pararosaniline groups (10.5 mol %) on the side-chains is based on a cooperative effect between photodissociation of the pararosaniline moiety and the induced acid dissociation of the carboxyl groups.[241]

A series of conjugates Nps-Lys$(Boc)_n$-p-[(cholestan-3β-yloxy)-carbonyl]benzyl have been prepared where n = 2 to 8. I.r. indicates an intermolecular β-structure for the higher members. These compounds showed a lower tendency to self-aggregation than the simple benzyl esters, aggregation and decreasing solubility running parallel.[242] C.d. spectral analysis of Dnp-Ala-Ala or D-Ala-Pro-D-Ala-p-nitroanilides indicates that they have a very low preference for β -turn formation. This can be related to the lack of antibacterial activity of [D-$Val^{1,1}$]- and [D-$Val^{1,1}$,$Phe^{4,4}$]-gramicidin S, of which they are simple less sterically hindered models.[243]

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5
β-Lactam Antibiotic Chemistry

BY A. V. STACHULSKI

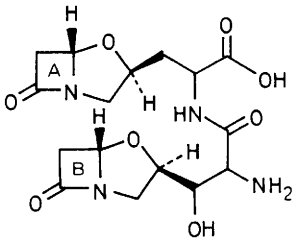
1. Introduction

In preparing this chapter I have generally retained the sections and sub-divisions used by Dr. Brennan in the previous two reports. Thus monocyclic β-lactams, which again form the largest section, are classed as azetidin-2-ones and grouped according to the ultimate bond forming step. Syntheses of azetidinone intermediates for the preparation of carbapenems or other bicyclic systems will also be found here unless the synthesis of the bicyclic system was completed. I have expanded the section on 'Mechanistic Studies Related to Biological Activity' to include more work on degradation studies, as these are important characteristics of potential antibiotics.

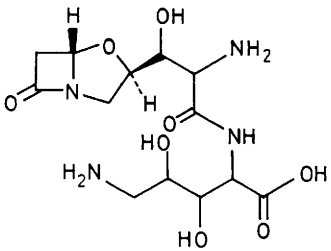
Reviews published in 1986 include an account of naturally occurring monobactams, *viz*. 3-amino-azetidin-2-one *N*-sulphonic acids [1], and of the biosynthesis of carbapenems.[2] Miller has reviewed his synthesis of azetidinones from hydroxamates.[3] Further synthetic reviews relate to the synthesis of penems[4], the use of ^{2}H and ^{3}H nuclear magnetic resonance in β-lactam biosynthesis[5] and more general aspects of β-lactam synthesis[6] and chemistry.[7]

2. New Natural Products

The only report of new naturally occurring β-lactams has been the disclosure of clavamycins[8], members of the clavam antibiotics isolated from *Streptomyces* species. Clavamycin A (1) is a bis-β-lactam, whereas clavamycins B (2) and C (3) may be regarded as (1) lacking, respectively, ring A or ring B. Clavamycins D, E and F, which have structures (4), (5) and (6) differ only in the amino-acid residue R. Clavams (1) and (4) possess antifungal activity but no antibacterial activity or β-lactamase inhibition.[9] Although not a β-lactam, the isoxazole derivative lactivicin (7) isolated from *Empedobacter* and



(1)



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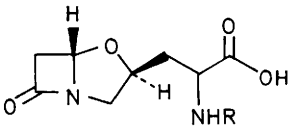
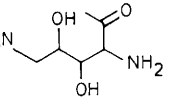
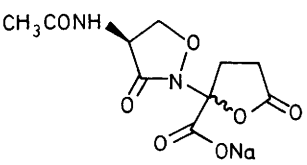


(3) R = 

(4) R = L-Valyl

(5) R = L-Alanyl

(6) R = N^{δ}-Acetylornithyl



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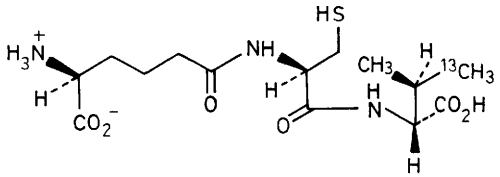

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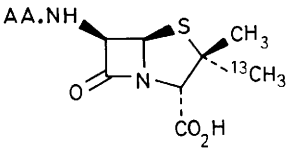
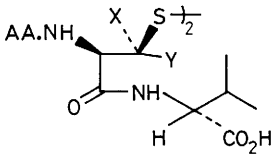


(9) AA = δ-(L-α-aminoadipoyl)



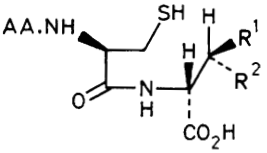
(10) X = D, Y = H

(11) X = H, Y = D

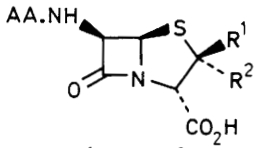
Lysobacter culture filtrates[10] shows strikingly similar properties, namely activity against both Gram-positive and -negative bacteria, affinity for penicillin binding proteins and susceptibility to β-lactamases.

3. Biosynthesis

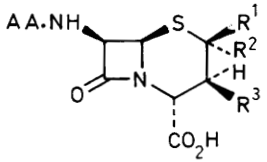
The Oxford group has continued to elucidate the finer details of penicillin biosynthesis and to probe the tolerance of the isopenicillin N synthetase (IPNS) enzyme for unnatural substrates. Conversion of the stereospecifically ^{13}C-labelled peptide (8) obtained from (2S,3S)-[4-^{13}C] valine with IPNS from Cephalosporium acremonium gave 2α- ^{13}C isopenicillin N (9) in over 95% conversion and with complete retention of stereochemistry on forming the C-S bond.[11] Replacement of valine with isoleucine gave a similar result. The tripeptides (10) and (11), stereospecifically deuteriated at the cysteine 3-position, were converted by IPNS into isopenicillin N with complete retention of the 3-pro-R-hydrogen and complete loss of the 3-pro-S-hydrogen.[12] It is now clear that substantial variations are possible in the valine residue of the δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine (LLD-ACV) tripeptide precursor. Thus the O-methyl-D-threonine derivedprecursor (12) on incubation with IPNS gave the 2α-methoxy product (13)[13]; interestingly the isomer (14) was not converted. With unsaturated peptides, the product was dependent on the location of the double bond.[14] The peptide (15) gave rise to the mixture of cephams (16) and (17), whereas (18)gave no β-lactam product. Vinylglycine analogue (19) afforded the 2α-(hydroxymethyl)penam (20) while the closely-related (21) gave a mixture of the corresponding penam (22) and the exomethylene cepham (23). The dehydropeptide (24) gave no β-lactam product. Further studies using $^{18}O_2$ revealed that the oxygen in the hydroxylated products was derived from cosubstrate dioxygen[15] in the enzymic conversion and an iron-oxo species was proposed to account for this, allowing for both 'desaturative' products (the vinyl cephams) and the hydroxylated ones. Only the 'desaturative' mode, leading to penam, cepham, and dienyl products was observed with the allene-containing tripeptides (25) and (26).[16] Thus (25) gave a mixture of penams (27) and (28) and cepham (29), while (26) gave cephams (30) and (31) and the diene (32). It was already known that some variation of the δ-(L-aminoadipoyl) moiety was possible, and two groups[17,18]have reported the enzymic cyclization of phenylacetyl-L-cysteinyl-D-valine to benzylpenicillin, using in these casescell-free



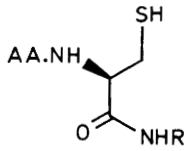
(12) R^1 = Me, R^2 = OMe
(14) R^1 = OMe, R^2 = Me
(15) R^1 = $CH_2CH{=}CH_2$, R^2 = H
(18) R^1 = $(CH_2)_2CH{=}CH_2$, R^2 = H
(25) R^1 = H, R^2 = $CH{=}C{=}CH_2$
(26) R^1 = H, R^2 = $CH_2CH{=}C{=}CH_2$



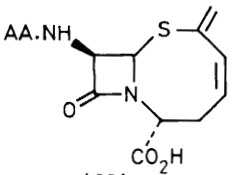
(13) R^1 = Me, R^2 = OMe
(20) R^1 = H, R^2 = CH_2OH
(22) R^1 = Me, R^2 = CH_2OH
(27) R^1 = H, R^2 = $CH{=}C{=}CH_2$
(28) R^1 = $CH{=}C{=}CH_2$, R^2 = H



(16) R^1 = R^3 = H, R^2 = $CH{=}CH_2$
(17) R^1 = $CH{=}CH_2$, R^2 = R^3 = H
(23) R^1 = R^2 = H, R^3, H = $=CH_2$
(29) R^1, R^2 = $=C{=}CH_2$, R^3 = H
(30) R^1 = H, R^2 = $CH{=}C{=}CH_2$, R^3 = H
(31) R^1 = $CH{=}C{=}CH_2$, R^2 = R^3 = H



(19) R = D-$CH(CO_2H)CH{=}CH_2$
(21) R = D-$CH(CO_2H)C(Me){=}CH_2$
(24) R = $C(CO_2H){=}CMe_2$



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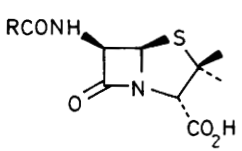
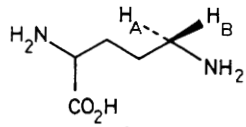
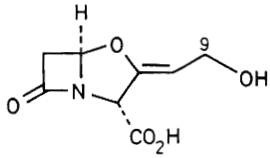


(33)



(34) a; H_A = ^3H, H_B = H
b; H_A = H, H_B = ^3H



(35)

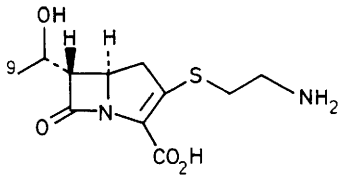
extracts of Streptomyces clavuligerus. Cyclization of δ-(D-α-aminoadipoyl)-L-cysteinyl-D-valine (DLD-ACV) has been reexamined[19]; the conclusion was that, in the presence of the expandase enzyme which subsequently converts the first-formed penicillin N to deacetoxycephalosporin C, this tripeptide is cyclized three times more slowly than 'natural' LLD-ACV. The implication is that isopenicillin N is less inhibitory than penicillin N to IPNS. Enzymatic acylation [20] of 6-aminopenicillanic acid (6-APA), catalysed by the enzyme Acyl-CoA: 6-APA acyl transferase, led to penicillins of type (33) only for aliphatic carboxylic acids from 6 to 8 carbon atoms, as their CoA esters.

The sterospecifically tritiated ornithines (34a) and (34b) were incorporated into clavulanic acid (35) such that the 5-pro (R) hydrogen was retained and became the 9-pro(S) hydrogen in the product.[21] Intermediate transamination and reduction steps are implicated. The C-9 methyl group of thienamycin (36) has been shown to be derived from the methyl group of methionine with net retention of configuration.[22]

4. Penicillins and Cephalosporins

As before, this section includes the chemistry and preparation of the parent penam and cephem ring systems, including molecules synthesized as β-lactamase inhibitors. A steroselective synthesis of penams of type (37) was achieved [23] by addition of appropriate ketenes to the 2-(methylseleno)-Δ^2-thiazolines (38) followed by tri-n-butyl tin hydride reduction of the product. In a similar manner, a series of 2-(methylthio)penams (39) was prepared by addition of ketenes to 5-(methylthio)-2-phenyl-Δ^2-thiazolines.[24] Application of Corey's "one-pot" oxidation of primary alcohols to t-butyl esters to known 6α-hydroxymethyl or -formyl penicillins led [25] to a series of 6α-carboxy and-carbamoyl penicillins (40) which had weak antibacterial activity. The 6α-succinimido-oxy penicillin (41) proved to be an effective intermediate for the preparation of a range of 6α-substituted penicillins[26] bearing oxygen, nitrogen or carbon substituents, eg., methoxy, formamido, cyano or vinyl; though (41) was stable in itself it underwent ready nucleophilic displacement of the good 6α-leaving group.

Penicillin sulphoxides continue to be much employed in various transformations. The ready oxidation of penicillin V



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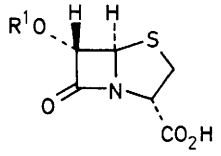
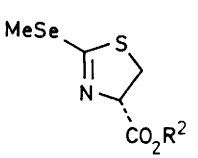


(37)



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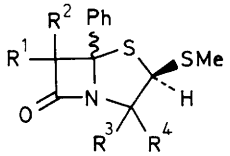

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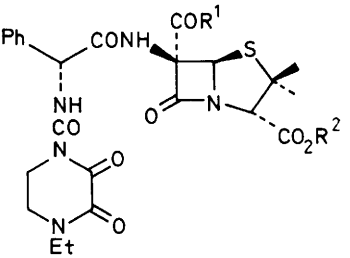
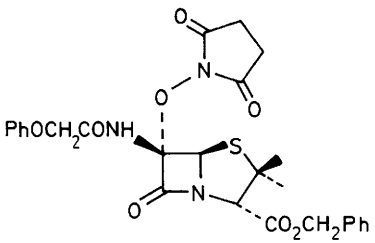
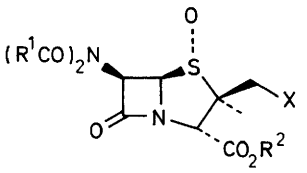


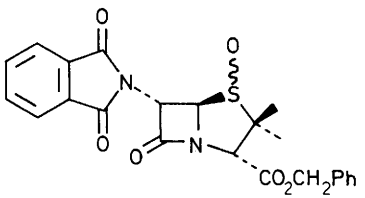
(40) R^1 = ONa, OAlkyl, NH_2



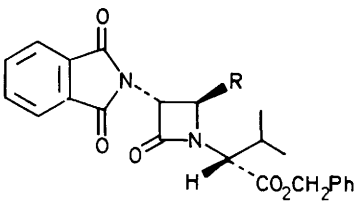
(41)



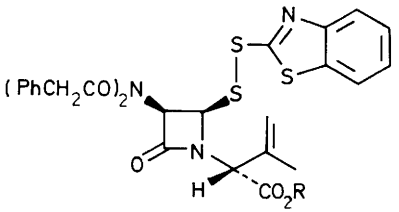
(42) X = H, Cl



(43)



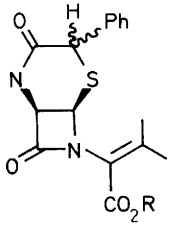
(44) R = $S(CH_2)_2CO_2Me$
(45) R = F



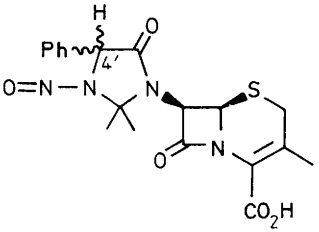
(46) R = CH_3, CH_2CCl_3

to its 1β-sulphoxide in a two-phase system (CH_2Cl_2/H_2O_2) with no added acid is claimed to be novel.[27] The β-sulphoxide has long been known to be the major oxidation product from a 6-acylamino-penicillin, but 6-(diacylamino) penicillins could be made to yield almost exclusively the α-sulphoxides (42) by appropriate choice of conditions[28]; zinc-mediated reduction then gave the unusual 6-amidopenicillin-1α-sulphoxides. Similarly, cephalosporin-1α-sulphoxides may be prepared in excellent yield from 7-(diacylamino) cephalosporins.[29] The sulphoxide (43) was transformed in three steps into the secopenicillanate (44) in 68% overall yield; treatment of this material with trimethyloxonium tetrafluoroborate led to the 4-fluoroazetidinone (45).[30] The azetidinone disulphide (46), itself obtained from a penicillin sulphoxide, on treatment with Et_3N afforded the 3,8-dioxo-5-thia-2,7-diazabicyclo [4.2.0] octane (47) [31] (75%). A previous conversion of a penicillin G 1β-sulphoxide ester into cephalexin gave a diastereoisomeric mixture of products; conversion to the mixture of N-nitroso-imidazolidinyl intermediates (48) allowed the isolation of the pure desired 4'R-diastereoisomer[32] which was converted to optically pure cephalexin. Penicillin sulphones continue to be of interest as β-lactamase inhibitors, and a new synthesis of sulbactam (6,6-dihydropenicillin-1,1-dioxide) from 6-APA has been reported.[33] Reaction of the 2β-halomethylpenam (49a) with sodium azide gave a mixture of 2β-azidomethyl penam (49b) and a 3β-azido cephem[34]; separation was more easily affected after oxidation to sulphone (49c).

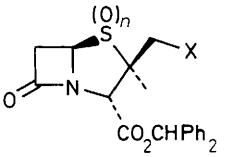
The reduction of methyl 6-mono-or dibromopenicillanates by zinc generally gave cleanly the 6,6-dihydropenicillanate,[35] but with Zn-AcOH in acetonitrile or ethyl acetate the dibromo compound gave mainly the 1,4-thiazepine (50). Oxidation at sulphur suppressed the rearrangement. 6β-Halopenicillanates were obtained in >90% yield by SN2 displacement on a 6α-[(fluorosulphonyl)oxy] penicillanate using tetrabutylammonium halides.[36] A number of 6α-allylpenicillanates (51) could be obtained from 6-bromo-penicillanates using allyltributyltin under free-radical conditions.[37] Replacement of a 6-halogen by a 6-hydroxyethyl group under Grignard conditions, a well-known reaction, has been studied for a variety of halogens, solvents and sulphur oxidation states;[38] up to 98% of the 6β-(8R)-compound (52) was obtained. When a similar Grignard intermediate was quenched with ethyl formaldoxime,[39] the 6α-(ethoxyamino)methyl compound (53) resulted; 6α-



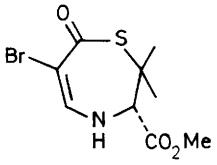
(47)



(48)



(49) a; X = Br or Cl, n = 0
b; X = N_3, n = 0
c; X = N_3, n = 2



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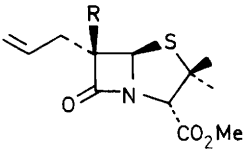

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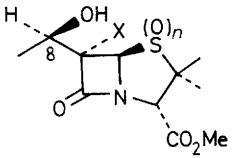

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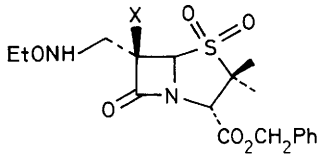

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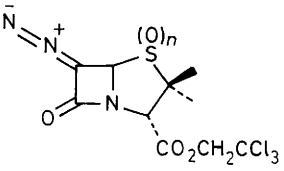
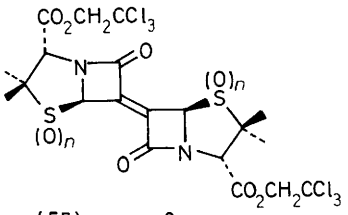
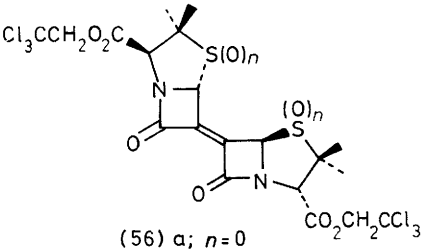


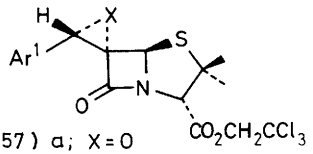
(54) a; n = 0
b; n = 1
c; n = 2



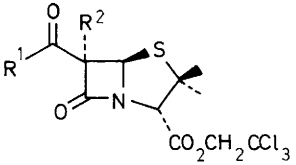
(55) a; n = 0
b; n = 1
c; n = 2



(56) a; n = 0
b; n = 1
c; n = 2



(57) a; X = O
b; X = NAr^2



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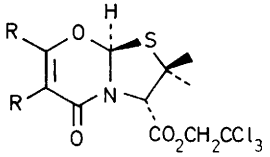


(59)

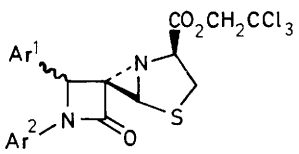
(aminomethyl) penicillin sulphones are also β-lactamase inhibitors.

A number of reports on the preparation and chemistry of 6-diazopenicillanate esters such as (54a) appeared during the year; for preliminary results see vol.17. The sulphoxide (54b) and sulphone (54c) were efficiently obtained[40] from the corresponding 6β-phenylacetamido compounds by N-nitrosation followed by thermolysis, and their chemistry investigated, many reactions being parallel to those of (54a). All three S-oxidation levels of the diazopenicillanate gave moderate yields of mixtures of the (E)- and (Z)-dimers,[41] (55a-c) and (56a-c), on treatment with copper bis(acetoacetonate). Lewis acid-mediated reaction of (54a) with aromatic aldehydes and imines gave, respectively, modest yields of spiro epoxides (57a)[42] and spiro aziridines (57b)[43] together with a number of rearrangement products resulting from C(5)-C(6) bond cleavage. Under similar conditions (54a) reacted with ketones to give 6α-alkyl-6β-acylpenicillanates (58), together with oxazinone rearrangement products (59).[44] When the reaction between (54a) and aromatic imines was performed thermally, a more complex rearrangement resulted[45], yielding spiro-aziridine-azetidinones (60); on heating at reflux in toluene these adducts were transformed to azetidinones (61).

Another class of penicillin-derived β-lactamase inhibitors possesses a 6-methylene substituent, for example the 6-(2-pyridyl) methylene compound (62)[46] obtained in four steps (as its potassium salt) and 40% yield from allyl 6α-hydroxypenicillanate. A Wittig-type synthesis on allyl 6-oxo penicillanate[47] proved to be a general method for the preparation of 6-sulphonyl-, 6-sulphinyl- and the known 6-acetylmethylene penicillanates of type (63). The 6-acetyl methylene compound was still the most effective inhibitor. 1-3-Dipolar cycloaddition of diazomethane to the methylene group gave spiropyrazoline penicillanates. In view of the instability of the acetylmethylene compounds in biological fluids, pro-drug forms have been sought[48] with modest success.

Treatment of 6-acylaminopenicillin esters with hydroxylamine opened the β-lactam ring; subsequent Lossen-type rearrangement followed by closure with N, N-diethylamino acetylene[49] gave ring-enlarged products (64) which were biologically inactive.

Both penicillin and cephalosporin sodium or potassium salts were efficiently esterified by alkyl bromides in MeCN[50] if an appropriate crown ether was added. No $\Delta^3 \rightarrow \Delta^2$ isomerisation was noted for the cephalosporins, and esterification of cephem free



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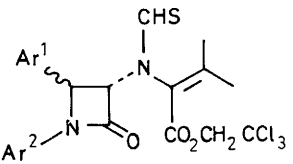

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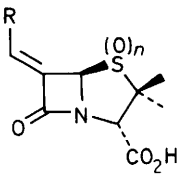
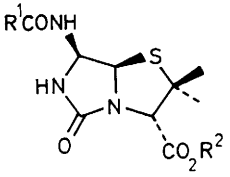


(62) R =  , $n = 2$

(63) R = R^1SO_2, R^1SO or R^1CO, $n = 0$



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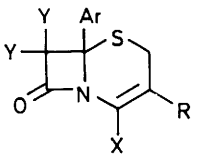

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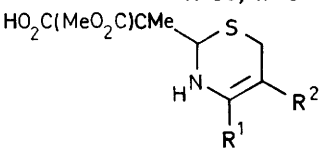

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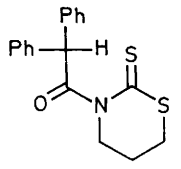

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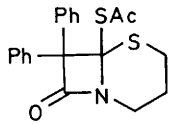

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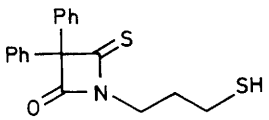

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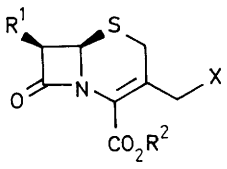
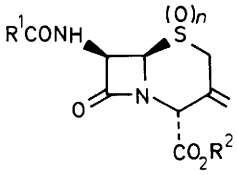


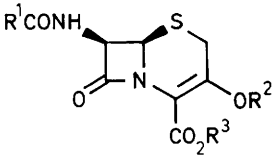
(70) X = OH

(71) X = I



(72) $n = 1$

(73) $n = 0$



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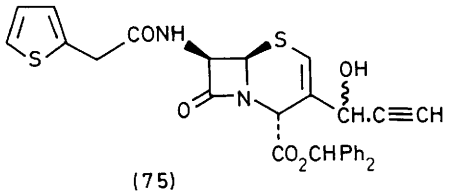

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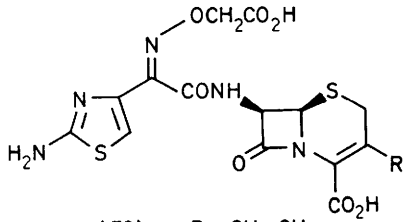
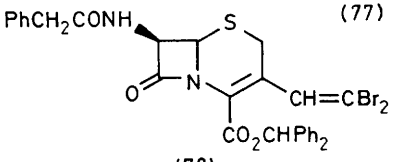


(76) a; R = $CH{=}CH_2$

b; R = $CH{=}CHal_2$

(77) R = $C{\equiv}CH$



(78)

acids using sodium hydrogen carbonate[51] in DMF-dioxan has been advised for the same reason. The t-butyl ester of 7-aminocephalosporanic acid (7-ACA) has been made by transesterification[52] from t-butyl acetate.

Two reports have described the synthesis of the ceph-3-em ring system from thiazine precursors by ketene-imine condensation [53] to afford a series of 6-arylceph-3-ems (65) or by intramolecular closure of the intermediates (66) using 1-benzotriazolyloxytris (dimethylamino) phosphonium hexafluorophosphate.[54] When the thiazine derivative (67) was irradiated at low temperature in the presence of acetyl chloride and triethylamine, the 6-S-acetylcepham (68) was isolated[55]; (67) by itself gave the corresponding 5-mercaptocepham, but this was not isolable; on workup the thioxo-β-lactam (69) was produced. Treatment of a 4-unsubstituted ceph-3-em with diazomethane followed by heating gave the 4-methyl derivative.[56]

Modification of the C(3)-acetoxymethyl group of 7-ACA is frequently important. A new approach to C(3)-(hydroxymethyl) ceph-3-ems (70)[57] calls for treatment of a 7-ACA derivative with trimethylsilyl iodide, followed by displacement with trifluoroacetate and hydrolysis at pH7; lactonisation was minimal. C(3)-Iodomethyl ceph-3-ems (71) could be isolated in >80% yield by a two-phase NaI displacement from the 3-chloromethyl compounds [58], or used directly in further reactions. 3-Exomethylenecephams *eg*. (72), (73) are also useful intermediates; they have now been found to be directly available from, respectively, ceph-3-em sulphoxides by Zn-NH_4Cl reduction[59] or from 3-chloroceph-3-ems by electrolysis.[60] Hydrolysis of a (3'-peptidyl) ceph-3-em by β-lactamase *in vitro* led to release of an antibiotic peptide and significant antibacterial activity resulted[61]; see also section 9.

Mitsunobu-type reaction of 3-hydroxyceph-3-ems led to 3-alkoxy compounds (74) in 60-90% yield[62]; C-alkylated by-products were easily separated. Treatment of a 3-formylceph-2-em ester with an acetylenic Grignard reagent led to the 3-(propyn-1-ol)yl-ceph-2-em (75)[63]; this was transformed to a variety of 3-alkenyl- and 3-heterocyclyl derivatives, none showing appreciable activity. A 3-formylceph-2-em intermediate was again used in the preparation of some 3-(2,2-dihalovinyl)ceph-3-em acids (76b)[64], after isomerization of the double bond; like the parent vinyl compound

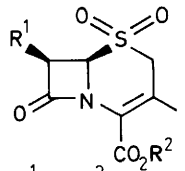
(76a) these showed good broad-spectrum antibacterial activity and oral absorption. The related 3-ethynyl compound (77)[65] was prepared from the 3-dibromovinyl sulphoxide (78)by elimination using four equivalents of n-butyllithium, followed by reduction, Delft cleavage and re-acylation.

The 3-methylceph-3-em 1,1-dioxides (79a) and (79b) were surprisingly rearranged to the Δ^2-compounds (80a) and (80b) by 5% palladium-charcoal[66]; cognate sulphides or sulphoxides were unaffected. Normally the ceph-3-em double bond is unreactive towards electrophiles, but it was known that the 4-hydroxymethyl derivative (81) reacted with bromine in methanol to give two isomeric adducts. These have now been confirmed [67] as (82a) and (82b) following conversion to penam sulphoxides and nuclear magnetic resonance studies.

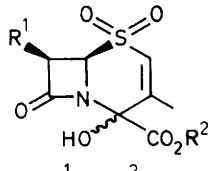
5. Penems

The general methods for the synthesis of the penem ring system are now well established; frequently penicillin derivatives or derived azetidinones are the starting materials. Rhodium-catalysed addition of a diazoacetic acid ester to a protected penicillanate afforded, after double-bond isomerization, the azetidinone (83).[68] After further transformations the usual intramolecular Wittig reaction delivered the 2-carboxypenem (84). More commonly the penems bear a 6-hydroxyalkyl side chain. A series of 2-aminoalkyl analogues (85)[69] and of 2-(heterocyclyl) mercaptoalkyl analogues (86)[70] were similarly prepared by the Wittig route. Another common strategy, the trialkylphosphite-mediated intramolecular cyclization of a thiol ester onto an oxalimide, was used to make a series of 2-(aminoalkyl) penems[71] similar to (85). The chiral bicyclic azetidinone (87) was a useful intermediate for one of the latter bearing a 6-(1'-hydroxy-1'-methyl) ethyl side chain. Normally the thiol ester required for this procedure is obtained by a displacement reaction on a 4-acetoxyazetidinone; a recently discovered alternative involves treatment of a penicillin-derived disulphide with a carboxylic anhydride and triethyl phosphite[72] (Scheme 1).

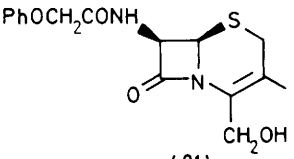
Ring contraction of 2-thiacephems to penems is also a well-known reaction, but suffers from lack of stereochemical control at the azetidinone-sulphur junction. However, the 1,1-dioxides



(79) a; R^1=H, R^2=CH_2Ph
b; R^1= $PhOCH_2CONH$,
R^2= $CHPh_2$



(80) a; R^1=H, R^2=CH_2Ph
b; R^1= $PhOCH_2CONH$,
R^2=$CHPh_2$



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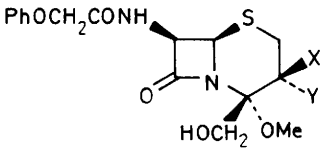
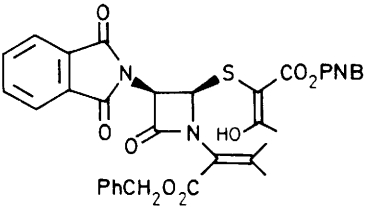
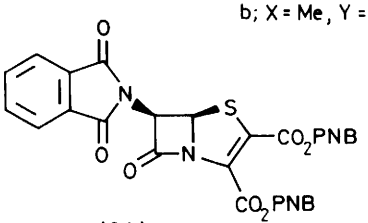


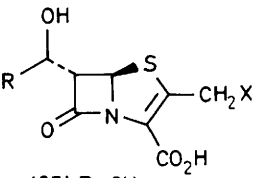
(82) a; X = Br, Y = Me
b; X = Me, Y = Br



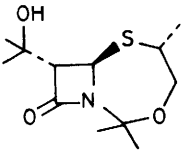
(83)



(84)



(85) R = CH_3
(86) R = H, CH_3



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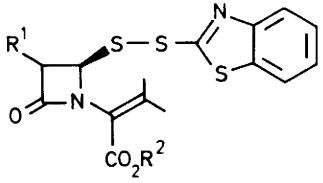
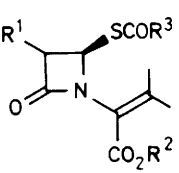
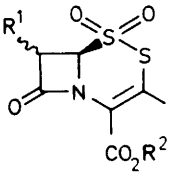
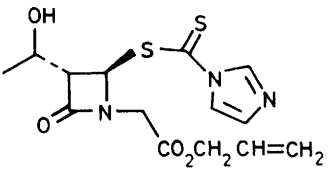




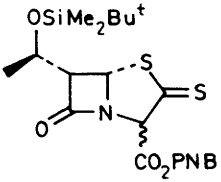


(88)

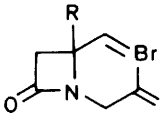
Scheme 1



(89)



(90)



(91) R = H
(95) R = Me

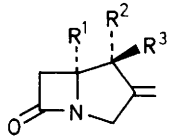
(88), readily available from 2-thiacephems by peracid oxidation,[73] underwent facile high-yielding thermal loss of SO_2 to afford 5 (R)-penems with no loss of stereochemistry. Normally the 6-hydroxyalkyl group is protected by a silyl moiety during penem synthesis, but tetrahydropyranyl[74] has been shown to be practicable.

The known base-catalysed closure of intermediates of type (89) was used to prepare a series of 2-(heterocyclyl)alkylthiopenems[75] having broad-spectrum antibacterial activity; previously described methods were also used to prepare a series of 2-N-heterocyclic and 2-mono-N-alkyl penems[76] as well as some 2-(quaternaryammonium) methylpenems.[77] The known thione (90) was a useful intermediate for a 2-(2-fluoroethyl)thiopenem[78] and other halo-analogues.

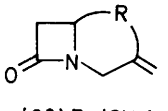
6. Carbapenems and Related Systems

This section includes carbapenams, carbapenems, carbacephams and carbacephems. Some newer routes to these systems highlight the increasing use of radical and organometallic procedures in synthesis. For instance, photochemically induced cyclization[79] of azetidinone (91) afforded either carbapenem (92) or carbacepham (93) according to the concentration: under thermal conditions, employing tri-n-butyl tin hydride, only (93) was produced. Using palladium acetate instead, the carbaceph-1-em (94)resulted, plus a dimeric product. The 4-methylazetidinone(95) gave the 1ß-methyl carbapenam (96) as the major product under thermal or photochemical conditions. Similarly, a palladium acetate-mediated cyclization of a 1-allyl-4-ethynyl azetidinone[80] afforded the bis methylene-carbapenam (97).

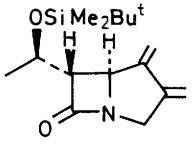
In a synthesis of a carbapenam from methyl (±)-pyroglutamate[81] the ß-lactam was closed using Ohno's method in the final step (Scheme 2). Intramolecular SN2 displacement of the iodo-compound (98a) using Triton B as base afforded the desired carbapenam (80%); remarkably the C-3 epimer (98b) gave only polymeric material.[82] In a conceptually similar approach, treatment of azetidinone (99) with benzeneselenenyl bromide gave carbapenam (100), an example of a general synthesis of nitrogen heterocycles.[83] A lengthier approach began with a diazopenicillanate [cf. (54a)] which was transformed in several steps to the 4-iodomethylazetidinone (101). Treatment of this material with dimethyl 2-(methylthio) fumarate,[84] using diphenylmethyl potassium as base, formed two ring bonds in one step to give the epimeric mixture of carbapenams (102a) and (102b). A 3-unsubstituted azetidinone was similarly cyclized.



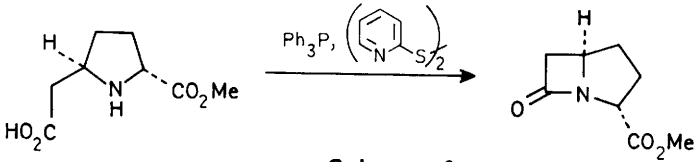
(92) $R^1 = R^3 = H$, $R^2 = Me$
(96) $R^1 = R^3 = Me$, $R^2 = H$



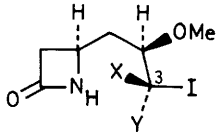
(93) $R = (CH_2)_2$
(94) $R = CH{=}CH$



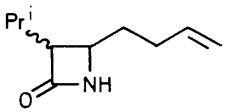
(97)



Scheme 2



(98) a; $X = H$, $Y = CO_2Me$
b; $X = CO_2Me$, $Y = H$



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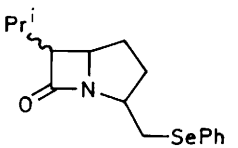

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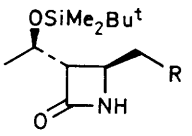
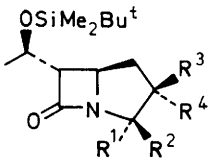
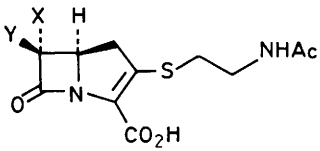


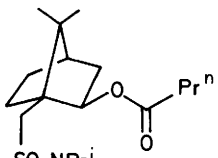
(101) R = I
(108) R = C≡CSPh



(102) a; $R^1 = R^3 = CO_2Me$
$R^2 = H$, $R^4 = SMe$
b; $R^2 = R^4 = CO_2Me$,
$R^1 = H$, $R^3 = SMe$



(103) X = Et, Y = H
(107) X = OMe, Y = Et



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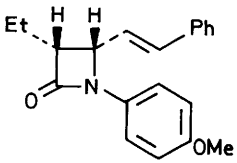

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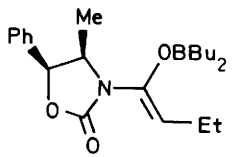

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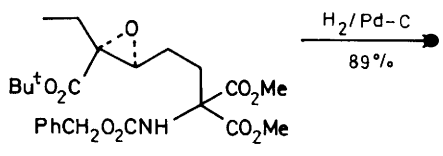
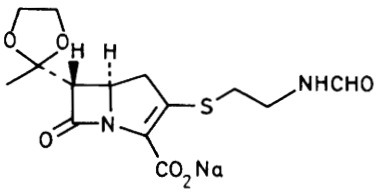


Scheme 3

Two asymmetric syntheses of the carbapenem antibiotic (+)-PS-5 (103) have been described. In one case[85] an isoborneol unit was employed as a chiral auxiliary; the anion of ester (104) was treated with a cinnamaldimine to give, in high enantiomeric excess, azetidinone (105) which was transformed into (103) using known methodology. Evans employed an aldol addition of the boron enolate (106)[86] to generate the required stereochemistry of the two chiral centres in (103), the synthesis being again completed by established methods from a later azetidinone intermediate. Further examples of syntheses of chiral azetidinones will be found in section 7. A synthesis of 6-methoxy-6-epi-PS-5(107) from a protected aminomalonate has been reported;[87] a key step was the generation of a pyrrolidine intermediate by hydrogenolysis (Scheme 3), followed by completion of the carbapenam skeleton in two steps and further modification. The derivative was more stable to renal dehydropeptidase than PS-5 but much less antibacterially active.

A synthesis of thienamycin (36) from 6-APA has been reported; the 4-(phenylthio)ethynylazetidinone (108) was a key intermediate.[88] The well-known rhodium catalysed carbene insertion into an azetidinone NH bond developed by the Merck group was used to prepare the thienamycin analogue (109) which had no antibacterial activity.[89] (±)-6-Epithienamycin, having the cis-carbapenem stereochemistry [cf.(36)] was obtained from the trans-ketone (110); halogenation followed by ketone reduction and dehalogenation gave the required precursor (111).[90] Various cycloaddition reactions of carbapenems have been reported; for instance addition of CH_2N_2 to a protected thienamycin precursor afforded mainly (112) which on thermolysis gave the cyclopropane (113).[91] The deprotected materials were essentially inactive. In a similar vein the 6-ethylidene compounds (114a) and (114b) readily available from natural olivanic acids underwent cycloadditions at the exo-double bond with CH_2N_2 and acetonitrile oxide.[92] Lead tetraacetate oxidation of the 3-(ethylthio)carbapenam (115) gave the 3-acetoxy-3-(ethylthio)derivative which could be transformed to either a 3-acetoxy- or a 3-(ethylthio)carbapenem.[93] In view of their increased enzymic stability, there is growing interest in 1-methylcarbapenems. The resolved (S)-alcohol (116) was converted to the optically active diazoketone (117) and thence to a biologically active 1-methyl-trans-carbapenem.[94] Partial synthetic modifications of thienamycin have been described bearing monothioacetal[95] or cycloalkylthio[96] groups at C(2). A fuller account of a synthesis



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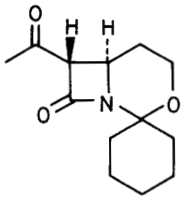

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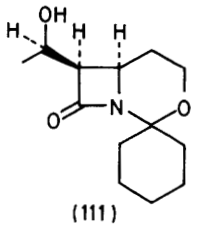
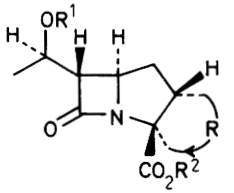
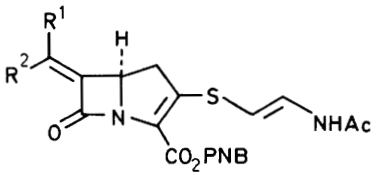


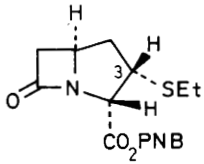
(111)



(112) R = $CH_2N{=}N$
(113) R = CH_2



(114) a; R^1 = H, R^2 = Me
b; R^1 = Me, R^2 = H



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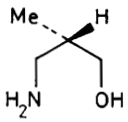

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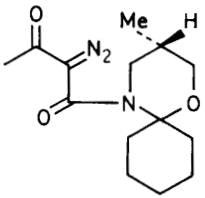

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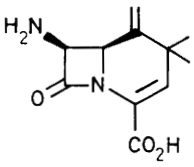

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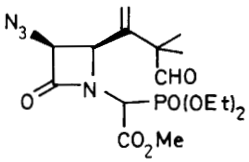

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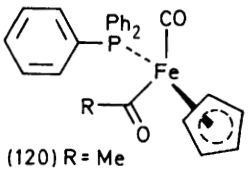
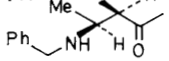
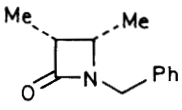


(120) R = Me
(121) R =





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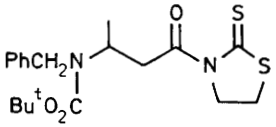
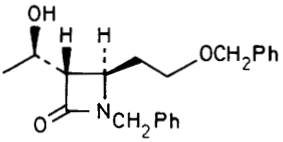


(123)



(124)

of dethiathienamycin has been published.[97]

A synthesis of a (±)-1-methylene-2,2-dimethylcarbacephem (118) has been described; 2,2-dimethyl-4-pentenal was converted into the azetidinone (119) and thence by known methodology to (118).[98]

7. Azetidinones

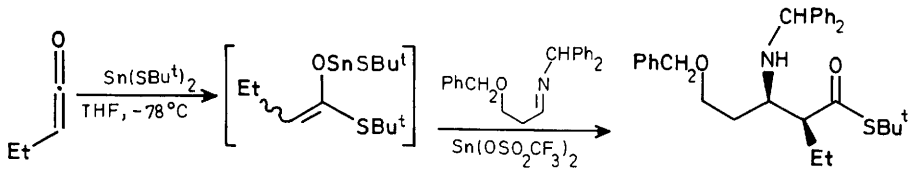
This section includes both synthetic methods for azetidin-2-ones, grouped according to the bond(s) formed in the ring-forming step, and their chemistry.

1-2 bond-forming Reactions

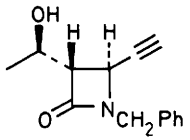
There is continuing interest in the use of chiral iron complexes for the synthesis of β-lactams. For instance, the acyl complex (120) was transformed in five steps into (121) with very high stereocontrol, the nitrogen being introduced as lithium benzylamide; mild oxidation (Br_2, -78°C) delivered the (3R,4S)-(-)-azetidinone (122).[99,100] Other 1-benzyl-2,3-dialkyl analogues were similarly made. Alternatively, β-amino iron acyl complexes were available by addition of the enolate of (120) to nitrones[101] followed by Ti(III) reduction; in this case bicyclic products were also accessible.

Other procedures have employed stereoselective aldol-type reactions to generate defined C(2) and C(3) stereochemistry (*cf.* references 85 and 86 above). For example, the *N*-acylthiazolidine-2-thione (123), after conversion to its tin enolate, underwent reactions with aldehydes in ⩾95% diastereoselectivity. Subsequent ring closure using 2-chloro-1-methylpyridinium iodide afforded the thienamycin precursor (124).[102] Another approach involved generation of a tin (II) thioester enolate by addition to a ketene, Scheme 4; this species added in highly *anti* - selective fashion to an imine.[103] Closure of the azetidinone by mercury (II) trifluoroacetate afforded a PS-5 precursor. Similarly, a boron thioester enolate (*cf.* reference 86) plus imine condensation was used in the construction of (125), a precursor of 1β-methylcarbapenems.[104]

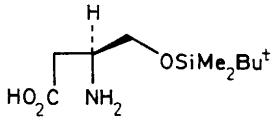
The inherent chirality of amino-acids, particularly aspartic acid, is useful in azetidinone construction. For instance, *N*-benzyloxycarbonylaspartic acid anhydride was converted in three steps to the β-amino-acid (126) and conventionally cyclized



Scheme 4



(125)



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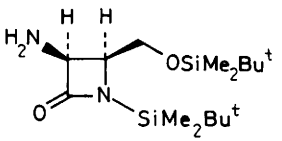

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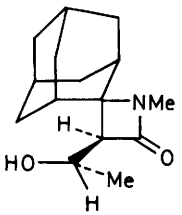

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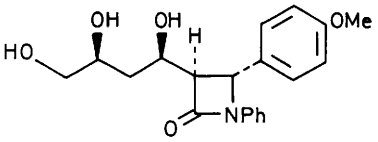

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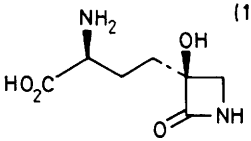

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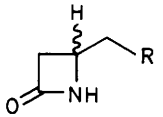
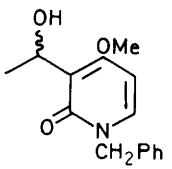
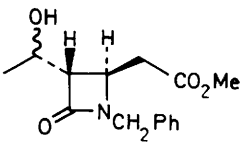


(131) a; R = CH_2OAc
b; R = CO_2CH_2Ph
c; R = $CONR^1R^2$, $COSR^1$, COR^1



(132)



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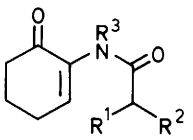

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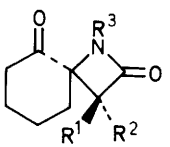

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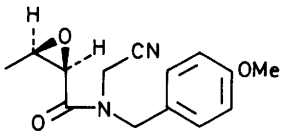

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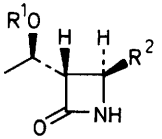
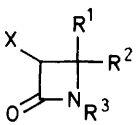


(137) $R^1 = SiMe_2Bu^t$, $R^2 = CH_2NH_2$
(138) $R^1 = H$, $R^2 = SO_2Ph$



(139)

(cf.Scheme 2) to an azetidinone.[105] A 3-amino group was introduced by nitrosation and reduction giving largely the 3,4-cis-stereochemistry (127), as a route to monobactams. Similar methodology was used to convert the anhydride to precursors of epi-PS-5 and carpetimycin.[106] A photo-Wolff rearrangement on an N-benzyloxycarbonyl D-aspartic acid half ester followed by the same cyclization procedure was used to prepare a known thienamycin precursor.[107]

Another well-known procedure for closing the 1-2 bond, the action of a Grignard reagent on a β-aminoester, was used to prepare the spirocyclic azetidinone (128)[108] and the trihydroxy compound (129)[109]. In the latter case 2-chloro-1-methylpyridinium iodide gave a higher cyclization yield; in both syntheses the desired intermediates were generated by cycloaddition of a nitrone to an αβ-unsaturated ester followed by hydrogenolysis. Baldwin had previously reported a synthesis of tabtoxin (see vol.17 in this series) and has now given full details of a synthesis of the derived tabtoxinine β-lactam (130) by a similar route.[110] An N-(1H-tetrazol-5-yl)-azetidin-2-one was obtained by dicyclohexylcarbodi-imide condensation but was biologically inactive.[111]

3-4 bond-forming reactions

Photolytic methods continue to be employed for this mode of ring closure; in particular, the conversion of 2-pyridones to azabicyclo [2.2.0] hexanes. Thus fuller details have appeared on the synthesis of azetidinones (131a), (131b) and (131c) by this route.[112,113] Similarly, the racemic 3-(1-hydroxyethyl)-4-methoxy-2-pyridone (132) was converted by photolysis, acidification and methanolysis into the carbapenem precursor (133) in 35% overall yield.[113] Enantioselection could be achieved on irradiation of a 4-menthoxy-2-pyridone; the intermediate bicyclic diastereoisomers were separable and thus the individual chiral methyl esters corresponding to (131b) were obtained.[114] Further investigation of the photolytic conversion of the enamides (134) to spiro-β-lactams (135) has shown that R^1 and R^2 should not both be hydrogen and that the corresponding cyclopent-2-enones also work[115], but with loss of diastereoselection.

Two reports have appeared on the use of epoxides for this step. In one, (2R,3R)-potassium 2,3-epoxybutyrate was converted to

(136) which was cyclized using lithium hexamethyldisilazide; further steps gave (137).[116] In another, L-threonine was used as the chiral source and a sulphone-stabilized carbanion employed, leading to (138).[117] Electrolysis of various haloacetamides led to β-lactams of type (139) in good yield.[118]

4-1 bond-forming reactions

All the reactions reported involved displacement of a C-4 leaving group by N-1, using intramolecular alkylation or the Mitsunobu reaction. Thus a stereocontrolled addition of an imide boron enolate (*cf.* reference 86) to acetaldehyde led after further transformations to the mesylate (140) which was cyclised to the azetidinone using potassium carbonate in 94% yield.[119] Diastereoselective aldol reactions also featured in the synthesis of intermediates for β-lactams (*R*)-(141) and (142), employing a β-silylenolate in the latter case.[120,121]

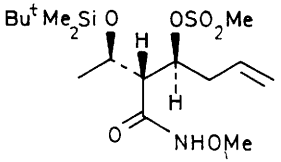
Treatment of the *N*-benzylhydroxamate of 3-bromo-2-bromomethylpropanoic acid with NaH (two equivalents) gave the 3-methylene-2-azetidinone (143) which was used in an alternative route to (130).[110] *N*-Monosubstituted 2-(bromomethyl)propenamides were cyclized to similar compounds.[122] The tartaric acid-derived intermediates (144) were efficiently cyclized only *via* the tosylates.[123] Treatment of *O*-sulphonate (145) with base gave a 2:1 mixture of azetidinones (146) and (147); the latter was formed by 1,2-acyl migration (Scheme 5).[124,125] Deprotection and reacylation of (146) gave potent antibacterial agents. Total syntheses of (-)-nocardicins A and G were achieved *via* t-butyl 3-aminonocardicinate (148) which was obtained using Mitsunobu cyclization.[126] The same cyclization was used to prepare a series of *N*-(1H-tetrazol-5-yl)-monobactams having good antibacterial activity [127,128]; the simpler *N*-(1H-tetrazol-5-yl)-azetidin-2-one mentioned earlier[111] was also accessible through mesylate alkylation of N-1.

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 1-4 bond formation

Only one example of this class has been reported: treatment of



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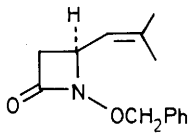

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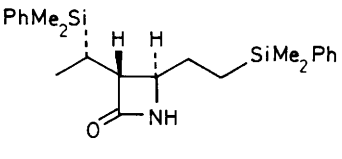

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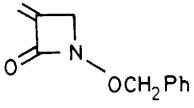

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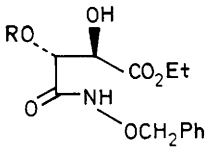

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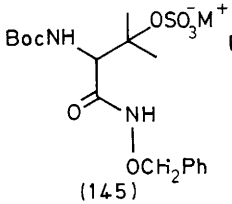

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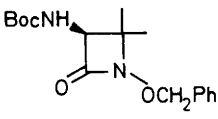

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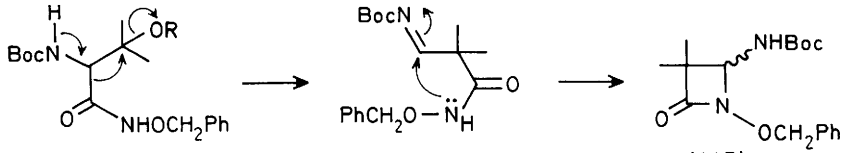
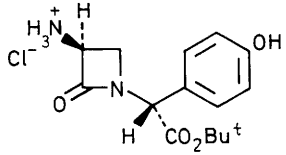


Scheme 5

(147)



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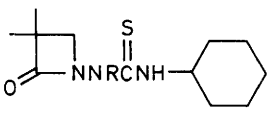
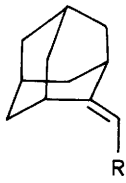
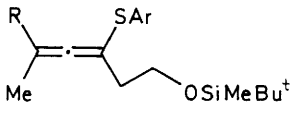


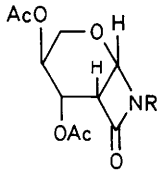
(149) R = H, Me



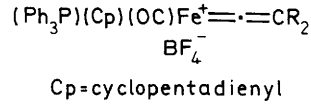
(150) R = Aryl



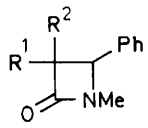
(151) R = Me
(152) R = Me_3Si



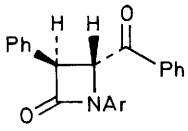
(153)



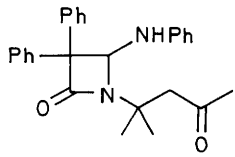
(154) a; R = H
b; R = Me



(155) a; R^1, R^2 = =CHPh
b; $R^1 = R^2$ = Me



(156)



(157)

β-chloropivaloyl chloride with 3-(cyclohexylthio)semicarbazides gave the β-lactams (149).[129]

[2+2] Additions

2-3 and 4-1 bond formation

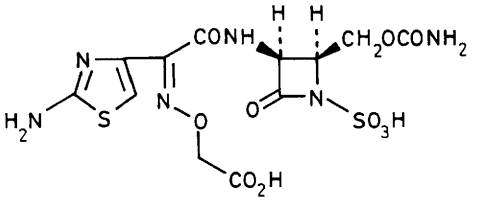
All the examples of this class involved addition of an isocyanate to a double bond. Thus addition of chlorosulphonyl isocyanate (CSI) to olefins (150) gave spiro β-lactams similar to (128); the regiochemistry depended on the aryl substituent.[108] CSI addition to allenyl sulphides (151) and (152) was used to prepare, respectively, a (±)-carpetimycin A precursor[130] and the thienamycin precursor (110).[131] The high-pressure addition of sulphonyl and acyl isocyanates to glycals has been further investigated; single enantiomers of β-lactams such as (153) are available.[132,133] Simple alkyl and aryl isocyanates are unreactive.

1-2 and 3-4 bond formation

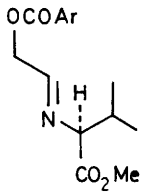
Many examples of this class have again featured ketene-imine or enolate-imine condensations; these will be mentioned in detail only where new features of chemistry or stereocontrol were present. A rather different approach was the addition of iron (II) vinylidenes (154a) and (154b) to imines, generating complexes which were oxidised to the azetidinones (155a) and (155b).[134] A photolytic [2+2] cycloaddition with a quinoxalin-2-one was used to obtain a 4-(trifluoromethyl)azetidin-2-one.[135] Addition of a metal acetylide to nitrones afforded the trans- 4-benzoyl analogues (156).[136] Variations on the imine portion were seen in the addition of ester enolates to nitriles[137] and to sulphenimines[138]; in the latter case the bulky S-trityl sulphenimines gave better yields.

Three reports featured the use of partially reduced pyrimidines as the imine portion; in two cases the expected fused bicyclic structures were obtained[139,140] but a 1,4-dihydropyrimidine was subsequently ring-opened to (157).[141]

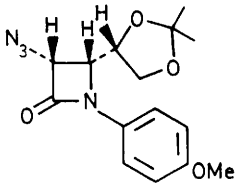
Stereocontrol is frequently effected by appropriate substitution of the imine. Thus a synthesis of the monobactam antibiotic carumonam (158) employed the imine (159) derived from L-valine; addition of a protected glycine mixed anhydride gave the desired 3,4-cis stereochemistry and the desired diastereoisomer was separated. The N-substituent was later removed by electrolysis.[142] Addition of diketene to chiral α-substituted imines was used to



(158)



(159)



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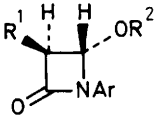

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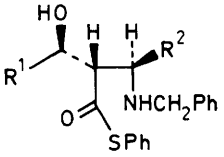
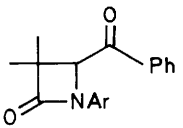
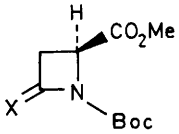


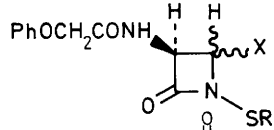
(162)



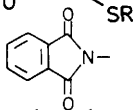
(163)



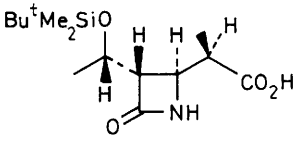
(164) a; X = H_2
b; X = O



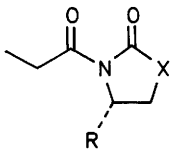
(165) a; R =



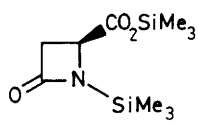
b; R = OR^1, NR^1, SO_3^- *etc.*



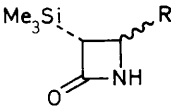
(166)



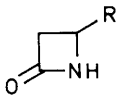
(167) R = Pr^i, X = O
(168) R = Et or Pr^i, X = S



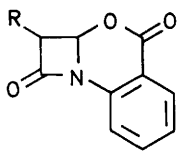
(169)



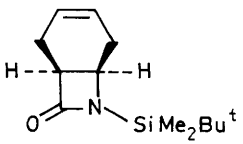
(170) a; R = β−CO_2H
b; R = α,β−OAc



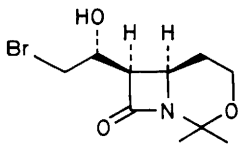
(171) a; R = $CH_2C{\equiv}CH$
b; R = CH_2CO_2Et
c; R = $CH_2COCH_2CO_2Me$



(172)



(173)



(174)

prepare 3-(1-hydroxyethyl)azetidin-2-one intermediates with a 3,4-trans-relationship[143,144] for carbapenem synthesis. In a similar way the β-lactam (160) was obtained from addition of the imine of D-glyceraldehyde acetonide to azidoketene and its absolute configuration ascertained as (3R,4R).[145] Cinnamaldimines were already known to give predominantly cis- azetidinones; using 2-methylcinnamaldimines, a series of β-lactams was obtained with at least 90% of the 3,4-cis isomers.[146] Ozonlysis of the products followed by Baeyer-Villiger oxidation gave 4-acetoxyazetidinones. By contrast, aryl formimidates gave almost exclusively the trans products (161).[147] Ketene addition to monoimines of benzil gave exclusively cis-4-benzoyl-β-lactams which were isomerized to the trans-isomers in varying degrees by base.[148]

The enolate-imine condensation may be used in a one or two step mode; cf. references 103 and 104. Normally the lithium enolates of chiral 3-hydroxybutanoates condense with imines in a one-step mode to give 3,4-cis-β-lactams. Using a boron thioester enolate, however, trans-products could be preferentially obtained (4:1 or better)[149]; the intermediates (162) were not directly cyclized but first hydrolysed then cyclized by Ohno's procedure. Reaction of N-(α-methoxyphenacyl)anilines with methyl lithioisobutyrate afforded, among other products, the azetidinone (163).[150]

Other derivatives prepared by these methods include a series of N-(2-phenyl-2-hydroxyethyl)azetidinones[151], various monobactams exhibiting some β-lactamase inhibition[152,153], further monobactams [154] and simpler azetidinones.[155]

Chemistry of azetidinones

A novel azetidinone synthesis involved the ruthenium tetroxide oxidation of azetidine (164a), ultimately derived from L-methionine: the β-lactam (164b) was obtained in 73% yield.[156]

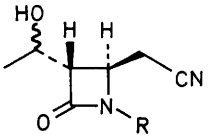
Various methods of N-substitution of azetidinones have appeared; thus Miller has published full details of his synthesis of thiamazins[157] (see vol.18). Treatment of 1-unsubstituted azetidinones with bis(phthalimido)sulphide gave N-thiophthalimido derivatives (165a); reaction with oxygen, nitrogen, sulphur and carbon nucleophiles then gave various N-(substitutedthio) compounds (165b).[158] Reaction with 1-benzyl-5-fluoro-1H-tetrazole afforded a series of N-(tetrazol-5-yl)azetidin-2-ones[159], and procedures for N-acylation[160] and electrochemical N-alkylation [161]

have also appeared.

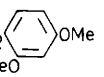
Also important are procedures for transformations at the 4-position, in particular by reaction of 4-acetoxyazetidinones with various nucleophiles. For example, Lewis acid mediated condensation of the appropriate 4-acetoxy precursor with a boron enolate of a carboximide (cf. reference 86) afforded the 1β-methylcarbapenem precursor (166) in 73% yield with 99% diastereoselection.[162] Similarly, reaction of 4-acetoxyazetidin-2-one itself with the boron enolate of the chiral amide (167) or the tin enolate of (168) proceeded with at least 90% diastereoselection.[162,163] In the latter case introduction of a C-3 acetyl group followed by reduction gave an intermediate very similar to (166). Another synthesis of (166) by an intermediate very similar to (168) has been published.[164] Lewis acid mediated reaction of (3S,4S)-4-acetoxy-3-phenylacetamido-2-azetidinones with some heterocyclic nucleophiles gave 4-substituted analogues of low antibacterial activity.[165] Treatment of the N, O-bistrimethylsilyl compound (169) afforded acid (170a); lead tetraacetate oxidation then gave a mixture of 4-acetoxy compounds (170b) which could be transformed to other 3-trimethylsilyl-4-substituted derivatives.[166] Other groups may be displaced; thus 4-(phenylsulphonyl)azetidin-2-one with a propargyl Grignard reagent afforded (171a) in 87% yield[167]; further transformations gave the carbapenem intermediates (171b) and (171c) via, respectively, alkenylstannane and β-phenylthio ester derivatives.[167,168] A 4-(methylthio)azetidinone was transformed into the bicyclic system (172).[169] Direct 4-benzoyloxylation of 4-unsubstituted azetidinones with t-butyl perbenzoate and a copper catalyst proceeded in up to 59% yield.[170]

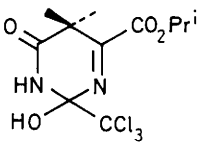
Oxidation of optically active (173) gave a dialdehyde which underwent a regiospecific aldol condensation; further transformations led to (174), a precursor of 5,6-cis-carbapenems.[171] Hydrolyses of nitriles (175a) and (175b) led to Melillo's lactone, a thienamycin intermediate.[172] Oxidation of some 3-vinylazetidin-2-ones using $PdCl_2$-CuCl-O_2 gave very largely the 3-formylmethyl products.[173]

The rearrangement of various 4-carboalkoxy-N-hydroxy-2-azetidinones available by the Mitsunobu reaction was studied[174]; 3-unsubstituted analogues with a carbodiimide gave acrylamides, while a 3,3-dimethyl analogue on treatment with trichloroacetonitrile and base gave the pyrimidine (176). Treatment of N-hydroxy-β-lactams with diethyl bromomalonate and base gave carbinolamines (177); the [1,2] anionic rearrangement shown (Scheme 6) was proposed.



(175) a; R = CH_2Ph

b; R = CH_2 



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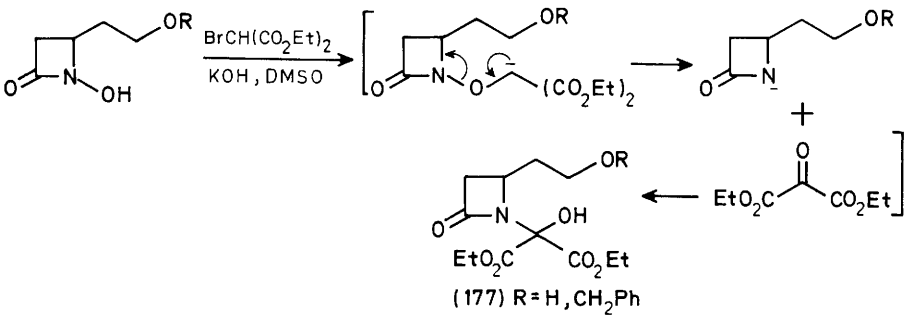
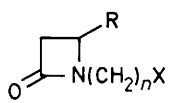
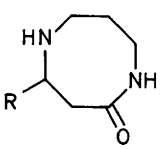


(177) R = H, CH_2Ph

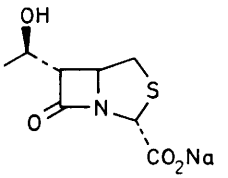
Scheme 6



(178) X = Br or Cl



(179)



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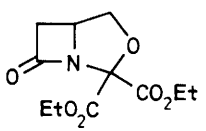
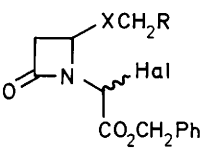


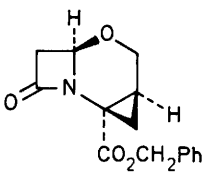
(181)



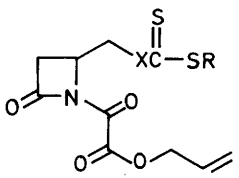
(182) a; X = O, R = $CH{=}CH_2$

b; X = O, R = $C{\equiv}CH$

c; X = CH_2, R = $CH{=}CH_2$

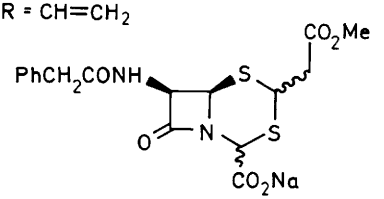


(183)



(184) a; X = S

b; X = O



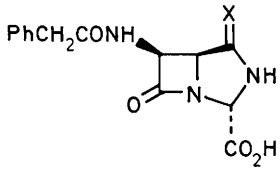
(185)

[175] Hydrolysis of two (spiro-adamantyl)azetidin-2-ones[108] was used to prepare β-alanines difficult of access.[176] Catalytic reduction of 3,4-disubstituted homochiral β-lactams with D_2 provided a series of labelled homochiral peptides by cleavage of the 1-4 bond.[177] N-(Halogenoalkyl)azetidin-2-ones (178) have been used as intermediates in the synthesis of various Homalium alkaloids bearing two 1,5-diazacyclooctane rings [178] and of (±)-dihydroperiphylline, having a 13-membered ring[179]; thus ammonolysis of (178) (n=3) readily gave (179).

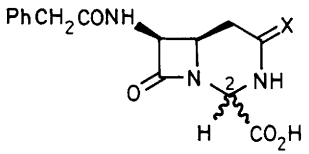
8 Major Structural Variants

This section includes both β-lactam derivatives not described earlier and other systems in which the β-lactam has been replaced by a grouping designed to exhibit similar properties.

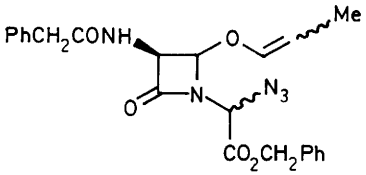
The 4-(iodomethyl)azetidin-2-one(101) was also transformed, in five steps, into the isopenam(180) which had very little antibacterial activity.[84] Mitsunobu closure of diol (177), R=H, gave a 3-oxacepham system, and the isoxapenam (181) was obtained by a similar sequence.[175] Palladium catalysed cyclization of the halo-compounds (182a-c) gave, after elimination of hydrogen halide, a variety of oxacephams, homooxacephams and carbacephams, bearing fused cyclopropanes or double bonds, eg. (183) from (182a).[180] 3-(Alkylthio)isocephems and -iso-oxacephems were available by cyclization of (184a) and (184b) with triethyl phosphite; the corresponding cis-7-acylaminoanalogues were also prepared but were less active than the related 'natural' cephalosporins.[181] The 3-thiacepham (185) was prepared by a modification of a previous synthesis of the 7-unsubstituted analogue but had weak antibacterial activity.[182] A series of azapenam and -cepham derivatives was prepared using the previously described intramolecular cycloaddition of olefinic azides, in both the 6(7)-unsubstituted and 6(7)-acylamino series. The 6(7)-unsubstituted compounds were inactive; the 2-azapenams (186a) and (186b) were similarly inactive but the 3-azacephams (187 a-c) possessed weak activity against Gram-positive bacteria.[183] Related azapenem and azacephem analogues were inactive. Similarly, the penicillin V-derived olefinic azide (188) was converted to an antibacterially inactive 1-oxa-3-azaceph-2-em.[184] The 1-selenapenem (189) was obtained in two steps from azetidinone (190); it was four-fold less active than the sulphur compound; a similar intermediate was converted



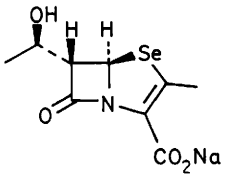
(186) a; X = O
b; X = $CHCO_2Me$



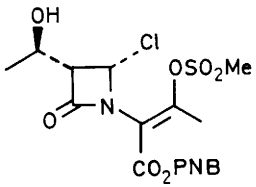
(187) a; X = $CHCO_2Me$, C-2 epimers
b; X = O, α − CO_2H
c; X = O, β − CO_2H



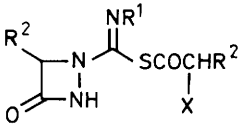
(188)



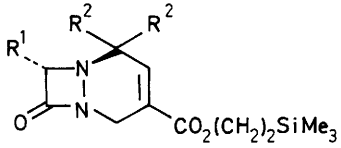
(189)



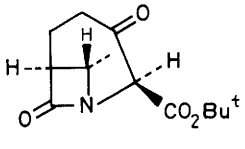
(190)



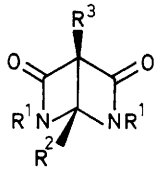
(191)



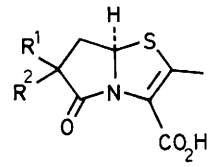
(192) a; $R^1 = R^2 = Me$
b; $R^1 = H$, $R^2 = Me$
c; $R^1 = Me$, $R^2 = H$



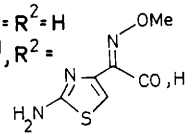
(193)



(194)



(195) a; $R^1 = R^2 = H$
b; $R^1, R^2 =$



to a 2-selenacephem which gave the undesired 5-epimer on ring contraction.[185]

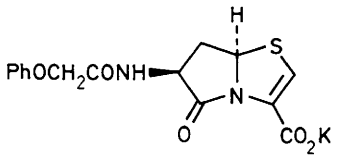
A number of reports have appeared on 1,2-diazetidin-3-ones. Several analogues of type (191) were prepared by the action of semicarbazides on α-haloacyl halides.[129] 1,2-Diazetidin-3-ones substituted at N-1 were readily alkylated at N-2, but attempted elaboration of the products to bicyclic β-lactams by eventual aldol condensation failed.[186] However, the 6-aza-1-carbaceph-2-ems (192a-c) were accessible *via* intramolecular Horner-Emmons reaction.[187] Reaction of 1-benzhydryl - 1,2-azetidin-3-ones with arylsulphonyl isocyanates gave 3-carboxamides of no antibacterial but slight antifungal activity.[188]

The novel 'anti-Bredt' 1,3-bridged β-lactam (193) was accessible from a 3,4-disubstituted azetidin-2-one by eventual carbene insertion into the NH bond.[189] Analogous to the photoconversion of 2-pyridones to β-lactams[112-114], photolysis of pyrimidinium-4-olates was found to generate bis (β-lactams) of the type (194).[190]

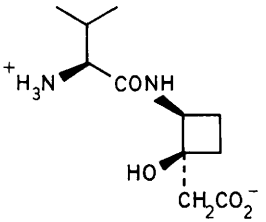
Analogues in which a γ-lactam replaces the β-lactam continue to be described. Full details of the synthesis of three γ-lactam analogues of carbapenicillanic acids have appeared (see vol.18).[191] Two groups have prepared γ-lactam analogues of penems, in either the 2-Me [(195a) and (195b)][192] or 2-H series(196).[193] Both (195b) and (196) showed slight antibacterial activity. Some γ-lactam carbapenem analogues very closely related to (195b) were prepared, one showing slight antibacterial activity.[194] An interesting carbacyclic system which exhibited some biological activity was the cyclobutanol (197) synthesised from an azetidinone precursor[195], and another synthesis of a carbocyclic analogue of a 1-oxapenam has appeared.[196]

9 Mechanistic Studies on Mode of Action and Degradation

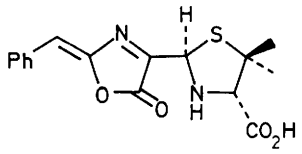
Although an oxazolone-thiazolidine intermediate is almost universally accepted as the first stage in acid-catalysed penicillin degradation, such molecules are usually too unstable to be isolated. However, from α-bromopenicillin G the stable oxazolone (198) was isolable on acidolysis; it probably arose by loss of HBr from the hypothetical intermediate.[197] N-Formyl-D-penicillamine (199) was formerly regarded as a minor aqueous degradation product of penicillins, but it has now been shown to be formed from some 6-acylaminopenicillins in up to 46% yield



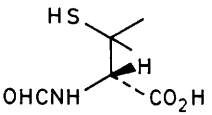
(196)



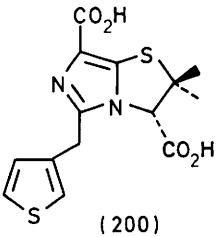
(197)



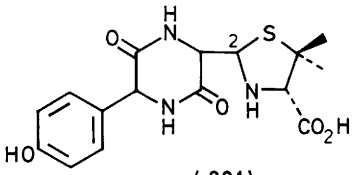
(198)



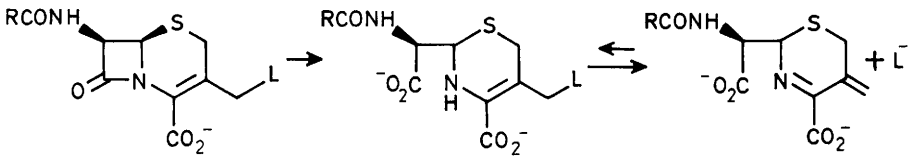
(199)



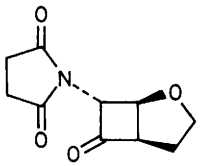
(200)



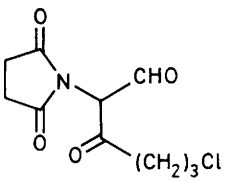
(201)



Scheme 7



(202)



(203)

(2% aqueous solution, 7 days, initial pH5-7).[198] From α-amino and α-ureidopenicillins it was formed in less than 1% yield. Temocillin, a 6α-methoxy-α-carboxypenicillin, was degraded mainly to the penillic acid in aqueous acid; loss of methanol could then occur to give the fused imidazole (200).[199] The 6α-substituent doubtless blocked the 'normal' formation of penicilloic acid. Amoxycillin piperazine-2,5-dione (201), a known metabolite of amoxycillin *in vivo*, was found to epimerise at C-2, more readily at low pH[200]; similar behaviour is well known for penicilloates.

Two studies have further examined the chemical and enzymic hydrolyses of cephalosporins bearing a 3' leaving group, Scheme 7. The enamine is now firmly established as a discrete intermediate, the leaving group being subsequently expelled in a non-enzyme catalyzed step[201]; indeed addition of thiols at pH7 to the imine regenerates the enamine.[202] The position of the equilibrium is dependent on the pKa of the thiol as well as pH. As expected, poorer leaving groups (*eg.* Scheme 7, L=OH) underwent the second step much more slowly, subject to acid-base catalysis; better leaving groups (*eg.* L=pyridinium) gave the imine even at pH7. A detailed report of the products and kinetics of carumonam (158) degradation have appeared.[203] The use of ^{13}C-labelled acetate has shown that the hydrolysis of cephalothin proceeds by both acyl- and alkyl-oxygen fission in the 3'-acetate group.[204]

Cyclobutanone analogues of β-lactams are considered capable of inhibiting transpeptidase or β-lactamase enzymes; in a model study, (202)[196] was readily cleaved by acid to (203).

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

This appendix includes a number of semisynthetic β-lactams, prepared by methods involving no new chemistry of the β-lactam nucleus, and with biological activity the chief goal. It is hoped that this appendix will increase the usefulness of the chapter to practising chemists in the area, particularly in the pharmaceutical industry. Inevitably the distinction between compounds listed here and those mentioned in preceding sections may seem arbitrary in some cases.

β-Lactam	Ref
6α-Formamido penicillins	205
6α-Methoxy sulphopenicillins	206
A series of 6α-methoxy ureidopenicillins	207
Some pyrazolinone penicillins	208
Ureidopenicillins bearing catechol groups	209,210
A novel biphenyl-ureidopenicillin	211
Sodium 6-[[3'-methyl-5'-(o-nitrophenyl)-isoxazol-4-yl]carboxamido] penicillanate	212
Two heterocyclyl-amino ampicillin analogues	213
7α-Formamido cephalosporins and their sulphoxides and 7α-formamido oxaceph-3-ems	214,215
Some 3-vinylceph-3-em analogues	216,217
A series of (3'-quaternary ammonio) cephalosporins	218
Various 3-substituted (2-aminothiazol-4-yl)ceph-3-em analogues	219
Some (3'-isothiazolyl) cephalosporins	220
Dipeptidyl cephalosporins	221
Further aminothiazolyl cephalosporins and monobactams	222, 223,224
Aminooxazolyl and aminooxadiazolyl cephalosporins	225,226
Furoylureidocephalosporin sulphoxides and sulphones	227
Twelve 7-substituted 7-amino-3-methyl-ceph-3-em analogues	228
Some (6-substituted-3-formamido)coumarinyl cephalosporins	229
N-(1H-Tetrazol-5-yl) monobactams	230
Novel aminoheterocyclic monobactams	231

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6
Metal Complexes of Amino Acids and Peptides

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

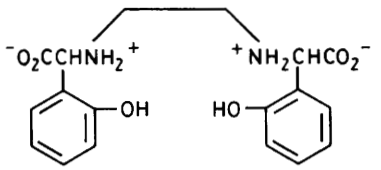
1 Introduction

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 1986. The formation, properties and structures of metal complexes with thioether-containing amino acid or peptide ligands have been the subjects of two reviews.[1,2] Other relevant books, reviews or dissertations deal with the biochemistry of chromium in relation to the glucose tolerance factor,[3] hydrogen bonding in metal-aminocarboxylate complexes investigated by X-ray diffraction,[4] circularly polarised luminescence studies of lanthanide amino-polycarboxylate complexes,[5] ^{17}O and ^{14}N nuclear magnetic resonance studies of paramagnetic metal amino acid and peptide complexes,[6] isomer discrimination in the reactions of cobalt complexes with amino acids,[7] and the chemical, biochemical and medical aspects of vitamin B_6 pyridoxal phosphate.[8]

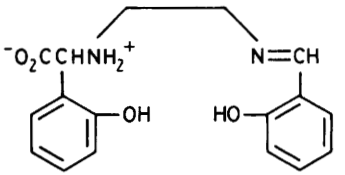
2 Amino Acids

Solid State Studies.- As in the previous year there have been many reports in the 1986 literature describing the synthesis, structures and properties of metal-amino acid complexes. The greater availability of X-ray diffraction techniques is reflected in the increased number of structures reported, particularly in the case of copper(II) complexes.

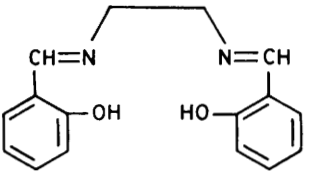
Metals of the First-Row d-Block.- The ligand ethylenebis(o-hydroxyphenylglycine), (1), abbreviated H_4-EHPG, has previously been studied as a model for the metal binding site of the iron transport protein human serum transferrin. In view of the fact that vanadium has been used as an e.s.r. probe for transferrin and that this protein may bind vanadium under conditions of vanadium toxicity it was relevant to investigate complexes of this metal with the model ligand. The vanadium(IV) complexes $NH_4[VO(H\text{-}EHPG)].H_2O.EtOH$ and


$^{-}O_2CCHNH_2^{+}$
OH
$^{+}NH_2CHCO_2^{-}$
HO

(1)


$^{-}O_2CCHNH_2^{+}$
OH
N=CH
HO

(2)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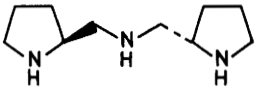
N=CH
HO

(3)


N
H
N
H
N
H

(4)


N
CONHCH$_2$CO$_2$H

(5)

$VO(H_2\text{-EHPG}).3\frac{1}{2}H_2O$ and the vanadium(V) complexes $Na_3[VO_2(EHPG)].3H_2O$ and $VO(H\text{-EHPG}).H_2O$ have therefore been isolated and characterised using nuclear magnetic resonance, uv-visible, e.s.r. and i.r. spectroscopy.[9] During the course of this work it was observed that vanadium(V) promoted a stepwise oxidative decarboxylation of H_4-EHPG to give oxovanadium(IV) complexes of the ligands N-[2-(o-salicylideneamine)ethyl](o-hydroxyphenyl)glycine, H_3-EHGS (2), and N,N^1-disalicylideneethylenediamine, H_2-salen (3). A mechanism for this reaction is proposed and the oxovanadium(IV) complexes $Na[VO(EHGS)].1\frac{1}{2}H_2O.CH_3OH$ and VO(salen) as well as the oxovanadium(V) complex $VO(EHGS).1H_2O$ have all been isolated and characterised. The X-ray crystal structures of some of the above complexes have been determined and the results of these (and i.r.) studies are summarised in Table 1 which also includes corresponding data for the catecholate complex $[VO(cat)_2]^{2-}$ for comparison.[10]

Table 1 Structural details for vanadyl complexes

Complex	Metal Coordination Sphere	Ligand Donor Atoms	$\nu(V{=}O)$/ cm^{-1}	V=O/ Å	V-O$^-$/ Å
$[VO(H\text{-EHPG})]^-$	Distorted octahedral	$2N,2CO_2^-,O^-$	948	1.607	1.950
$[VO(EHGS)]^-$	Distorted octahedral	$2N,CO_2^-,2O^-$	952	1.606	1.946
VO(salen)	Square pyramidal	$2N,2O^-$	981	1.88	1.922
$[VO(cat)_2]^{2-}$	Square pyramidal	$4O^-$	921	1.616	1.956

The vanadium(III) complexes $V(L\text{-Ser})_2Cl.2H_2O$ and $V(L)(HL)Cl_2.xH_2O$ (HL ▫ L-Thr, L-Leu; x = 2 or 3) have been prepared and their structures proposed on the basis of i.r., electronic and c.d. spectra, magnetic susceptibility and thermogravimetric measurements.[11] A number of complexes of the type $VOL_2.mH_2O$ (HL = picolinic acid N-oxide, 4-hydroxy-7-trifluoromethyl-3-quinolinecarboxylic acid, quinaldic acid, 2-pyrazinecarboxylic acid; m = 0, 2) have been prepared and their i.r. spectra and magnetic susceptibilities reported.[12] Series of deuterated (C-2 labelled) amino acid complexes of formulae $[Cr(en)_2L]S_2O_6.xH_2O$ (en ▫ 1,2-diaminoethane; L ▫ Gly, DL-Ala, L-Ser, DL-Thr, DL-Hse, L-Leu, DL-Met, DL-Phe; x = 0,1), $[Cr(1,3\text{-pn})_2L']S_2O_6.yH_2O$ (pn = propanediamine; L' ▫ Gly, DL-Ala;

y = 1 and 0.5), cis-α-[Cr(edda)Gly].$2H_2O$ (edda = ethylenediamine diacetate) and $[Cr(NH_3)_5L^2](ClO_4)_3$ (L^2 ▫ Gly, Ala) have been prepared and studied by ^2H nuclear magnetic resonance spectroscopy.[13] The range of isotropic shifts in these spectra shows that ^2H nuclear magnetic resonance is very sensitive to environmental changes and the decomposition of the bidentate amino acid complexes via monodentate species has been studied by this technique. The crystal structure of the complex cis-α-[Cr(edda)-Gly].$2H_2O$ has also been reported. The complexes $Cr(L\text{-}Asp)_2.3H_2O$ and $Cr(L\text{-}Ala)_3.H_2O$ have been separated into geometric isomers by cation exchange chromatography.[14]

Octahedral complexes of iron(III) containing phthalimide as primary ligand and the amino acids Met, Glu, Cys, Arg, His and anthranilic acid as secondary ligands have been prepared and examined by a variety of spectroscopic (electronic, i.r., Mössbauer) and other (conductometric, magnetic) techniques.[15]

All four optical isomers of the complex $[Co(Sar)Hbg]^{2+}$ (Hbg ▫ biguanide) have been isolated and characterised by their electronic, c.d. and ^1H nuclear magnetic resonance spectra.[16] Rates of racemization at both the cobalt(III) (Δ ⇌ Λ) and at the asymmetric nitrogen (R ⇌ S) centres in these isomers have been investigated. There are five possible geometrical isomers of the complex ion $[Co(NO_2)_2(Gly)_2]^-$. Two of these isomers, the cis$(NO_2)_2$-transNN and the cis$(NO_2)_2$-cisNN have been isolated as K^+ salts and the latter optically resolved.[17] The X-ray crystal structure as well as the electronic and c.d. spectra of the $K(-)^{c.d.}_{450}$-$[Co(NO_2)_2(Gly)_2]0.5H_2O$ isomer have been reported and the absolute configuration determined to be Δ(R).

In order to investigate the selective exchange of the methylene protons of a glycinate ligand, the complex [Co(S,S-proam)picgly]Cl (S,S-proam ▫ S,S-bis(2-pyrrolidinylmethyl)-amine, (4); picgly = N-(2-picolinoyl)glycine, (5)) which contains S,S-proam as a chiral auxiliary to direct attack by OH^- preferentially on one of the glycinate methylene protons was synthesised.[18] The crystal structure of one of the geometrical isomers of [Co(S,S-proam)picgly]Cl.H_2O in which the central H atom of the proam ligand points towards the pyridine ring (as opposed to the CO_2^- group) of the picgly ligand has been determined.

The complexes $(+)_{577}$-$[Co(AMM)(Me_2\text{-}2,3,2\text{-}tet)]ClO_4.H_2O$ and $(-)_{546}$-$[Co(AMM)(Me_4\text{-}2,3,2\text{-}tet)]Br.3H_2O$ (AMM = α-amino-α-methyl-

malonate; Me_2-2,3,2-tet ▫ 4R,6R-dimethyl-1,9-diamino-3,7-diazanonane; Me_4-2,3,2-tet = 6R,8R-dimethyl-2,5,9,12-tetraazatridecane) undergo decarboxylation under acidic conditions to give complex mixtures containing R-Ala and S-Ala ligands in ratios of 34:66 and 83:17 respectively.[19] In an effort to account for the difference in stereoselectivity on decarboxylation the detailed stereochemistries of the AMM complexes have been established by X-ray crystallographic studies. The results suggest that the stereoselectivity arises not from the coordination geometry of the AMM ligand but rather from steric influences of the tetramine ligand. Furthermore the complexes [Co(R- or S-Ala)(Me_4-2,3,2-tet)]$^{2+}$ are remarkably labile and release the amino acid ligands with retention of the asymmetric carbon centres under very mild basic conditions. An X-ray crystal structure of the complex $(-)_{546}$-[Co(R-Ala)(Me_4-2,3,2-tet)]$Br_2.3H_2O$ however, shows that the very high lability of the complex is not due to any unusual stereochemical features associated with the alanine ligand.[20] Three complexes of formulae [CoL]X (L = S,S'-ethylenebis-L-homocysteinate, X = Br or ClO_4; L = S,S'-trimethylenebis-L-homocysteinate, X ▫ ClO_4) and [CoL'$_2$]Br (L' ▫ L-ethioninate) have been prepared and separated into geometrical isomers by Sephadex G-10 chromatography.[21] Electronic, c.d. and ^{13}C nuclear magnetic resonance spectra are reported for all the isomers.

Some optically active cobalt(III) complexes, [Co(L)X]$^{2/3+}$, containing 1,4,8,11-tetraazacyclotetradecane(L) and the bidentate ligands R-propylenediamine, S-Ala or acetylacetonate (X) have been prepared and separated into isomers by column chromatography.[22] Enantiomers or diastereoisomers of the complexes CoL_3 (L ▫ Gly, D-Ala, L-Ala, β-Ala) have been separated on anion exchange resins in the Sb-D-tartrate form and the crystal structure of mer-Δ-Co(β-Ala)$_3$ isolated in this way has been determined by X-ray diffraction.[23] Cobalt(III) complexes of Gln, Asn, Glu, Asp and Pro have been prepared by peroxide oxidation of the cobalt(II) complexes.[24]

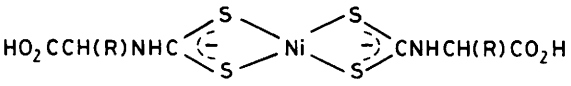
A number of square planar nickel(II) complexes with dithiocarbamate derivatives of amino acids have been prepared according to the reaction in equation (1).[25] On the basis of spectroscopic (electronic, i.r., ^1H nuclear magnetic resonance)

$$Ba(S_2CNHCHRCO_2) + NiCl_2 \longrightarrow Ni(S_2CNHCHRCO_2)_2 + BaCl_2 \quad (1)$$

data these complexes are assigned structures in which the ligands are coordinated to the metal through their sulphur atoms (6). The crystal and molecular structure of $Ba(S_2CNHCH_2CO_2).3H_2O$ has also been reported. The reaction of $Ni(\beta\text{-Ala})_2(H_2O)_2$ with glucose-type aldoses in methanol leads to products containing N-glycoside ligands.[26] On the basis of magnetic moments, electronic absorption and c.d. data these complexes are assigned octahedral structures in which the ligands behave as tridentate N,2O(OH, CO_2^-) donors. The synthesis and reflectance spectra of the complexes $M(Gly)HL.nH_2O$ (M = Ni, Co; HL = gluconic and pangamic acid; n = 2 or 3) have been reported.[27]

The crystal and molecular structure of the complex $Cu(SMC)_2$ (SMC = S-methyl-L-cysteinate) has been determined.[28] The structure consists of trans square planar $Cu(N\text{-}O)_2$ units which are linked by weak Cu-O bonds to give a two dimensional polymer. The reaction of CuO with L-lysine in boiling water results in oxidation of the amino acid, followed by cyclisation leading to the product bis(3,4,5,6-tetrahydropicolinate)copper(II) (7).[29] The crystal structure of the complex octahydrate has been determined. The geometry about the copper is a (4 + 2)-elongated octahedron which contains two centrosymmetrically related bidentate ligands and two water molecules at relatively long distances from the metal cation. The complexes $[Cu(Lys)_2](HgI_3)_2$, prepared using both L- and DL-Lys as starting materials, have been reinvestigated by chiral gas chromatography and a previous report claiming spontaneous resolution during precipitation of the complex of DL-Lys has been refuted.[30] The crystal and molecular structure of the complex $Cu(L\text{-}Arg)_2(CH_3CO_2)_2.3H_2O$ has been reported.[31] The simultaneous dehydration and dimerisation of $[Cu(Ac\text{-}\beta\text{-}Ala)_2].2H_2O$ (blue) has been studied by isothermal thermogravimetry and the results interpreted to indicate a two stage loss of water, with dimerisation being concurrent with loss of the second water molecule.[32] The crystal structure of the complex $[Cu_2(\beta\text{-}Ala)_2(dpp)_2].2dpp.2H_2O$ (dpp = diphenyl-phosphate) which contains carboxylato-bridged dimeric units[33] and of $[Cu(H_2O)_6].2dpp.2Gly$, which contains uncoordinated Gly[34] have been reported.

The crystal structure of Cu(L-Asp)(1,10-phen).$4H_2O$ shows that the complex consists of mononuclear, elongated, rhomic, octahedral units in which the four closest ligand atoms are O ($\alpha\text{-}CO_2^-$) and 3N (Asp,phen) with the $\beta\text{-}CO_2^-$ group and a water



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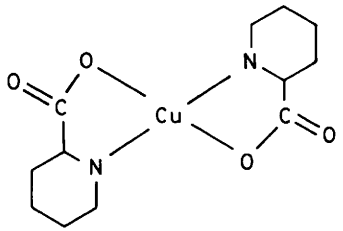

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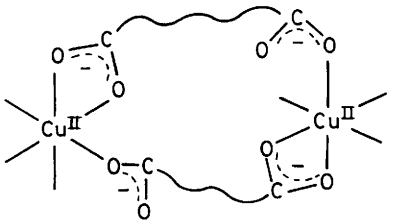

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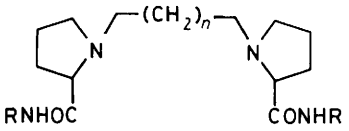


(9) R = H or Me

n = 0 or 1

molecule occupying the axial positions of the octahedron.[35] The tridentate behaviour of the amino acid ligand in this complex contrasts with the bidentate role of L-Asp and L-Glu ligands in previously reported copper(II) complexes containing these ligands.[36] Generally these complexes are polymeric as anhydrous or low hydrate species but are monomeric when heavily hydrated. The N-protected aspartate complexes $[Cu(Z\text{-}Asp)H_2O]_n \cdot 0.25nNaClO_4$, $[Cu(Ac\text{-}Asp)H_2O]_n.nH_2O$ and $[Cu(Bz\text{-}Asp)H_2O]_n \cdot 1\frac{1}{2}nH_2O$ have been synthesised and characterised by e.s.r., electronic and magnetic data.[37] The complexes exist as magnetically isolated dinuclear units having tetracarboxylate-bridged structures. They all readily react with 2,2'-bipyridyl (bipy) to give ternary complexes and the crystal structure of one of these i.e. $[Cu(Z\text{-}Asp)bipy]_2.2\frac{1}{2}H_2O.\frac{1}{4}NaClO_4$ has been determined. The structure consists of two crystallographically independent $Cu_2(Z\text{-}Asp)_2(bipy)_2$ units in which the four metal ions exhibit severely distorted square pyramidal geometries. The copper ions in each unit are doubly bridged by the carboxylate groups (α is monodentate, β is bidentate) of two aspartate ligands (8).

The N-dansylglycine (H_2L) complexes, $Cu(HL)_2.2CH_3OH$, $Cu(HL)_2.4H_2O$, $Cu(HL)_2(py)_2.H_2O$ and $CuL(bipy).CH_3OH$, the last of which contains a deprotonated amide ligand, have been synthesised and characterised by thermogravimetric, spectroscopic and magnetic measurements.[38] The crystal structures of the ligand (H_2L) and the amine adducts have also been determined. In $Cu(HL)_2(py)_2.H_2O$ the geometry about the metal is tetragonal pyramidal with two carboxylate oxygen atoms and two pyridine nitrogen atoms in the basal plane and a water ligand occupying the apical position. In $CuL(bipy)CH_3OH$ the geometry about the metal is a tetrahedrally distorted square pyramid in which the bidentate ligands occupy the basal plane and the methanol the apical position. A number of copper(II) complexes with a number of potentially tetradentate diamide ligands (9) have been synthesised and characterised by electronic, c.d., i.r. and e.s.r. spectroscopy.[39]

The Cu_A site in cytochrome c oxidase is thought to involve a pseudotetrahedral $CuN_2(His)S_2(Cys)$ unit having substantial Cu(II)-thiyl character. Attempts to mimic this site using the bridged L-cysteinethiolate ligand $[^-SCH_2CH(CO_2CH_3)NHCH_2\text{-}]_2$ have been reported.[40,41] The reaction of this ligand $(S\sim N)_2$ with copper(II) complexes proceeds by either direct ligand replacement or by redox decomposition depending on the copper(II) starting

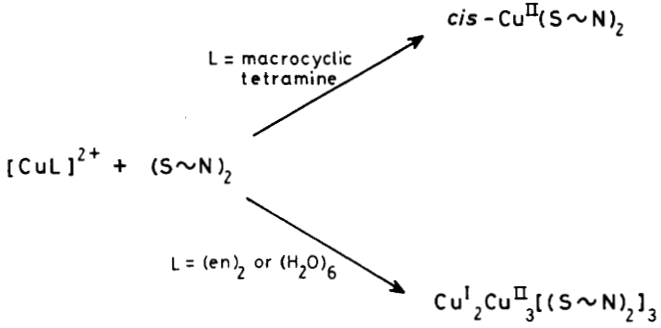
material, Scheme 1. The crystal structures of the complexes cis-$Cu^{II}[SCH_2CH(CO_2CH_3)NHCH_2-]_2$, a stable copper(II) aliphatic dithiolate and of $Cu^{I}_2Cu^{II}_3\{[SCH_2CH(CO_2CH_3)NHCH_2-]_2\}_3.2ClO_4.H_2O$ have been determined. Both complexes contain tetrahedrally distorted, planar cis-$Cu^{II}S_2N_2$ units (10) which have Cu-S bond distances (2.237-2.266 Å) similar to those obtained by EXAFS for cytochrome c oxidase (2.27 Å). The unusual e.s.r. and electronic spectroscopic features of the Cu_A protein site are however not reproduced in the monomer. In the mixed valence species the cis-CuS_2N_2 units are arranged so as to create triangular S_3 ligation for copper(I) (11).

Four structural types of amorphous or crystalline compounds of general formula $[M(H_2O)_x][M'Cdta].yH_2O$ (M ◘ M' = Cu, x = 4, y = 0; M = Mn, Co, Ni or Zn; M' ◘ Ni or Cu, x = 5, y = 1; M ◘ Cu, M' ◘ Ni, x = 4, y = 3; M ◘ Mn, Co or Zn, M' = Zn or Co, x = 4, y = 5) and containing the Edta-type ligand 1,2-cyclohexanediamine-N,N,N',N'-tetraacetate have been prepared and the crystal structure of a compound from each structural type determined.[42] Two hexa-coordinated sites are found in each structure, an anionic site containing hexadentate CDTA ligands and a cationic site which contains aqua ligands with oxygen atoms from the neighbouring anionic site completing the 6-coordination.

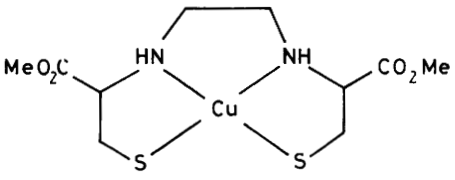
Reaction of $Cu(Gly)_2$, formaldehyde and benzaldehyde phenylhydrazone results in the formation of complex (12) the dihydrate of which has been structurally characterised by X-ray diffraction.[43]

Other Metal Ions.- Magnesium(II) complexes of L-aspartate and L-glutamate have recently been used as chemotherapeutic agents. The role of the dicarboxylic amino acids is apparently to transport the metal ion through membranes as well as across the blood-brain barrier. An X-ray diffraction study of $Mg(L\text{-}Asp)(H_2O)_2.H_2O$ (13) which exists only in strongly basic aqueous solution, has provided the first structural information on these complexes.[44] In this complex the amino acid is coordinated in a fac tridentate fashion, with two water molecules and a carboxylate oxygen atom from a neighbouring molecule completing octahedral coordination about the metal.

The intercalation of L-His, L-Lys and L-Arg by γ-zirconium phosphate has been investigated using X-ray diffraction, electron microscopy, i.r. absorption and thermogravimetry.[45]



Scheme 1



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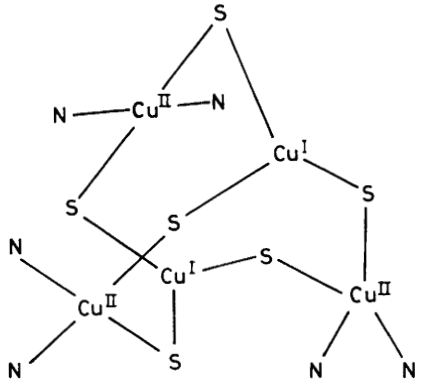
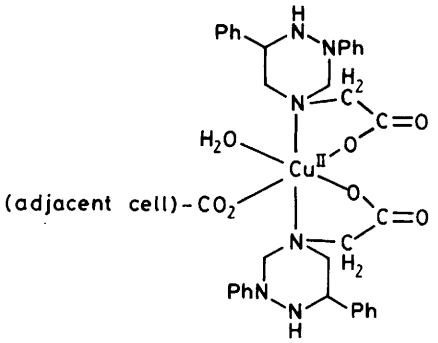


(11)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H
N
NPh
H2
C
N
C=O
H2O
O
Cu II
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O
C=O
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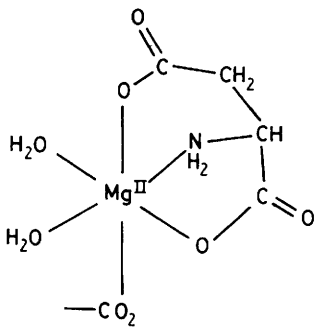
H2
PhN
N
H
Ph

(12)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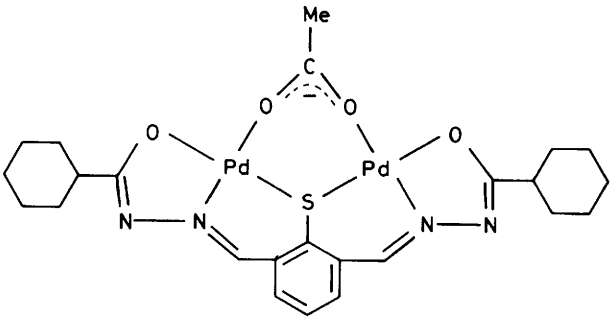
H2O
N
H2
CH
Mg II
C
O
H2O
O
—CO2

(13)


Me
C
O
O
O
Pd
Pd
O
S
N
N
N
N

(14)

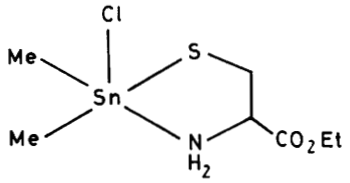
Seventeen new complexes of formulae [Pd(bipy)AA]Cl.xH_2O (x = 1-3; AA ◘ Gly, L-Ala, L-Phe, L-Leu, L-Pro, L-Ser, L-Lys, L-Gln, L-Asn, L-His, L-Met, L-Tyr, L-Trp, L-Glu) and Pd(bipy)AA' (AA' ◘ Cys,Asp) have been synthesised and characterised (microanalysis conductance, electronic and ^1H nuclear magnetic resonance spectra).[46,47] Some of these complexes show growth inhibition against various tumour cells and have LD_{50} values lower than those of cisplatin.

The complex (14) which contains a binucleating ligand (L) and an acetate bridge reacts with Gly-OEt.HCl in the presence of base to give the complex $LPd_2(Cl)(NH_2CH_2CO_2Et)$ in which the acetate bridge has been displaced by two separate ligands. The crystal structure of this complex has been determined.[48] The reaction of Zeise's salt $[PtCl_3(C_2H_4)]^-$ with β-alanine gives the trans-(N,olefin) product $PtCl(\beta\text{-Ala})C_2H_4$ for which the crystal and molecular structure has been reported.[49]

The crystal structures of the ruthenium(III) chelates $[Ru(NH_3)_4Gly](PF_6)_2$ and $[Ru(NH_3)_4Gly\text{-}NH](PF_6)_2$ (Gly-NH = deprotonated glycinamide) have been determined and the results compared with those for analogous cobalt(III) chelates.[50] The C-O and C-N bonds distances in the Ru(III)-glycinamido complex are similar to those in the free amide while in the case of Co(III) chelates the former bond is longer and the latter shorter. Photolabile ruthenium(II) complexes of the type rac-$[RuL_2(AA)]ClO_4.nH_2O$ (L ◘ bipy or phen; AA ◘ Gly, N-Me-Gly, N-Ph-Gly) have been prepared and their structures in solution investigated by ^1H nuclear magnetic resonance spectroscopy.[51] The X-ray crystal structure of rac-$[Ru(bipy)_2Gly]ClO_4.2H_2O$ shows that the Ru-N bond trans to O is shorter than the other Ru-N bonds.

Three series of organotin(IV) cysteamine compounds of formulae $SnR_2(Cl)SCH_2CH_2NR'_2$, $SnR_3(SCH_2CH_2NR'_2)$ and $SnR_2(SCH_2CH_2NR'_2)_2$ in which R ◘ Me, Et, n-Bu, N-Oct, Ph and R' ◘ H, Me, Et have been synthesised and investigated by ^{13}C, ^{15}N and ^{119}Sn nuclear magnetic resonance spectroscopy.[52] The results are compared with those for the ethyl cysteinate complex $Sn(Me)_2(Cl)[NH_2CH(CH_2S^-)CO_2Et]$ (15). Depending on the balance of electronic and steric effects the aminothiol ligands in the above series may be mono- or bi-dentate, chelating or bridging.

In view of the increasing use of cadmium as a nuclear magnetic resonance probe for Ca^{2+} binding sites in proteins such



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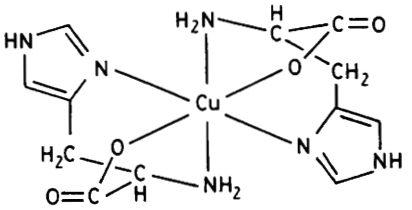

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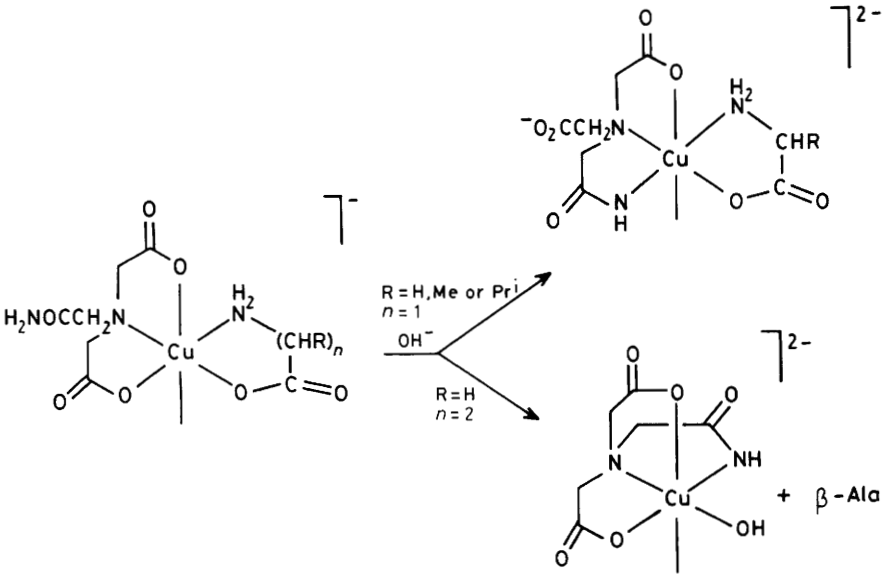


Scheme 2

as parvalbumin, calmodulin and insulin and in order to understand structural effects on ^{113}Cd chemical shifts, the complexes $Cd(Gly)_2.H_2O$ and $Cd(Me_4tu)_2(NO_3)_2$ (Me_4tu = 1,1,3,3-tetramethyl-2-urea) have been investigated by single crystal oriented ^{113}Cd nuclear magnetic resonance.[53] Treatment of $Br_3SnRe(CO)_5$ with Leu-OMe in dioxane at 20°C gave fac-$BrRe(CO)_3$(Leu-OMe) and a number of complexes containing other amino acids or their derivatives have been isolated by similar substitution reactions.[54]

Solution Studies.- There is a large number of papers describing structures, reactions and stabilities of metal-amino acid complexes in solution.

Structures in Solution.- Vanadyl complexes of formula $VO(S)_3(HL)_3$ containing monodentate amino acid (HL) ligands have been characterised in DMSO and CH_3OH(S) solutions at room temperature and at 77K by e.s.r. spectroscopy.[55] A number of chromium(III) complexes containing C2-deuterated amino acid ligands have been prepared and their structures in solution investigated by ^2H nuclear magnetic resonance spectroscopy.[13] This technique can be used to identify the mode of coordination of the amino acid and also to distinguish between diastereoisomers.

The ^{13}C nuclear magnetic resonance spectra of the six geometrical isomers of $[Co(edma)_2]^+$ (edma ▫ ethylenediaminemonoacetate) and of the four geometrical isomers of Co(ida)edma (ida = iminodiacetate) in D_2O have been studied.[56] The positions of the ^{13}C signals of the glycinate-type rings are sensitive to the nature of the trans ligands and also to the geometry (mer or fac). Structural assignments are based on a combination of ^{13}C nuclear magnetic resonance and visible spectral data.

In view of the biochemical (transport of copper(II) in blood) and medicinal (treatment of Menke's disease) importance of copper(II)-L-histidine complexes much effort has been directed over the years towards establishing the structures of complexes formed between copper(II) and this amino acid under various conditions. While a range of species can exist depending on the pH only $Cu(L\text{-}His)_2$ is observed in near neutral aqueous solution. However despite its apparent simplicity no less than six different structures have at various stages been proposed for this complex. ^{13}C and ^1H nuclear magnetic resonance chemical shifts and relaxation times have been used to extract structural information and kinetic parameters for this complex in basic (pH 10.5, 10^{-3}M Cu^{2+}, 0.95M L-His) solution.[57] The carbon to metal distances found in this work point to the existence of a trans bis(tridentate) species, $Cu(L\text{-}His)_2$ (16). The

copper(II) L-histidine system has also been investigated by e.s.r. spectroscopy of frozen solutions (liquid N_2 temperature, 8 x 10^{-3}M Cu^{2+}, 0.1M L-His, pD 6.8 and 7.3).[58] The e.s.r. data at pD 6.8 point to the existence of a complex containing a square-planar Cu(II)-N_4 unit suggested to be either $Cu(L\text{-}His)_2$ in which the L-His is bidentate (2N) or the more favoured $Cu(L\text{-}His)_4$ in which L-His is monodentate and imidazole-bonded to the metal. The spectrum at pD 7.3 is consistent with the presence of an additional species which may result from deprotonation of an axial ligand or which may be due to a change in the square planar coordination from one 4N-donor set to a different 4N donor set. It is however emphasised that the species in the frozen state differ from those in the liquid state even under otherwise identical conditions and it has been shown that four histidine ligands can bind to copper(II) in the former state under conditions where only two are bound in the latter.

Potentiometric, e.s.r. and electronic absorption spectra of solutions containing Cu^{2+} and L-α-alaninehydroxamic acid, $CH_3CH(NH_2)CONHOH$ (HL), show that this ligand acts as a 2N-donor, and that while dimeric complexes exist in the pH range 4-6 only the species CuL_2 exists in the pH range 7.4-8.3.[59] The mechanism of complex formation between Ni(II) and glycinehydroxamic acid has been investigated by stopped-flow methods.[60] The complexes formed between Cu(II) and 4-amino-2,2,6,6-tetramethylpiperidine N-oxide-glycinate, a nitroxide labelled amino acid have been studied by e.s.r. spectroscopy.[61]

Reactions.- When heated in solution the oxovanadium(V) complex VO(EHPG) (see earlier text and structures 1-3) undergoes a stepwise oxidative decarboxylation of ligand to give the oxovanadium(IV) complexes $[VO(EHGS)]^-$ and VO(salen). A mechanism for this novel vanadium(V)-assisted reaction is proposed.[9] The electrochemistry of the oxovanadium(IV) and (V) complexes of EHPG (1) in various solvents has been investigated by cyclic voltammetry and these complexes are shown to be related by electron transfer-chemical step mechanisms.[62] The kinetics and mechanism of the replacement of glycinate in $VO(Gly)_2$ by oxalate in aqueous solution have been investigated by stopped-flow methods.[63]

The kinetics of the acid-catalysed ring opening of the geometric isomers of $Cr(L\text{-}Asp)_2$ have been investigated at 25-45°C.[14] A mechanism involving rapid pre-equilibrium

protonation of a carbonyl group followed by rate determining opening of the chelate ring has been proposed for this reaction.

In basic solution (pH ~ 14) the complex $[Co(en)_2(\beta\text{-Ala})]^{2+}$ (en ▫ ethylenediamine) undergoes ring opening to give cis-(70%) and trans-$[Co(en)_2OH(\beta\text{-Ala})]^{+}$. Both of these isomers hydrolyse to cis-$[Co(en)_2(OH)_2]^{+}$ which undergoes isomerisation to a cis ⇌ trans equilibrium mixture.[64] Rate constants are reported for all of these processes as well as for the ring closure and isomerisation of cis-$[Co(en)_2OH(\beta\text{-Ala})]^{+}$. The Hg(II)-promoted and base hydrolysis of the complexes cis-$[Co(en)_2X(\beta\text{-Ala-OR})]^{2+}$ (X ▫ Cl, Br; R ▫ H, Me, Pr^i) have been investigated.[65] The products of the Hg^{2+}-promoted hydrolysis are cis-$[Co(en)_2H_2O(\beta\text{-Ala-OR})]^{3+}$ (80% for R = H, 90% for R = Me, Pr^i) and the chelated amino ester complex $[Co(en)_2(\beta\text{-Ala-OR})]^{3+}$. The products of base hydrolysis are cis-$[Co(en)_2OH(\beta\text{-Ala/OR})]^{n+}$ (90% for β-Ala, n = 1; 32% for β-Ala-OPr^i, n ▫ 2) and $[Co(en)_2\beta\text{-Ala}]^{2+}$. Rate constants and product analysis for these systems are described. The kinetics and mechanisms of the hydrolysis of the ester groups in the complexes $[Co(en)_2\beta\text{-Ala-}OPr^i]^{3+}$, cis-$[Co(en)_2(H_2O)\beta\text{-Ala-}OPr^i]^{3+}$ and its hydroxo conjugate base have been investigated and the results where possible are compared with those of the analogous glycine ester complexes.[66] In the hydrolysis of cis-$[Co(en)_2H_2O(\beta\text{-Ala-}OPr^i)]^{3+}$ ^{18}O tracer experiments provide evidence for a mechanism involving nucleophilic attack by the aqua ligand (surprising in view of the very weak nucleophilicity of this ligand) on the acyl group. The kinetics and mechanism of the aminolysis of $[Co(en)_2\beta\text{-Ala-}OPr^i]^{3+}$ by Gly-OEt have also been investigated.[67]

Complexes of the type $[N_4Co(AA\text{-OR})]^{3+}$ (N_4 = a 4N donor set, AA-OR ▫ chelated amino ester) have previously been evaluated for use in peptide synthesis, but conflicting results have been reported regarding racemization during such syntheses. The aminolysis of $[Co(en)_2L\text{-Phe-OMe}]^{3+}$ by L-Phe-OMe and L-Phe-OBu^t has therefore been studied.[68] Contrary to previous reports it is shown that these reactions are accompanied by appreciable racemization which makes the reagents unsuitable for the synthesis of biologically active peptides. The kinetics and mechanisms of substitution of the glycinate ligand in the complex $[Co(en)_2Gly]^{2+}$ by ethylenediamine (pH ≈ 12, 60-70°C) are reported.[69]

Ternary complex formation between $[Ni(NTA)(H_2O)_2]^{-}$

(NTA = nitrilotriacetate) and the amino acids Gly, Ala, β-Ala, L-Val and L-Phe has been studied by stopped-flow spectrophotometry as a function of pH.[70] The formation and dissociation of nickel(II)-histidine complexes have been studied by polarography.[71]

The decomposition of H_2O_2 in the presence of copper(II)-amino acid complexes has been investigated by polarography at 25°C and the catalytic sequence Cu^{II}(bipy)(L-Pro) > Cu^{II}(phen)(L-Pro) > Cu^{II}(bipy)(L-Thr) > Cu^{II}(phen)(L-Thr) (bipy = 2,2'-bipyridine, phen = 1,10-phenanthroline) has been observed.[72] The exchange of the amino acid ligand by H_2O_2 seems to be the critical factor in determining reactivity. A number of binary and ternary copper(II)-amino acid complexes of formulae $Cu(AA)_2$ and Cu(bipy)AA which catalyse the disproportionation of superoxide have been investigated by differential pulse voltammetry.[73] Complexes of general formula $[Cu(H\text{-}ADA)AA]^-$ (H-ADA = N-acetamidoiminodiacetate, AA = Gly, D/L-Ala, Val, β-Ala) behave differently upon deprotonation of the uncoordinated amide group.[74] In the Gly, D/L-Ala and Val complexes a carboxylate ligand is displaced by the deprotonated amide group while in the case of the β-Ala complex dechelation with loss of the amino acid ligand occurs, Scheme 2. The kinetics of oxidation of cysteine by the tris[2-(2-pyridyl)ethyl]amine-copper(II) cation have been investigated and the results compared with those for the analogous complex containing only one methylene group between the amino and pyridyl groups.[75] The reduction potentials of both complexes have been measured and mechanisms for the redox reactions are reported.

The hydrolysis of a number of amino acid esters both in the absence of and in the presence of metal ions has been investigated. Copper(II) promotes the base hydrolysis of Gly-O(4-NO_2Ph) by a factor of ~ 3 x 10^4, and of Leu-O(4-NO_2Ph) by ~ 4 x 10^5 at 25°C.[77] Coordination of amino acid esters (AA-OR) in the complexes $[Pd(pip)_2AAOR]^{2+}$ (pip = piperidine, AAOR = Gly-OMe, Gly-OEt, L-Ala-OEt, L-Cys-OMe, L-His-OMe and ethyl picolinate) lead to rate enhancements of up to 4.9 x 10^7 for base hydrolysis.[78]

The reaction of ethionine (H-Eth) with Pd(II) in aqueous solution has been studied by potentiometric and spectrophotometric methods.[79] The species $[PdCl_3(H_2\text{-}Eth]$, $[PdCl_2(Eth)]^-$, $[PdCl(OH)Eth]^-$, $[Pd(H\text{-}Eth)_2]^{2+}$ and $Pd(Eth)_2$ have been reported to exist in the pH range 1-5.5 and kinetic studies show that the

complexes $[PdCl_4]^{2-}$ and $[PdCl_3(OH)]^{2-}$ play an important rôle in complex formation. Allyl and allyloxycarbonyl amino acid derivatives are selectively deprotected by the palladium-catalysed hydrostannolysis by Bu_3SnH.[80]

The micellar promoted stereoselective hydrolysis of N-acylamino acid esters containing D- or L-Ala, D- or L-Phe or D- or L-Leu residues in the presence of $Ru(III)(NH_3)_5AA$ catalysts (AA = His, Z-His or Z-Leu-His) ligands has been investigated.[81] The electrooxidation of the oxo-bridged species $[(bipy)_2(H_2O)Ru(III)]_2O$ generates a Ru(III)-Ru(IV) dimer which oxidises alcohols, sugars and amino acids.[82] The pertechnetate ion oxidises L-cysteine in strongly acid solutions to give a yellow species which is proposed to be a technetium(V)-cystine complex. In the presence of a large excess of L-Cys, however, this changes to a violet Te(V)-Cys complex.[83] The luminescence of uranyl ions in aqueous $HClO_4$ is weakly quenched in the presence of amino acids. However prolonged photolysis at 77K leads to decarboxylated C-centred radicals e.g. $^+NH_3\dot{C}HPr^i$ in the case of non-sulphur amino acids and S-centred radicals in the case of Cys, Met and cystine.[84] The terbium luminescence from a terbium-angiotensin II complex is enhanced when the complex is excited at 259 nm (Phe band) or 280 nm (Tyr band).[85] The 259 nm enhancement is similar to that observed for a Tb-Phe complex when excited at the same wavelength but the 280 nm enhancement is only ~ 34% of that observed for a similar excitation of a Tb-Tyr complex. These results are considered in relation to the structure of angiotensin II.

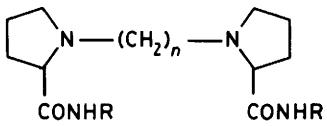
Equilibrium Constants.- The factors which influence complex stability in aqueous solution have been discussed for a range of metal ions (Mg^{2+}, Ca^{2+} and divalent first row d-block ions) and a range of ligands including some amino acids and peptides.[86] Formation constants are reported for complexes of iron(III) with glycinate, iminodiacetate, β-hydroxyethyliminodiacetate, N,N-di(hydroxyethyl)glycinate, nitrilotriacetate and triethanolamine at pH < 3, I ◘ 1M $NaClO_4$ and 25°C.[87] The interactions of methioninehydroxamic acid with Fe(III),[88] of glycinehydroxamic acid with Ni(II) (both in the absence of and in the presence of pyridoxal)[60] and of L-alaninehydroxamic acid with Cu(II)[59] have been studied by potentiometric methods at 25°C, I ◘ 0.15M NaCl and formation constants have been determined.

Equilibrium constants as well as enthalpies and entropies of formation are reported for the complexes $[Ni(Gly)_n]^{(2-n)+}$ (n ▫ 1-3),[89] AgL, $[AgL_2]^-$ and $[Ag(HL)]^+$ (L ▫ Gly or β-Ala),[90] in water and a dioxane-water mixture at 25°C, I ▫ 3M $LiClO_4$. The order of formation enthalpies and entropies for the Ni(II)-Gly complexes viz. $\Delta H_1^\circ > \Delta H_2^\circ > \Delta H_3^\circ$ and $\Delta S_1^\circ > \Delta S_2^\circ > \Delta S_3^\circ$ are discussed in terms of metal-ligand bond distances in the relevant complexes. Addition of dioxane was found to increase the formation constants.

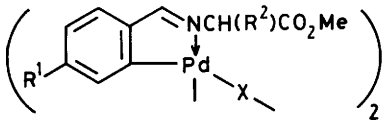
Protonation constants, copper(II)-complex formation constants and amide ligand deprotonation constants have been measured for the potentially tetradentate L-prolinamide derivatives represented by structure (17).[91] Stability constants have also been reported for ternary Cu(II)-edma-AA complexes (edma ▫ ethylenediamine-N-acetate, AA = Gly, L-Ala, L-Avl, L-Phe or L-Trp).[92] The tendency to form mixed ligand complexes decreases in the order Trp > Phe > Avl ≈ Ala ≈ Gly, which may be due to metal ion-aromatic ring interactions and decreased hydration in the case of the first two amino acids. Stacking interactions between the aromatic groups of the primary (L) and secondary ligands (L') have been invoked to explain the enhanced stability of ternary complexes $M^{II}(L)L'$ (M ▫ Mg, Ca, Mn, Co, Ni, Cu, Zn; L = 5'-cytidine monophosphate, L' ▫ Gly, oxalate, His, histamine) containing aromatic as opposed to aliphatic secondary ligands (L').[93]

Ternary equilibria in solutions containing Cu(II), His and Asn, Gln or Ser have been reinvestigated by potentiometric methods at 37°C and the computer simulated distribution of Cu(II) in blood plasma has been redetermined in the light of the new formation constants.[94] Formation constants for Cu(II)-edta-AA complexes (AA = Gly, Try, Phe, Val, Ala, Ser) at 37°C and I ▫ 0.15M $NaClO_4$ show that Cu(II) distribution in blood plasma is altered by administration of edta (e.g. in chelation therapy) and that ternary complexes of the type investigated must be taken into consideration when building up blood plasma models to assess the sequestering ability of polyaminocarboxylate ligands.[95] A new computer program POLET (84) in FORTRAN which determines stability constants log β_{pqrs} and unknown indices p,q,r,s of up to quaternary complexes $M_pL_qY_rH_s$ from potentiometric data has been developed and its validity has been verified using literature and simulated data.[96]

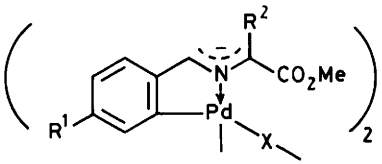
Stability constants are reported for ternary complexes of



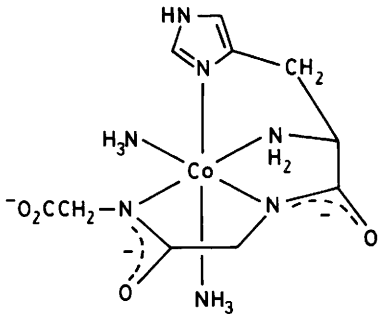
(17) R = H or Me
n = 2 or 3



(18) R^1 = H, Pr^i, OMe, or NO_2
R^2 = H, alkyl, or aryl
X = Cl or OAc



(19) R^1 = H, Pr^i, OMe, or NO_2
R^2 = H, alkyl, or aryl
X = Cl or OAc



(20)

the type M(II)-L-A (M = Cu, Ni; L = L-dopa = 3,4-dihydroxyphenylalanine, L-Tyr, L-Phe, L-dopn = 3,4-dihydroxyphenylethylamine) at 25°C, I = 0.2M KCl.[97] In the ternary complexes an O,O binding mode of L-dopa is highly favoured.

Protonation constants and MeHg(II)-complex formation constants have been determined for a number of selenohydryl compounds including selenocysteine and selenopenicillamine.[98] Using the result for selenocysteine as an estimate for the formation constant of the MeHg(II)-glutathione peroxidase complex and from the known formation constants of the MeHg(II)-glutathione and -haemoglobin complexes it is predicted that in red blood cells between 1.6 and 47% of the selenol groups of glutathione peroxidase are complexed by MeHg(II) at concentrations of the latter ranging from 1×10^{-6} - 5×10^{-5}M.

References to other formation constant work as well as methods, conditions and comments where appropriate are listed in Table 2.

Schiff Bases.- Ortho-palladated imines of α-amino acid esters (18) are deprotonated by triethylamine to give metallo-1,3-dipoles (19) which have been isolated as cycloadducts with N-phenylmaleimide.[126] The kinetics of formation and reactions (transamination and dephosphonylation) of Schiff bases derived from pyridoxal 5'-phosphate and 2-amino-3-phosphonopropionic acid both in the absence and presence of Al^{3+}, Ga^{3+} and Zn^{2+} have been investigated by potentiometric and nuclear magnetic resonance (^{1}H, ^{31}P) methods.[127] A number of cobalt(II) complexes with Schiff base ligands derived from L-amino acids (AA = Ala, Val, Phe, His) and salicylaldehyde (sal) or pyridoxal (pdx) have been synthesised by template reactions.[128] The complexes Co(sal-AA) and Co(pdx-AA) are high spin, five- or six-coordinate and bind O_2 to give monomeric or dimeric adducts, which have been investigated as oxidation catalysts for various organic substrates. Some octahedral nickel(II) complexes with Schiff base ligands derived from salicylaldehyde and amino acids (2-, 3- and 4-Abu, β-Ala, DL-Asn and DL-Gln) have been prepared and characterised.[129] A method has been developed for the fluorometric determination of Mg(II) by salen (3) in the presence of amines or amino acids in basic solution.[130] Salicylaldehyde formed by hydrolysis of (3) reacts with the amine or amino acid to give a Schiff base which then complexes with the cation.

Table 2 Formation constant measurements for metal-amino acid complexes

cation	ligand, complexes	method, conditions, comments	Ref.
Be^{2+}	iminodiacetate (ida), N-Me-ida, N-Et-ida, N-Pr-ida (L); $[Be(OH)L]^-$.	potentiometry, T = 25°C, I = 0.5M $NaClO_4$.	99
Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}	5'-cytidine phosphate or cytosine and Gly, His, oxalate or histamine; 1:1 and 1:1:1 complexes.	potentiometry, T = 35°C.	93
Cr^{3+}	L-Asp, DL-Met, DL-Eth; binary and ternary complexes.	potentiometry, T = 25°C, I = 0.1M $NaClO_4$.	100
Cr^{3+}	L-Glu, DL-Abu, L-Ser, L-Thr and DL-Met or DL-Eth; binary and ternary complexes.	potentiometry, T = 25°C, I = 0.1M $NaClO_4$.	101
Cr^{3+}	amino acids, nicotinate; ternary complexes.	spectrophotometry, pH = 4, I ◻ 0.1M KNO_3.	102
Cr^{3+}, Al^{3+}, Th^{4+}	Leu,Ser,Thr (AA); $[M(AA)]^{2+}$ $[M(AA)_2]^+$, $M(AA)_3$, M ◻ Cr,Al; $[Th(AA)]^{3+}$.	ionophoretic method, T = 35°C, I = 0.1M $HClO_4$.	103
Mn^{2+}, Cu^{2+}, Zn^{2+}	Ala,Pro,Thr,Arg,Asp; 1:1 and 2:1 complexes.	spectrophotometry.	104
Co^{2+}	ascorbic acid (H_2L), Gly, Ser,Ala,His,Glu (AA); Co(AA)HL, $[Co(AA)_2HL]^-$, $[Co(AA)L]^-$, $[Co(AA)_2L]^{2-}$.	potentiometry, spectrophotometry, T = 25°C, I ◻ 0.1M KCl.	105
Co^{2+}, Ni^{2+}	gluconic or pangamic acid (H_2L) and Gly, Ala, Val, Ser,Lys or His (HA); M(A)HL.	spectrophotometry.	106
Ni^{2+}	Gly; 1:1, 2:1, 3:1 complexes.	calorimetry, T ◻ 15,25,35°C, I = 0.5, 1.0, 1.5M KNO_3; heats of formation, thermodynamic parameters.	107
Ni^{2+}	ATP and Asp, Glu or His; ternary complexes.	spectrophotometry, T ◻ 20°C, I ◻ 0.1M $NaClO_4$.	108

cation	ligand, complexes	method, conditions, comments	Ref.
Ni^{2+}, Cu^{2+}	4-OMe-picolinate N-oxide and Gly,Ala,Pro,OH-Pro; ternary complexes.	potentiometry at various temperatures; enthalpies and entropies of formation.	109
Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}	Edta and Gly; $[M(Edta)Gly]^{3-}$.	calorimetry, T = 298K, I = 1.5M KNO_3; thermodynamic parameters.	110
Ni^{2+}, Cu^{2+}	nitrilotris(methylene-phosphonic acid) and Gly, Ala,Val,Trp,Phe,β-Ala or His.	potentiometry, spectrophotometry, magnetic relaxation, T ◘ 25°C.	111
Cu^{2+}	picolinate N-oxide and Gly,Ala,Phe,Pro,OH-Pro, cat,bipy or phen; 1:1:1 ternary complexes.	potentiometry at various temperatures; enthalpies and entropies of formation.	112
Cu^{2+}	Ala-Met, Ala-Avl, Gly-Sar, Gly-Asn, Gly-Val; binary complexes: Gly-Asn and 13 amino acids; ternary complexes.	T = 293, 303, 313K, I = 0.1M $NaClO_4$; thermodynamic parameters.	113
Cu^{2+}	amino acids.	potentiometry, ion selective electrodes.	114
Cu^{2+}	5-NO_2-salicylate and amino acids.		115
Cu^{2+}	Gly,Ala,Val,Leu,Asp,Glu, Phe (AA) and salicylate (L); binary and ternary Cu(II)-AA-L complexes.	potentiometry, 25°C I = 0.1M KNO_3.	116
Cu^{2+}	2-N,N-diethylaminomethyl-benzimidazole (HL) and Gly, Ala, β-Ala, Pro or ida(AA); CuL, CuL_2, CuL(AA).	potentiometry, T ◘ 25,35,45°C, I ◘ 0.1M $NaClO_4$ in 50% EtOH/H_2O.	117
Cu^{2+}	bipy and Pro,Abu,Val or Thr; ternary complexes.	temperature jump methods, T = 25°C, I ◘ 0.1M $NaClO_4$.	118

cation	ligand, complexes	method, conditions, comments	Ref.
Cu^{2+}	piperidine-2-carboxylate and Gly,Ala,Phe,Pro, OH-Pro,bipy,cat or phen.	potentiometry, T = 25,35,45°C, I = 0.1M KNO_3; thermodynamic parameters.	119
Zn^{2+}, Pb^{2+}	Gly,Ala,Val,Asp,Glu, Tyr and nta or ida; binary (1:1, 2:1, 3:1) and ternary complexes.	potentiometry, T = 25°C, I ◘ 0.1M KNO_3.	120
Pd^{2+}	ethionine (H-Eth): $PdCl_3(H_2\text{-Eth})$, $[PdCl_2(Eth)]$, $[PdCl(OH)Eth]^-$, $[Pd(Eth)_2]^{2+}$, $Pd(Eth)_2$.	potentiometry, T = 25°C, I = 0.16M Cl^-, pH 2.8-4.8 .	79
Ag^+	Cys.	potentiometry, T ◘ 25°C.	121
Cd^{2+}	Leu (L); CdL_2, $[CdL_3]^-$.	polarography, T ◘ 25°C, I ◘ 0.1M $NaClO_4$.	122
Cd^{2+}	Glu, Avl, Ile; 1:1, 2:1, 3:1 complexes.	polarography.	123
Cd^{2+}	pyridoxinate and Trp, Ile, Asp, Lys or Val; ternary complexes.	polarography.	124
Cd^{2+}	Gly,Ala,Phe,Ser, S-Me-Lys, Met, Asp, His, Ac-Lys, Ac-pe, peptides.	potentiometry.	125

Miscellaneous.- The cytotoxicity of a series of complexes of formula cis-$PtCl_2(NH_2Bu^t)$AA (AA = L-, D- or DL-Ser, L- or D-Phe, L-Leu, L-Ala, L-Met, L-Asn) against L1210 leukemia cells have been investigated and compared with that of cisplatin and $K[PtCl_3(NH_2Bu^t)]$.[131] Complexing of anticancer platinum drugs with macromolecular carriers such as polysaccharides or polyamino acids reduces their toxicity while maintaining a high therapeutic activity.[132]

The uptake of Ca^{2+} by brain cortex slices incubated in vitro was found to be enhanced by N-Me-DL-Asp, L-Glu and DL-Hcy which act by opening voltage sensitive Ca^{2+} channels.[133] The relationship between metal ion coordination and hydrophobic or aromatic ring stacking interactions in metal-amino acid complexes has been discussed.[134] The effect of various amino acids (bidentate to hexadentate) on Zn(II) uptake by the pancreas has been studied using ^{65}Zn-radiolabelled complexes.[135] The ^{62}Zn-labelled complex Zn(edda) showed promise as a pancreas PCT (positron computed tomography) imaging agent.[136]

The R_f values of 78 Co(III) and Pt(II) complexes, some of which contain amino acid ligands, are found to depend on a number of factors including chelate ring size, the length of ligand side chains, the particular geometric isomer or the absolute configuration of a diastereoisomer.[137] Evidence for ternary complex formation between nucleotides, metal ions (Mn^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+}) and amino acid has been obtained from chromatographic behaviour and the retention data have been used to determine formation constants for nucleotide (AMP,ADP,ATP,GMP, GDP,GTP) - metal ion (Zn^{2+}, Cu^{2+}, Cd^{2+}) - Leu systems.[138] A Se-selective chromatographic detector based on mass spectrometry has been used to analyse trimethylsilylated amino acid mixtures.[139] The resolution of racemic amino acids (Pro, OH-Pro, aOH-Pro) and their N-benzyl derivatives on reversed phase packings in aqueous eluents containing chiral complexes of the same amino acids has been examined.[140] The resolution of amino acids by h.p.l.c. with equimolar concentrations of Cu(II) and a second optically active amino acid in the mobile phase has been described.[141] The isomers of binary and ternary tris-chelates of Co(III) containing Gly, Ala and Leu ligands have been separated by t.l.c.[142]

The catalytic activity of Cu(II)-amino acid complexes in the chemiluminescent reaction between phen and H_2O_2 is decreased

in the presence of proteins and this observation forms the basis of a method for the convenient determination of small quantities of protein.[143] An iodometric method which determines low levels of Cu^{2+} and Ni^{2+} following their reactions with α-amino acids is described.[144]

Other papers on metal-amino acid complexes describe an ion exchange method for their determination in sea water,[145] the analysis of potentiometric data for complexes (including amino acids) of selected components of radioactive waste (Cs^{+}, Sr^{2+}, Co^{2+}, La^{3+}, Eu^{3+}),[146] the possible therapeutic value of Fe(III), Co(II) and Ni(II) complexes of Asp, Glu and Met,[147] the effect of nucleic acids, amino acids (Lys, Thr, Trp, Ala) and proteins on copper nutrition,[148] the mucoprotective properties of amino acid derivatives such as 'captopril' and 'enalapril',[149] the use of tetrasulphophthalocyaninezinc(II) for the photooxidation of amino acids,[150] an electrochemical method for the preparation of pure amino acid chelates of Fe^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Cu^{2+} and Ca^{2+},[151] an ab initio approach to the influence of metal ions on the conformation of Ac-Ala-OMe,[152] the adsorption and desorption properties of ^{14}C-labelled Ala, ATP and Asp on metal chelating resins,[153] the influence of amino acids on the mobility of heavy metals in soil,[154] the mechanism of the reaction of $ZrOCl_2$ with collagen amino acids,[155] the reaction of isocyanatosilanes with amino acids,[156] an analysis of the reflectance spectra of 30 polycrystalline copper(II)-amino acid complexes,[157] ligand (interlattice) substitution in $[Co(NH_3)_6]_2(CO_3)_2$ by amino acids,[158] the ability of a solution mixture (L-Ala, L-His and L-Cys, with and without D-penicillamine) to dissolve Cu metal (foil) and Fe (steel) at 37°C and pH 7.4,[159] a literature data analysis of the effect of ionic strength on complex formation enthalpies,[160] the photodecarboxylation of cobalt(III) complexes containing 1-diethylenetriamine-3-propionate,[161] and the preparation and spectroscopic analysis of triorganotin derivatives of several polyaminocarboxylate ligands.[162]

3 Peptides

A variety of papers dealing with syntheses, structure, solution studies and reactions of metal peptide complexes have been published during 1986. Copper(II) complexes have attracted particular attention, but interesting studies have been carried out with palladium(II) and cobalt(III) derivatives.

Synthetic and Spectroscopic Studies.- The preparations of three cobalt(III) complexes of L-histidylglycylglycinate have been described.[163] In one complex $[Co(NH_3)(H_{-2}HisGG)]$ the peptide is coordinated as a quinquedentate ligand via the terminal NH_2, two deprotonated peptide nitrogens, the carboxylate and the imidazole group. In $[Co(NH_3)_2(H_{-2}HisGG)]$ the carboxylate is replaced by a second ammonia ligand giving the structure (20). The third complex has properties consistent with the superoxo binuclear complex $NH_4[H_{-2}HisGG)Co(O_2^-)Co(H_{-2}HisGG)]$. The structures of the first two complexes were derived from the complexes' electronic absorption, circular dichroism and ^1H and ^{13}C nuclear magnetic resonance spectra. The pK values for ionisation of the NH-1 proton of the imidazole ring at 25°C are 10.73 and 10.69 for $[Co(NH_3)(H_{-2}HisGG)]$ and $[Co(NH_3)_2(H_{-2}HisGG)]$ respectively. In the diammine complex (20) the pK of the pendant carboxylate group is 4.34.

Previous work by Hawkins et al.[164,165] has shown that tripeptide ligands are coordinated to Co(III) as quadridentates via the terminal NH_2, two deprotonated peptide nitrogens and terminal CO_2^- donors. A recent paper[166] deals with the attempted preparation and spectroscopic properties of a series of Co(III)-tripeptide complexes having a bulky imidazole group bound in the apical position. Detailed analysis of the nuclear magnetic resonance spectra establish that imidazole remains in the coordination sphere of the metal ion only in the Co(Gly-Gly-Gly) complex. Imidazole coordination is not observed with Co(Tyr-Gly-Gly) and Co(Leu-Gly-Leu). Both ^1H and ^{13}C nuclear magnetic resonance data indicate that quadridentate coordination of the tripeptide ligands occurs in the complexes.

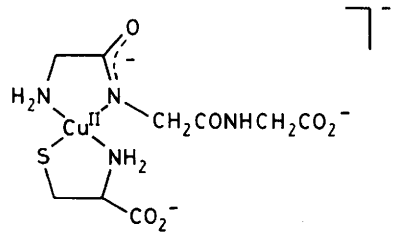
Plant-type ferrodoxins play important rôles in biological electron transport chains. Tetrapeptide 2Fe-2S complexes $[Et_4N]_{2n}[Fe_2S_2(Z\text{-}Cys\text{-}X\text{-}Y\text{-}Cys\text{-}OMe)_2]_n$ (X-Y = Ala-Ala, Pro-Leu, Thr-Val (n = 1), and Val-Val (n > 1)) have been prepared[167] by ligand exchange reactions from $[Et_4N]_2[Fe_2S_2(S\text{-}t\text{-}Bu)_4]$. The first three complexes exhibit chelation of the tetrapeptides with the 2Fe-2S cores and similar spectral and electrochemical properties, but the latter derivative has a non-chelating structure displaying different c.d. and absorption spectra and a negative shift (0.3V) of the redox potential. These differences arise due to steric hindrance from the side chains of the Val-Val ligands.

Interest in synthetic multidentate macrocyclic compounds is continually increasing. Among these compounds, cyclic peptides which owe their functional properties to their macrocyclic structure have attracted much attention in recent years.[168] Interaction of Cu(II) and cyclo-(Gly-His-Gly-His-Gly-His-Gly) a synthetic macrocyclic peptide has recently been investigated by a range of spectroscopic techniques[169] (electronic spectra, e.s.r. and ^{1}H and ^{13}C nuclear magnetic resonance). Two complexes were observed under different conditions of pH. At neutral pH the copper is bound by three imidazole nitrogens, while in basic solution four deprotonated peptide nitrogens act as donors. In basic solution it was possible to observe nuclear magnetic resonance spectra from the paramagnetic copper(II) complex, presumably as a result of the copper being firmly trapped within the cyclic cavity of the ligand.

E.s.r. evidence for the transient complex $[(Cys)Cu(II)(H_{-1}trigly)]^{2-}$, has been obtained,[170] in the reaction of Cys with $[Cu(II)(H_{-2}trigly)]^{-}$. The complex has a S-Cu(II) charge transfer band at 333 nm and is believed to have the structure (21). Similar ternary complexes involving a macromolecule such as serum albumin and a thiol have been suggested as intermediates in copper transport.[171]

Water-soluble metal complexes with paramagnetic peptides have not been reported in the literature, but spin-labelled amino-acids, peptides and proteins are of current interest. The interaction of glycylglycine substituted with 4-amino-2,2,6,6-tetramethylpiperidine N-oxide on the carboxylic acid function, with copper(II) in aqueous solution has now been studied in detail.[172] Strong magnetic exchange between the spin-labelled ligand and copper(II) is observed.

A variety of investigations have appeared dealing with metal-glutathione complexes. The e.s.r. spectra of rapidly frozen solutions of Fe(III) and glutathione showed the progressive reduction of the iron(III) with time and the transient presence of a $g \simeq 2$ radical signal.[173] The intermediate is believed to involve a high spin iron(II) centre weakly coupled to a sulphur radical. Relatively long-lived chromium(V) species have been reported[174] to be produced by the action of glutathione on carcinogenic chromium(VI). Other studies have dealt with the catalytic activity of a copper(II)-oxidised glutathione complex on the dismutation of superoxide in aqueous solution,[175] and the



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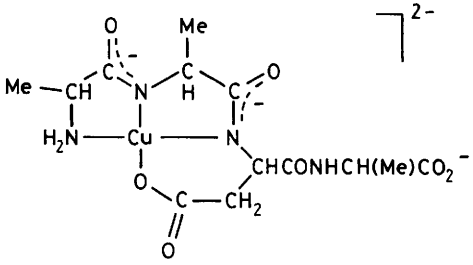

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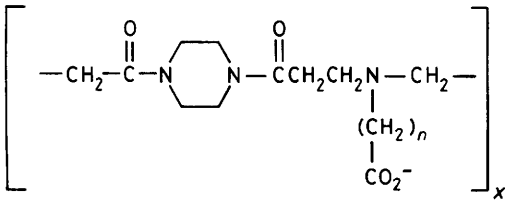
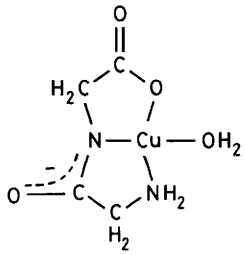


(23) L^1 ($n=1$)

(24) L^2 ($n=2$)



(25)

generation of hydrogen peroxide by the metal ion catalysed autoxidation of glutathione.[176]

Ligand-ligand interactions in mixed ligand metal complexes which lead to specificity or selectivity in ternary complex formation have attracted attention in recent years because of their possible biological relevance.[177] A recent paper[178] describes ^{1}H nuclear magnetic resonance studies of aromatic ring stacking in ternary Pd(II) complexes involving aromatic diamines and dipeptides with N-terminal aromatic amino acid residues. The ternary complexes have a planar N_4 coordination, and the fractional populations of the three staggered rotamers calculated from the coupling constants indicated that the rotamer capable of intramolecular ring stacking is favoured in Pd(L)(DA) (DA = bipy or bphen ▫ 4,7-diphenyl-1,10-phenanthroline-4',4"-disulphonate; L = Tyr-Glu, Tyr-Gly, Trp-Glu, Trp-Gly) as compared with Pd(L)(en) which does not involve stacking.

Solution Studies.- A variety of formation constant studies involving peptide ligands have been published, the bulk of these deal with copper(II) complexes. Formation constants have been reported at 25°C and I ▫ 0.10 mol dm^{-3} (KNO_3) for binuclear complexes of nine sulphur-containing dipeptides with Ag(I) and Cu(II), together with the formation constants of the parent Cu(II) complexes.[179] Dipeptides (HL) studied were Gly-L-Met, L-Met-Gly, L-Met-L-Met, L-Met-D-Met, Cys(Me)-L-Met, Cys(Me)-D-Met, L-Met-Cys(Me) D-Met-Cys(Me) and Cys(Me)-Cys(Me) [Cys(Me) = S-methyl-L-cysteine]. The major ternary complex with dipeptides of two sulphur-containing amino acids of the same chirality was $[AgCuH_{-1}L]^{+}$. When the amino-acid residues were of opposite chirality, ternary complexes were less stable by a factor of over 20 giving very dramatic stereoselectivity. The crystal structure of $[CuH_{-1}\{L\text{-Met-Cys(Me)O}\}]$ (L-Met-Cys(MeO) ▫ L-methionyl-S-methylcysteinate(1-)] was also determined. The copper atom is five-coordinate (square pyramidal) with no Cu-S interaction.

The effects of mixed-ligand complex formation on the deprotonation of amide groups in simple amides and peptides has been studied in some detail.[180] Previous investigations have shown that the presence of the B ligand leads to a significant increase in the pK for the equilibrium, $[CuAB] \rightleftharpoons [CuABH_{-1}]^{-} + H^{+}$, relative to that of the parent peptide

complex. In the present investigation the formation constants of a series of A ligands (glycinamide, glycylglycinamide and N-acetylhistidine) with copper(II) and of the mixed ligand complexes formed with the B ligands (glycine, 2,3-diaminopropionic acid, tiron, histamine, L-histidine and 2,2'-bipyridyl) have been determined. In the glycylglycinamide complexes of the type $[CuABH_{-1}]$ the B ligand is coordinated to a considerable extent via two equatorial sites, while the bonding in the complex $[CuABH_{-2}]$ is mainly axial-equatorial. Interestingly in the parent complexes of copper(II) with N-acetylhistidine and with N-acetylhistamine, deprotonation and coordination of the amide group could not be detected. However, in the presence of B ligands containing an aromatic N-donor deprotonation of the amide group occurred with both ligands. Possibly the aromatic N-donors act as π-acceptor ligands thus increasing the Lewis acidity of the copper(II) centre.

Human fibrinopeptide A is a peptide containing 16 amino acid residues, Ala-Asp-Ser-Gly-Glu-Gly-Asp-Phe-Leu-Ala-Glu-Gly-Gly-Val-Arg. Fibrinogen is a dimer, the two halves being linked by disulphide bridges, and fibrinopeptides A and B, together with fibrin are produced by cleavage of fibrinogen by thrombin. The amino-terminal tetrapeptide fragment of human fibrinopeptide A (Ala-Asp-Ser-Gly) and derivative tetrapeptides with the β-carboxylate of the aspartate residue, and the hydroxy group of the serine residue blocked to prevent coordination to metal ions have recently been prepared.[181] Complexes with hydrogen ion and copper(II), and in the case of Ala-Asp-Ser-Gly with nickel(II), have been studied by a range of potentiometric and spectroscopic techniques. Formation constants of Cu(II) and Ni(II) complexes with Ala-Ala-Ala-Ala were also determined. The β-carboxylate group was shown to coordinate strongly to Cu(II) over the pH range 4-9 to give an unusually stable $[CuH_{-1}L)$ species (overall charge omitted). Fibrinopeptide A, which is present in locally high concentrations near the sites of bodily injury, would similarly be expected to coordinate strongly to Cu(II).

A recent study of the biologically active pentapeptide fragment of thymopoietin, Arg-Lys-Asp-Val-Tyr(H_3L) showed that the major complex formed with Cu(II) over the pH range 5-9 is the [CuL] species which has three nitrogen atoms coordinated to the metal ion.[182] At higher pH two further protons can ionise

to give $[CuH_{-1}L]$ and $[CuH_{-2}L]$, but neither of these appear to involve four nitrogen coordination as would be expected for normal polypeptides.[183] This behaviour presumably results from the presence of the side-chain of the Asp residue, and the possibility of coordination through the β-carboxylate group. In an attempt to assess the effect of Asp on position three, the tetrapeptide Ala-Ala-Asp-Ala(H_2L) has now been prepared,[184] and its interaction with Cu(II) studied potentiometrically and spectroscopically. The data obtained indicate that the $[CuH_{-2}L]$ species believed to have the structure (22) predominates over the entire pH range 5-11.5. The absorption maximum of the d-d transition (548 nm) is compatible with that expected for NNNO bonding, using an Asp-carboxylate oxygen. As a result of this study it can be assumed that the unusual stability of the corresponding NNN complex with Arg-Lys-Asp-Val-Tyr is due to β-carboxylate interaction which is even more marked than with Ala-Ala-Asp-Ala for conformational reasons.

Formation constants and thermodynamic parameters relating to copper(II) complexes of Gly-L-Phe, Gly-L-Tyr, L-Phe-Gly and L-Tyr-Gly have been determined pH-metrically and calorimetrically at 25°C.[185] From these data and the u.v., visible and e.s.r. spectra of the complexes, it has been established that in addition to metal-ligand coordination characteristic of simple aliphatic dipeptides, there are interactions between the d-orbitals of copper(II) and the 6π-electron system of the aromatic amino acid. In addition, interactions between the hydrophobic parts of the molecule, and between copper(II) and the phenolate group are also observed.

Copper(II) complexes formed by two polymers (23 ▪ L^1) derived from glycine and (24 = L^2) derived from β-alanine have been studied using aqueous solutions at different pH by e.s.r. and F.t.i.r. spectroscopy and by calorimetry at 25°C.[186] The spectroscopic data establish that the ligand L^1 forms a single complex in which coordination occurs via one amino nitrogen and one carboxylate group, with each copper coordinated by one repeat unit in the polymer. These views are consistent with the $\Delta H^{\ominus}$ value of 30 kJ mol^{-1} which is independent of pH, and is very close to that measured for a non-macromolecular model ligand. In the case of L^2, the e.s.r. spectra indicate the presence of two complexes, one involving only oxygen donors formed at low pH, while nitrogen becomes a donor at higher pH.

The copper(II) complex of glycylglycine [GlyGlyCu(OH_2)] (25) has been studied in aqueous solution by potentiometric titration, e.s.r. spectroscopy and polarography.[187] The pK_a of the coordinated water molecule is 9.31 from potentiometric titration. Reduction of the hydroxo complex is more difficult than reduction of the aqua complex. The electrochemical behaviour of a number of copper(II)-dipeptide complexes in water-solvent (CH_3CN, DMF and DMSO) mixtures has been studied.[188] Quasi-reversible reductions are observed and the effects of the solvents on the reduction potentials are discussed.

There is considerable interest in the properties of electrodes which have molecular surfaces. Such electrodes may catalyse electron transfers between the electrode and substrate molecules in solution. The preparation of a new kind of modified electrode with copper(II)-glycylglycine absorbed on the surface of glassy carbon has been described.[189]

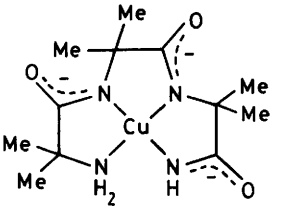
Stabilisation of trivalent copper is no longer considered unusual. A variety of donor groups including deprotonated peptide nitrogens have been used to stabilise this oxidation state. Tri-α-aminoisobutyryl amide, Aib_3a, forms three square-planar copper(III) complexes that are stable (25°C, in the dark) in aqueous solution from pH0 to 14,[190]

$$Cu^{III}(H_{-2}Aib_3a)^+ \underset{}{\overset{pK=0.25}{\rightleftharpoons}} Cu^{III}(H_{-3}Aib_3a) + H^+$$

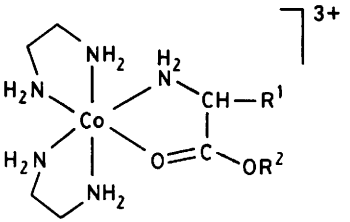
$$Cu^{III}(H_{-3}Aib_3a) \overset{pK=12.5}{\rightleftharpoons} Cu^{III}(H_{-4}Aib_3a) + H^+$$

where H_{-n} refers to the number of coordinated deprotonated nitrogens. The predominant form (pH 0.25 to 12.5) has the structure (26). In strong acid the terminal amide nitrogen is protonated, and coordination to the metal is by the amide oxygen. In strong base the amine nitrogen is deprotonated and remains coordinated to copper(III). These complexes show variable photochemical sensitivity upon irradiation into their ligand-to-metal charge transfer (LMCT) bands. The principal peptide oxidation products from photolysis of $Cu^{III}(H_{-3}Aib_3a)$ at pH5 and $Cu^{III}(H_{-4}Aib_3a)^-$ in 1.0M sodium hydroxide are substituted hydantoins which are proposed to form by a metal-assisted intramolecular nucleophilic reaction.

Interactions of terbium(III) with a synthetic γ-carboxy-



(26) $Cu^{III}(H_{-3}Aib_3a)$



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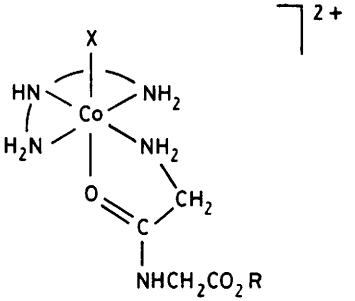
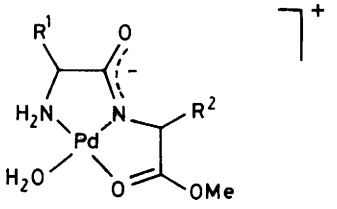


(28) X = Cl or NO_2



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glutamic acid containing loop corresponding to bovine prothrombin residues 17-23 (^{17}Gla-Cys-Leu-Gla-Gla-Pro-^{23}Cys) have been studied.[191] The results are compared with the metal ion binding properties of intact bovine prothrombin fragment 1, bovine prothrombin residues 1-39, and simple Gla- and Gla-Gla-containing peptides. The heptapeptide forms a 2:1 metal:peptide complex with dissociation constants of 5.2 x 10^{-6}M and 2.4 x 10^{-6}M for 1:1 and 2:1 metal:peptide complexes. It is concluded that the 17-23 structure plays a central rôle in determining the functional metal ion binding properties of the intact prothrombin molecule.

Reactivity.- The use of chelated amino acid esters in cobalt(III) complexes of the type (27) offer interesting possibilities for peptide synthesis. The cobalt(III) ion acts as both an N-protecting and activating group. A recent paper[192] describes a study of the acylation of the chelated amino acid ester complex $[Co(en)_2(l\text{-PheOMe})]^{3+}$ by other amino acid esters. The reaction of the cobalt complex with l-PheOMe and l-PheOBu^t in Me_2SO resulted in the formation of 18% of the racemised peptide d-Phe-l-Phe after removal of cobalt. The $[Co(en)_2(peptide)]$ complexes formed were also observed to undergo further racemisation of the amino acid residue bound to cobalt(III) when they were dissolved in neutral aqueous solution. It is suggested that this large degree of racemisation makes the use of these chelated cobalt(III) complexes unsuitable for the general synthesis of biologically active peptides. However, Tasker *et al.*[193] have previously reported that a number of peptides could be synthesised by this route without appreciable racemisation. Further investigations are required to fully define the potential of this method of peptide synthesis.

A number of cobalt(III) complexes of the general type *mer*-$[Co(dien)(dipeptideOR)X]^{2+}$ (X = Cl,NO_2) have been prepared and characterised.[194] These complexes have the general structure (28) in which the peptide is carbonyl-bonded to cobalt(III). Base hydrolysis of the peptide bond in the carbonyl bonded glycyl peptides was studied at 25°C and I = 0.1 mol dm^{-3}. The rate constants k_{OH} for peptide bond hydrolysis fall within the range 0.67-0.88 mol dm^{-3} s^{-1}. Base hydrolysis of the complexed peptide is *ca.* 2 x 10^4 times than for the uncomplexed peptide ligand at 25°C. The system provides an

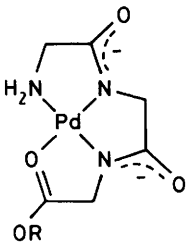
interesting model for the Zn(II)-dependent peptidases such as carboxypeptidases A and B.

The palladium(II)-promoted hydrolysis of the methyl esters of glycyl-L-leucine, glycyl-L-alanine and L-alanylglycine has been studied at 25°C and I = 0.1 mol dm^{-3} in the pH range 4-5.[195] At a 1:1 metal to ligand ratio, the peptide esters act as tridentate ligands, donation occurring via the terminal amino group, the deprotonated amide nitrogen, and the carbonyl group of the ester as shown in (29). Due to the high Lewis acidity of Pd(II), rapid hydrolysis of the ester function by water and hydroxide ion occurs. Rate constants k_{OH} and k_{H_2O} have been obtained for base hydrolysis and water hydrolysis of the coordinated peptide esters at 25°C. The rate constants for base hydrolysis are 3.4×10^6 dm^3 mol^{-1} s^{-1} (L-AlaGlyOMe), 6.4×10^6 dm^3 mol^{-1} s^{-1} (Gly-L-AlaOMe) and 2.3×10^7 dm^3 mol^{-1} s^{-1} (Gly-L-LeuOMe). Base hydrolysis of the coordinated peptide esters is at least 10^6 fold that of the free unprotonated ligand.

The palladium(II) promoted hydrolysis of the ester function of methyl, ethyl and isopropyl glycylglycylglycinate has also been studied in the pH range 4-5.[196] In this case complexes of the type (30) are formed, and rate enhancements for ester hydrolysis are also 10^6 fold when comparisons are made with the free ligand.

Crystal Structures.- The crystal structure analysis of the 2:1 adduct of cyclosarcosylsarcosine with copper(II) perchlorate shows that the independent unit is composed of six water molecules octahedrally coordinated to the Cu(II) ion, two tetrahedral perchlorate ions and four independent halves of cyclosarcosylsarcosine molecules lying on crystallographic centres of symmetry.[197] All available hydrogens of water molecules are involved in hydrogen bonding as donors and all carbonyl oxygen atoms of the cyclic peptide molecules function as acceptors. A similar crystallographic study[198] of the 2:1 adduct of cyclosarcosylsarcosine with silver(I) nitrate shows that the Ag(I) ion interacts directly with the carbonyl oxygen atoms of the peptide. The crystal is held together by strong coulombic interactions between the silver and the nitrate ions and by ion-dipole interactions between the silver ion and the peptide.

The activation of bleomycins (BLM), a family of



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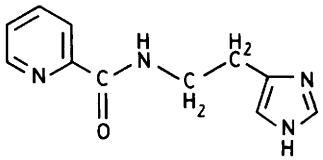
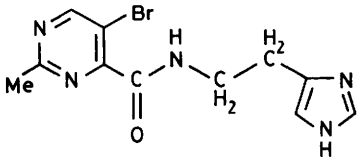


(31) PypepH



(32) PmpepH

glycopeptide antibiotics, in the presence of metal ions such as Fe(II) and Cu(II) and dioxygen, and subsequent catalytic cleavage of double-stranded DNA has attracted considerable attention in recent years.[199-201] Copper(II) complexes of two peptides, PypepH (31) and PmpepH (32) resembling fragments of the metal-chelating section of bleomycins have been isolated and their structures determined by X-ray crystallography.[202] The complex $[Cu(Pypep)(CH_3COO)]_2 \cdot 1.46\ H_2O$ has a coordination geometry around copper which is approximately square pyramidal. The acetate ions bridge between the two copper centres via one oxygen atom. In $[Cu(Pmpep)(CH_3COO)(H_2O)]$ the acetate ion is bidentate and the sixth coordination site is occupied by a water molecule. Due to the small "bite" of the acetate ligand, the coordination geometry around copper is highly distorted. In methanol and DMF solutions, both complexes give rise to a monomeric tetragonal Cu(II) e.s.r. spectrum. Similarities in various spectroscopic properties of the two complexes and Cu(II)-BLM raise questions regarding the structure of the coordination sphere of copper in Cu(II)-BLM proposed on the basis of preliminary structural data on the P3A peptide complex of copper.[203]

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