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Amino Acids and Peptides

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A Specialist Periodical Report

Amino Acids and Peptides
Volume 22

A Review of the Literature Published during 1989

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Preface

During the year under review, peptide science continued its relentless growth. Output still increases, beyond, it seems, control. What might loosely be called straight peptide chemistry has more than five thousand workers engaged in it full-time, producing around a thousand publications a year and flocking to international meetings in their hundreds. Furthermore, what is published on some topics is, as we all know, less complete (and sometimes, one suspects, less interesting) than that which is not, because commercial considerations impose paranoid secrecy. With the subject now so extensive, and its aficionados so numerous, the emergence of specialised societies was inevitable. Many countries have had semi-formal peptide discussion groups for some time (the UK *Peptide and Protein Group* was initiated under the auspices of the Chemical and Biochemical Societies as long ago as 1968), and an informal European Peptide Committee has been organising symposia for over thirty years. This movement has now gone a stage further with the formation of, and approval of governing statutes for, a *European Peptide Society*, in 1989; this was followed by similar formalitites for the birth of an *American Peptide Society* and a *Japanese Peptide Society*, both early in 1990. In only a few months the enrolment of the *European Peptide Society* had risen to over seven hundred, powerful evidence of the field's strength.

Balliol College, Oxford *John Jones*

Contents

Abbreviations

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

1
Amino Acids

BY G. C. BARRETT

1 Introduction

The literature that is oriented towards chemistry and biochemistry of amino acids is covered in this Chapter, which has, as usual, been confined to their occurrence, chemistry and analysis. Routine literature covering the natural distribution of well-known amino acids is excluded.

Commentary on some papers is brief, so that adequate discussion can be offered for other papers where more significant synthetic and mechanistically-interesting chemistry is reported. Patent literature is almost wholly excluded but this is easily reached through Section 34 of Chemical Abstracts. The Chapter is arranged into sections as used in all previous Volumes of this Specialist Periodical Report, and major Journals and Chemical Abstracts (to Volume 112, issue 11) have been scanned to discover the material to be reviewed.

2 Textbooks and Reviews

Several books[1,2] and Conference Proceedings Volumes[3] have appeared. Reviews cover N-hydroxyamino acids,[4] distribution of D-amino acids,[5] biosynthetic pathways in plants,[6] and natural amino acids as enzyme inhibitors.[7]

Recent IUPAC-IUB Joint Committee for Biochemical Nomenclature recommendations in a number of areas including amino acids, have appeared in Journals.[8]

3 Naturally Occurring Amino Acids

3.1 Isolation of Amino Acids from Natural Sources.- This Section was introduced to this Chapter last year even though it would be thought of as a routine aspect of the literature. The generation of artefacts through extraction procedures and the ever-more-sensitive analytical methods for amino acids, clearly increase the scope for erroneous conclusions on the presence of amino acids in natural sources.

Ultrafiltration using a membrane impervious to molecules of size >2KDa allows amino acids and small peptides to be separated from proteins that have been partly degraded using poly(hydroxyethyl methacrylate)-immobilized carboxypeptidase.[9] At the smallest scale level, amino acids can be isolated from proteins that have been separated by SDS-PAGE and electroblotting on to a poly(vinylidene fluoride) membrane, excised, and hydrolysed by gas-phase hydrochloric acid.[10] At the other end of the scale, isocratic "moving-withdrawal" chromatography is advocated for separation of amino acids,[11] and isolation of amino acids as their arenesulphonate salts has been studied.[12] High recoveries of air-labile amino acids can be achieved from acid hydrolysates conducted in microcapillary tubes free from air.[13] Development of a microwave acid hydrolysis method for proteins (e.g., requiring 6-8 min irradiation of a peptide attached to a Merrifield resin suspended in propanoic acid - conc HCl, using a domestic microwave oven)[14] has been reported.

Adsorption of glycine, aspartic acid and lysine to glass beads from solutions at three different pHs has been studied.[15] Protonated lysine is adsorbed more strongly than the others from acidic solutions. A review of preparative scale ion-exchange chromatographic separation of amino acids has appeared.[16]

3.2 Occurrence of Known Amino Acids.- Significant results sifted from the continuously expanding routine literature under this heading include a distinction between racemic, therefore contemporary, coded amino acids and other amino acids more recently acquired by dinosaur egg shells,[17] and a note (in a useful review of the distribution, stereochemistry, and stable isotope composition of amino acids in fossils and modern mollusc shells),[18] of the first observation of the occurrence of DL-glutamic acid in a Pleistocene-age Merceneria fossil shell.

Non-protein amino acids in meteorites, have been argued[19] to have formed from protein amino acids after decarboxylation and deamination, rather than indicative of any particular alternative living system based on amino acids.

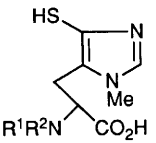
Non-protein amino acids from sources on this planet include (2S,3S)-3-hydroxyleucine, (2S,3R)-3-hydroxylysine, and Z-3-chlorodehydroalanine from HV-toxin M of the phytopathogenic fungus Helminthosporium victoriae.[20] Analogous results from higher species include the presence of β-tyrosine and N-methyl-β-bromotryptophan in Jasplakinolide, a novel antifungal anthelminthic 19-membered ketide-depsipeptide from the marine sponge Jaspis,[21] and β-D-aspartylglycine in the fish Aplysia

kurodai.[22] 4-Hydroxyisoleucine from fenugreek (Trigonella foenum-graecum) possesses (2S,3R,4S)-stereochemistry,[23] not (2S,3R,4R) as previously reported. The (2S,3S,4R)-diasteroisomer occurs in the form of its lactone as a moiety of funebrine from Quararibea funebris.[24]

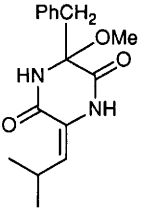
Ovothiols A-C (1) are natural π-N-methyl-4-mercaptohistidines that are shown in the pre-1986 literature erroneously as τ-methyl isomers.[25]

3.3 New Natural Amino Acids.- This heading covers derivatives and near-relatives, not only the amino acids themselves. The first example of a natural α-methoxy-α-amino acid derivative, megasporizine (2) from Penicillium megasporum NHL 2977,[26] is a member of the dioxopiperazine family, in this case a modified cyclo(phenylalanyl-leucyl). While this is not a rare type of natural product, nevertheless the phenylalanine - leucine combination is most unusual. The β-hydroxy-L-α-amino acid derivative (obafluorin, 3) is a useful broad-spectrum antibiotic.[27] A unique C_{20} β-amino acid "Adda" (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid, is a moiety of the cyclic penta- and heptapeptide cyanobacteria hepatoxins, modularin and microcystin-LR, respectively.[28,29]

3.4 New Amino Acids from Hydrolyzates.- "Dehydrobutyrine", O-methylthreonine, and N-methylasparagine are not new, but are unusual company for 14-chloro-2-hydroxy-3-amino-4-methylpalmitic acid and another nine known amino acids in pawainaphycin C, a cardioactive cyclic peptide from the blue-green alga Anabaena BQ-16.[30] Marine organisms are also represented as sources of Dolastatin 15 (a depsipeptide from the marine mollusca Indian Ocean sea hare Dolabella auricularia), that contains the hitherto unknown phenylalanine biosynthetic product (4; "dolapyrrolidone").[31] Theonellamide F, a dodecapeptide from the marine sponge Theonella contains seven common amino acids and (2S,3R)-3-hydroasparagine, (2S,4R)-2-amino-4-hydroxyadipic acid, p-bromo-L-phenylalanine, (3S,4S,5E,7E)-3-amino-4-hydroxy-6-methyl-8-(p-bromophenyl)-5,7-octadienoic acid, and a bridging amino acid, τ-L-histidinoalanine, not previously encountered in proteins.[32] A C-2 tryptophanyl - N^{im}-histidinyl linkage, with the tryptophanyl residue also linked through C-6 to the β-carbon of a substituted leucyl residue, is a notable feature of the cyclic octapeptide moroidin, from Laportea moroides, a bush prevalent in Eastern Australian rain forests.[33]



(1) Ovothiol A; $R^1 = H$, $R^2 = H$
Ovothiol B; $R^1 = H$, $R^2 = Me$
Ovothiol C; $R^1 = Me$, $R^2 = Me$



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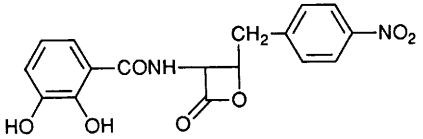

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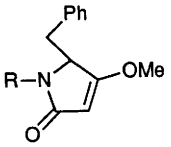

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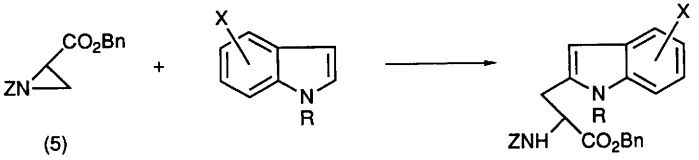

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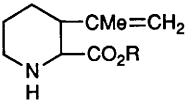
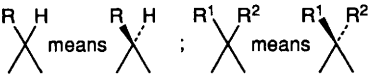


(7)

Reagents: i, $NaBH_4$; ii, NH_4OH

Scheme 1

Three-dimensional features of molecules are depicted throughout this Chapter as follows: horizontally-ranged atoms and bonds and ring atoms are to be understood as being in the plane of the paper; substituent atoms and groups attached to these are to be understood to be ABOVE the page if ranged LEFTWARDS and BELOW the page if ranged RIGHTWARDS



4 Chemical Synthesis and Resolution

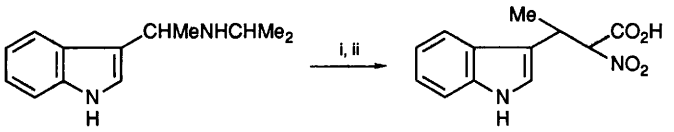
4.1 General Methods for the Synthesis of α-Amino Acids. – All standard general methods, some in new formats, are represented in the recent literature. Many of the general methods used in the area of asymmetric synthesis (next Section, 4.2) are also applicable in general synthetic routes to α-amino acids.

Reviews have appeared of aminocarbonylation,[34] synthesis of hydroxylated amino acids from epoxy- and aziridino-pyranoses,[35] and β-lactams as synthons for amino acids.[36]

Alkylation of glycine derivatives and near relatives is as popular as ever, with Schiff bases providing most of the non-routine interest. Thus, γ-allenic α-amino acids are obtained by alkylation of $Ph_2C{=}NCHLiCO_2Me$ with allenic phosphonates $(EtO)_2P(O)OCHR^1R^2CR^3{=}C{=}CR^4R^5$ in the presence of a palladium(II) catalyst.[37] Acylation of $Ph_2C{=}NCH_2CO_2Et$ with an aroyl halide after deprotonation to the delocalized aza-allyl anion $[Ph_2C{\cdots}N{\cdots}CHCO_2Et]^-Na^+$, gives N-aroylaziridinecarboxylates.[38] Chiral N-benzyloxycarbonyl aziridines have been prepared from L-serine and used for the synthesis of optically-pure benzo-substituted tryptophans (5 → 6),[39] and a similar use ("amidoethylation") of N-tosylaziridine t-butyl esters involving their ring-opening with organocuprates has been reported.[40] The route to these aziridines is through Sharpless epoxidation of allyl alcohols to give optically-active glycidic esters, these being azidolyzed and treated with PPh_3.[41] Schiff bases "the other way round" such as $Me_3SiN{=}CHR^1CO_2R^2$, prepared from the keto-acid and $LiN(SiMe_3)_2/ClSiMe_3$,[42] and $Me_2C{=}CH(CH_2)_3N{=}CHCO_2R$,[43] have been used in α-amino acid synthesis through reduction in the former case (overall, amination of a keto-acid) and trans-selective cyclization to 3-propenylpipecolic acid esters (7) in the latter case.

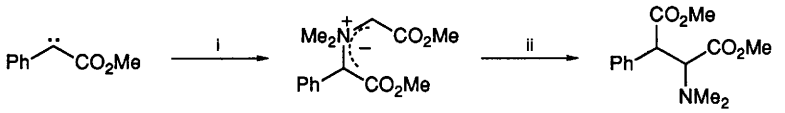
Carbonylation of amines and amides is represented by reaction of CO with carbenium immonium ions generated from N-hydroxymethylamides and imides, to give N-acylglycines, the dehydration - carbonylation process being recognisable as an extension of the Koch-Haaf reaction.[44]

N-Benzoyl-2-bromoglycine methyl ester is a well-known amino acid synthon, and undergoes substitution with alkylnitronate carbanions $R^1R^2NO_2C^-$ to give corresponding β-nitroalkylglycines, suitable substrates for elimination to "dehydro-amino acids".[45] 2-Ethoxyglycine derivatives $AcNHCH(OEt)CONHCH_2Ph$, prepared by the amidoalkylation reaction, undergo analogous substitution reactions.[46] Other standard methods are represented in the azlactone synthesis for the synthesis of β-alkylaminoalaninamides (Scheme 1)[47] and in the alkylation of methyl nitroacetate (Scheme 2) for the synthesis of β-methyltryptophan as a



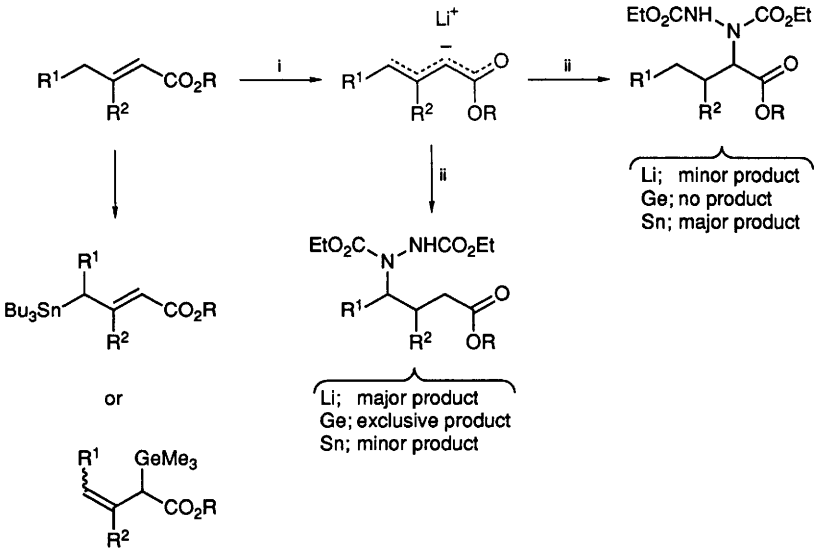
Reagents: i, $NO_2CH_2CO_2Me$; ii, H_2/Ni

Scheme 2



Reagents: i, $Me_2NCH_2CO_2Me$; ii, heat

Scheme 3



Reagents: i, LDA/HMPA; ii, $EtO_2CN{=}NCO_2Et$

Scheme 4

mixture of diastereoisomers.[48] The (2RS,3SR)-diastereoisomer crystallized out as the sole product as a result of epimerization in solution of the other diastereoisomer.[48]

Stevens rearrangement of the carbene - tertiary amine adduct in Scheme 3 is an ingenious alternative approach to using a glycine derivative in α-amino acid synthesis.[49]

Not too remote, structurally, from these glycine derivatives, is t-butyl N-(diphenylmethylene)oxamate, $Ph_2C{=}NCOCO_2Bu^t$, prepared from t-butoxalyl chloride and diphenyl ketimine. It reacts with phosphorus ylides to give "dehydro-amino acid" derivatives $Ph_2C{=}NC(=CR'R^2)CO_2Bu^t$, readily reducable to corresponding α-amino acid derivatives using sodium cyanoborohydride.[50] α-Oximino-esters $RC(=NOH)CO_2R$ are readily reduced to corresponding α-amino acid derivatives using $NaBH_4$ - titanium(III) chloride.[51]

These are novel details for standard approaches to α-amino acids, generally under the headings of amination of a carboxylic acid derivative or carboxylation of an amine. An example of the latter route is electrocarboxylation of imines with sacrificial metal anodes in membrane-free cells (e.g. $PhN{=}CHPh \rightarrow PhNHCHPhCO_2H$).[52] The "amination" approach is more widely represented, further examples including a new twist to the recently established use of azodicarboxylate esters as nitrogen source leading to very high regioselectivity in amination of lithium dienolates or Sn-masked or Ge-masked dienolates (Scheme 4) and giving α- or γ-amino acid derivatives.[53] A classical amino acid synthesis *via* α-azidoalkanoates can be completed by a one-pot conversion into the corresponding N-Boc-amino acid ester using H_2/Pd-C/Boc_2O.[54] Amination of α-keto-acids and esters is another classical route, new versions being the use of benzotriazole (BtH) for promoting the reaction of an amide with glyoxylic acid or one of its esters [$RCONH_2 + OHCCO_2Et + BtH \rightarrow RCONHCH(Bt)CO_2Et \rightarrow RCONHCH(NH_2)CONH_2$ with NH_3].[55] The transamination of phenylglycine with 2-oxoglutaric acid in the presence of N-dodecyl-pyridoxal chloride and of hexadecyltrimethylammonium chloride is the first example of mild non-enzymic transamination through the *in vivo* mechanism in the *absence* of metal ions.[56]

Less commonly-used general methods include the Ugi "Four Component Condensation" method, found to give an unexpected *cis*/*trans* distribution of products in a particular case.[57] Another route employing an isocyanide uses an aminocarbene - chromium(III) complex $(CO)_5Cr{-}CPh{=}N^+{=}CPhOCOPh$ + Bu^tNC to give C-aminoketenimines $ButN{=}C{=}CPhN{=}CPhOCOPh$, which cyclize to imidazolidin-5-ones in solution, or which add water

when treated with wet silica to give $Bu^tNHCOCPh(COPh)NHCOPh$ from which the corresponding amino acid can be obtained by hydrolysis.[58]

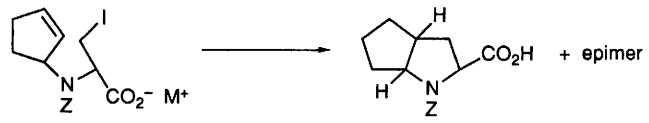
A standard hydantoin synthesis has been applied to the synthesis of 2,6-diaminopimelic acid, starting from piperidine-2,5-dicarbonitrile, and reacting it with $NH_4OH/(NH_4)_2CO_3$ at 100°C during 4 hours.[59]

At the start of this section, well-established uses of glycine derivatives in general synthetic methods for other α-amino acids have been discussed. Of course, modifications to side-chains of other simple α-amino acids should also be discussed here, insofar as they offer general synthetic routes, though a dilemma results from the way this Chapter has been organized over the years. "Specific Reactions of Amino Acids" (Section 6.3) covers such chemistry, and readers seeking coverage of this topic should scan both these parts of this Chapter in each Volume. β-Iodo-L-alanine, from L-serine, yields the corresponding alkylzinc iodide through ultrasonically-activated reaction with zinc, and then can be elaborated into 2-amino-4-oxoalkanoic acids with acyl chlorides.[60] Radical cyclization of N-substituted iodo-L-alanine derivatives using $Bu_3SnH/AIBN$ provides a route to ring-fused prolines (Scheme 5).[61]

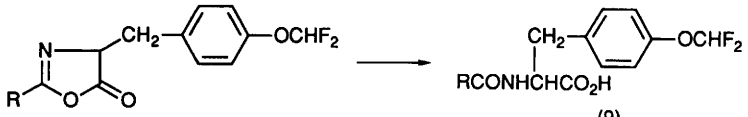
Creation of a carbanion α to the side-chain carbonyl group of β-methyl α-t-butyl N-benzyloxycarbonyl-L-aspartate[62] and the N-trityl-L-glutamic acid analogue[63] using 2.2 equivalents of lithium diethylamide (or lithium hexamethyldisilazide) at -78° can be followed by quenching with electrophiles, alkyl halides giving β-substituted aspartic acids and carbonyl compounds yielding γ-substituted glutamic acids.

4.2 Asymmetric Synthesis of α-Amino Acids.- There are some fascinating new approaches as well as equally satisfying studies that consolidate well-established methods. Several reviews[64-68] and Williams' book[2] are available. The reviews include a broad survey with 222 references,[65] some "Chemtracts" in which the work of Kunz and Pfrengle[66] and of Williams[67] is discussed, and reviews by Hegedus[68] of his own work, the use of chromium - carbene complexes in amino acid synthesis (see Vol.21, p.7).

Chirally-imprinted polymers are amazingly effective, all things considered, for some chiral recognition applications (see Section 7.5), and a crosslinked poly(styrene) imprinted through polymerization of the appropriate monomer mixture containing chiral additives, washed out from the polymer to create cavities containing salicylaldehyde and phenylboronic acid moieties, has been used in asymmetric synthesis of amino acids. The polymer-bound salicylaldehyde, converted into the salicylideneglycine Schiff base and complexed with nickel(II) ions and



Scheme 5



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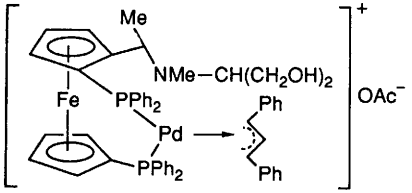

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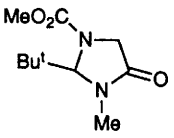

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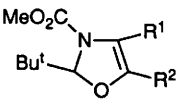

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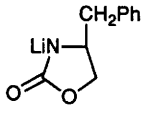

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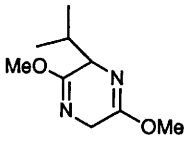

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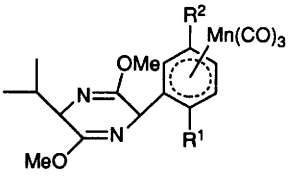

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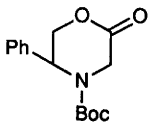

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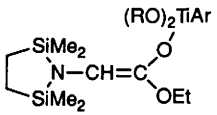


(17)

treated with acetaldehyde, gives L-threonine in slender enantiomeric excess, though 36% e.e. is obtained for a synthesis of L-DOPA on the same principle.[69]

To the familiar crop of papers reporting homogeneously-catalyzed asymmetric hydrogenation of acetamidoacrylates, using rhodium - chiral phosphine catalysts - Rh(chiral tetrasulphonated diphosphine),[70] cationic Rh(2S,4S-N-(t-butoxycarbonyl-4-[[bis(4'-methoxy-3',5'-dimethylphenyl)]phosphin]-2-([[bis](4'-methoxy-3'5'-dimethylphenyl)]methyl]pyrrolidine)$_2$ perchlorate [≡ Rh(NBD)$_2$ClO$_4$ for short],[71] for example. In the latter report, very high efficiency is claimed, but the literature on the general topic is still well-populated with disappointingly low enantioselectivities. Molecular graphics - molecular orbital calculations might come to the rescue, following an assessment through this approach, that for the system employing [Rh(S,S-chiraphos)]$^+$ X$^-$ as chiral homogeneous catalyst, 6 of the 8 possible modes of catalyzed H_2 addition to 2-acetamidocinnamates generate impossibly large atom - atom interactions.[72] A salutary warning arises in the observation that silica-bound chiral rhodium - phosphine complexes are capable of catalyzing ^1H - ^2H exchange during reaction of $^2H_2$ with (Z)-2-acetamido cinnamates in methanol.[73]

Catalytic reductive aminolysis of oxazol-5(4H)-ones also continues to disappoint in the same context, Ni - catalyzed reaction of the *p*-difluoromethoxybenzyl oxazolone (8 → 9) with H_2 in the presence of (S)-(-)-phenylethylamine giving less than 55% diastereoisomeric excess of the free acid, better than the 9 - 18% d.e. found for the same aminolysis product from hydrogenation of the corresponding azlactone and its *in situ* aminolysis by (S)-(-)-phenylethylamine.[74] On the other hand, Pd-catalyzed asymmetric allylic amination by benzylamine, of allylic substrates (RCH=CHCHRX → RCH=CHCHRNHCH$_2$Ph) in the presence of the diphosphine (10) and elaboration of the products into α-amino acids, gives better than 97% e.e.[75]

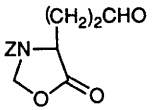
Seebach's group (*e.g.* Vol. 20, p.30) has demonstrated the high diastereoselectivity that can be achieved through alkylation of lithium enolates of 2-t-butylimidazolinones (11). Results for these substrates, derived from dipeptides by reaction with pivalaldehyde, have now been published in full.[76] A chosen enantiomer of an amino acid is obtainable through use an appropriate enantiomer of (11), and accounts of the preparation of β-arylalanines, ω-halogenoalkyl-α-amino acids, and aspartic acid, and studies of inversion of configuration by deprotonation (and α-^2H - α-^1H substitution) are given in further papers from this group.[77-79] Corresponding chiral 2-t-butyl N-methyloxycarbonyloxazolines (12; R^1 = R^2 = H, Me; R^1 = H, R^2 = CO_2Me)

can be prepared on a 100g scale from an enantiomer of serine or of threonine, a notable feature of the route being an electrochemically-operated key step.[78] These compounds undergo some standard reactions in a stereoselective mode, including Vilsmeier and Friedel-Crafts reactions at C-5 and cycloaddition to C = C (with attack from the face of the molecule remote from the t-butyl group).[79]

β-Methylphenylalanine[80] and β-methyltyrosine[81] enantiomers have been synthesised through the bromination and substitution of the chiral imide enolate (13) using the pivalic mixed anhydride of the appropriate (R)- or (S)-3-arylbutanoic acid.

The remaining papers under this heading are "asymmetric versions" of standard general methods of α-amino acid synthesis, starting either from the ingredients for constructing the -NH-CHR-CO- moiety itself, or starting from a substituted glycine. The latter group is represented by the well-established Schöllkopf synthon (14), shown to react with arene - $Mn(CO)_3$ cations to give correspondng dienyl-$Mn(CO)_3$ complexes (15) that could be regarded otherwise as D-arylglycine derivatives.[82] Another familiar chiral glycine synthon, the N-acylated 2S-phenyloxazinone (16), undergoes highly enantiospecific alkylation after conversion into its lithium enolate, as described in a most thorough overview.[83]

Aldol condensation of an aldehyde with the glycine ester - titanium carbohydrate complex (17; R' = a chiral 2-deoxyfuranose) gives D-threo-β-hydroxy-α-amino acids as predominant enantiomers. Use of the aldehyde (18) in this reaction gives (2R,3S,6S)-3-hydroxy-6-aminoheptanoic acid.[84] Synthesis of erythro-β-hydroxy-L-histidine using (R)-3-bromoacetyl-4-isopropyl-1,3-oxazolidin-2-one (19) as aldol partner for 4-formyl-τ-N-tritylimidazole has been reported.[85] Modest chiral induction is seen in condensation of chalcone with diethyl N-acetamidomalonate catalyzed by quaternary (-)-N-methylephedrinium salts to give (20).[86] Schiff bases formed with chiral carbonyl compounds are more familiar chiral glycine derivatives for use in asymmetric synthesis of α-amino acids. For example, the carbanion formed from the camphor - glycine t-butyl ester Schiff base (21) with n-butyllithium is capable of highly diastereoselective Michael addition to αβ-unsaturated esters (100% in the case of 2-alkylidenemalonates, to give anti-3-substituted D-glutamic acids).[87] Curiously, given the emphatic nature of this result, no stereoselection was observed in alkylation reactions with the norcamphor homologues.[88] In a related study of a synthesis of (S)-(+)-2,4-diaminobutanoic acid hydrochloride,[89] the chiral imine (22) provides poor levels of asymmetric induction through alkylation by $BrCH_2CN$, but 100% e.e. when Mg^{2+} or Bu_4N^+ salts are present. Synthesis



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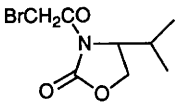

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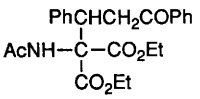

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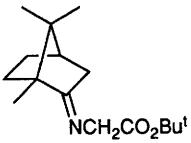

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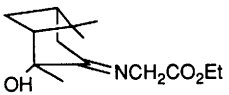

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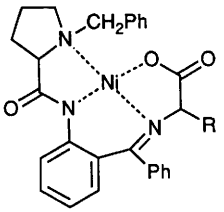

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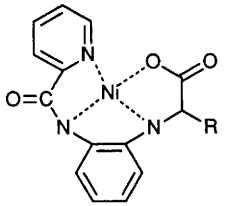

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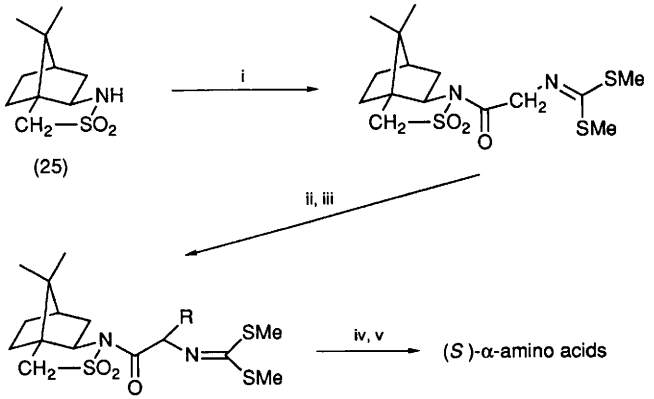


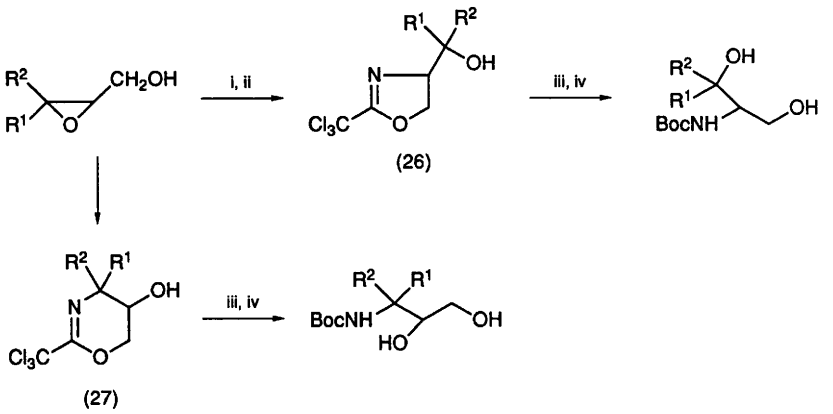
Reagents: i, $(MeS)_2C{=}NCH_2CO_2Me$, Me_3Al, toluene; ii, Bu^nLi, THF, –78°C, or $NaOH/Bu^n{}_4N^+HSO_4^-$; iii, RHal, HMPA; iv, 0.5M aq. HCl; v, aq.LiOH

Scheme 6

of α-allyl glycine has been investigated, through allylation of analogous chiral glycine imines (i.e. a chiral centre in the ester moiety as well as in the carbonyl compound used for imine formation). This involves "double asymmetric induction", by which the authors mean that the enantiomeric excess is contributed to by asymmetric induction involving two chiral centres.[90] Further development by its inventors, of asymmetric α-amino acid synthesis using the nickel-complexed glycine Schiff base (23) is described for the α-bromoglycine analogue (23; R = Br).[91] Substitution by carbanions and work-up to lead to L-aspartic acid and L-norleucine in enantiomeric purities 80 and 68%, represents some improvement over the usual technique whereby the carbanion is created in the chiral imine moiety, illustrated in the synthesis of (S)-5-(benzyloxy)tryptophan, (S)-α-allylglycine and (S)-β-(2-naphthyl)alanine.[92] Previous reports (Vol.21, p.12) have been extended further, using these synthons to give (S)-N-(α-naphthylmethyl)proline.[93] In this latter report, and in a study[94] of a new variant (24) of this synthon, alkylation of alanine analogues (e.g., 24; R = Me) has been included in an attempt to develop better routes to α-methyl analogues of common α-amino acids.

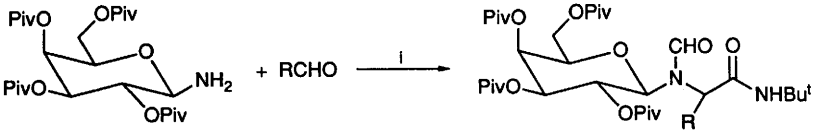
Phase-transfer alkylation of one of the simplest Schiff bases, e.g. $Ph_2C{=}NCH_2CO_2R$, in the presence of 0.2 equivalents of a cinchona alkaloid as chiral catalyst, gives 4-chloro-D-phenylalanine in >99% e.e., with 4-chlorobenzyl bromide as alkylating agent, but based on the fortuitous ease of removal of about 70% racemate by recrystallization.[95] Addition of cyanide ion to an imine derived from an enantiomer of a chiral benzylalkylamine using a cyanide - haemin copolymer complex gives α-amino acids in 80-95% e.e. after hydrolysis.[96] The e.e. is considerably higher than that of the same system in the absence of the haemin copolymer.

The cyclic chiral sulphonamide (25 in Scheme 6) is capable of generating 100% optically pure (S)-amino acids with return of the chiral auxiliary, when used to form the amide of the glycine-derived Schiff base $(MeS)_2C{=}NCH_2CO_2Me$, and reacted with n-butyllithium, then with an alkyl halide in HMPA.[97] Oxazolines are a masked form of Schiff base, and give optically-active β-alkyl-β-hydroxy-α-amino acids on hydrolysis when formed from α-keto-esters and methyl isocyanoacetate in the presence of a chiral (aminoalkyl)ferrocenylphosphinegold(I) catalyst.[98] Like many similar studies with initial promise, more examples are needed to establish whether there is any consistent efficiency for the process. Chiral oxazolines (26) and oxazines (27) formed from chiral 2,3-epoxyalkan-1-ols (Scheme 7) after conversion into trichloroacetimidate esters, can be converted into erythro-β-



Reagents: i, $CCl_3CN/DBU\text{-}CH_2Cl_2$, 0°C; ii, Lewis acid catalyst; iii, 2M-HCl in dioxan; iv, $(Boc)_2O/KHCO_3$-dioxan

Scheme 7



Reagents: i, Bu^tNC, HCO_2H, $ZnCl_2$, Et_2O-THF

Scheme 8

hydroxy-α-amino acids and α,α-disubstituted glycines, respectively, through routine oxidative cleavage.[99] An amination process is also represented in the use of the chiral pyridinophane-pyridoxamine analogue (28) for non-enzymatic transamination with 2-keto-alkanoic acids catalyzed by zinc(II) salts.[100] (R)-Amino acids are formed in modest preponderance, but a puzzling result is the finding that the macrocyclic sulphide from which (28) is prepared leads to higher e.e.s in the same process.

Photochemical amination of chiral silyl ketene acetals (29) with ethyl azidoformate gives N-ethoxycarbonyl-L-alanine esters with modest diastereoselectivity (70% as the best example so far).[101]

Use of carbohydrate templates for asymmetric synthesis of α-amino acids features in papers extending earlier studies. One report[102] describes a further use for 2,3,4-tri-O-pivaloyl-1-amino-α-D-arabinose, as the amine component of an otherwise standard Ugi synthesis (Scheme 8); another[103] involves a lengthy route from the 1,2-O-isopropylideneglucofuranose derivative (30) to β-hydroxy-α-amino acids through uneventful chemistry except for the replacement of the OH group of (30) by RNMe with the same configuration (through a double inversion sequence).

4.3 Synthesis of Protein Amino Acids and Other Naturally Occurring α-Amino Acids.- Standard methods are illustrated for a substantial number of the current papers under this heading, and a number of papers in the preceding sections would find an appropriate place here. However, readers seeking access to this topic should, in the interests of economy with space (as in previous Volumes), expect also to have to scan the other relevant Sections of this Chapter.

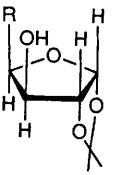
Fermentative production of protein amino acids continues to generate a substantial volume of literature, and mention is now made of representative reviews,[104] and papers on less-routine aspects. The success of a multi-enzyme system for D-amino acid synthesis with simultaneous co-enzyme regeneration is based on the high substrate specificity and thermostability of alanine racemase, and the low structural specificity but high stereoselectivity of D-amino acid transferases.[105] Access to certain amino acids through *in situ* processing of easily-available fermentation products (*e.g.* L-tryptophan formed from L-glutamic acid semi-aldehyde in 48% yield by reaction with refluxing aqueous phenylhydrazine) will become an increasingly attractive option.[106]

Natural non-protein α-amino acids include many with relatively simple structures, while others pose considerable synthetic problems.

Solutions to these problems also contribute to the development of generally applicable synthetic methods in organic chemistry, and new work meets the challenges with some style. At the simpler structural level, α-t-butyl N-Boc-L-aspartate is the starting point for a new synthesis of L-α-aminosuberic acid.[107] Only two previous (lengthy) stereospecific syntheses of this compound have been published, and these are now joined by the side-chain functional group conversion $-CO_2H \rightarrow -CON(OMe)Me \rightarrow -CHO \rightarrow -CH=CH(CH_2)_2CO_2H$ (the latter step employing the Wittig reagent $Ph_3P^+(CH_2)_3CO_2H\ Br^-$. All four configurational isomers of β-benzylglutamic acid have been prepared for screening as acyclic analogues of kainoids, starting with a serine-derived oxazoline for preparing the (2R,4R,5S)-isomer and its enantiomer, and starting with a $\Delta^{3,4}$-pyroglutamic acid derivative for preparing the other two stereoisomers.[108] Standard functional group elaboration of these starting materials is involved in these syntheses.

The apparently greater structural complexity of polyoxamic acid (2-amino-2-deoxy-L-xylonic acid) is in fact something of a benefit when the closeness in its structure to readily available monosaccharides is appreciated. Ring-opening by PhS^- of a 5-carbon hydroxylated aziridine, easily obtained from L-arabinose (Scheme 9) is a key step in new route,[109] and a nice example of a [3,3]-allylic trifluoracetimidate rearrangement is featured in another synthesis of this compound (Scheme 10).[110] Protected D-serinal (31) is a convenient starting point for such compounds, christened "glycosyl α-amino acids",[111] through elaboration of the aldehyde functional group, e.g. to (32) and familiar subsequent steps.

Cycloadducts formed from N-arylidene derivatives of simple α-amino acid esters with electron-deficient alkenes (e.g. 33 from methyl acrylate)[112] are easily elaborated into variously-substituted prolines, vying with an alternative approach, e.g. a synthesis of the C2-symmetric (2S,5S)-5-carboxyproline constituent of the marine alga Schizymenia dubyi, from (S)-1-benzyloxy-2-benzoylamino-5-hexene (Scheme 11).[113] (3R,5R)-Carbapenam-3-carboxylic acid has been synthesized from D-glutamic acid and found to be enantiomeric with the natural product from Serratia Erwinia.[114] Smaller and larger non-aromatic nitrogen heterocyclic α-amino acids are represented in an optimized preparation of nicotianamine by trimerization of (S)-2-azetidine-2-carboxylic acid,[115] and in a stereoselective synthesis of $\Delta^{4,5}$-pipecolic acids through a [3,3]σ-rearrangement (Scheme 12).[116] (3S)-Carboxy-(4S)-hydroxy-2,3,4,5-tetrahydropyridazine, an unusual component of Luzopeptin A, has been synthesized via the chiral epoxide (34 in Scheme



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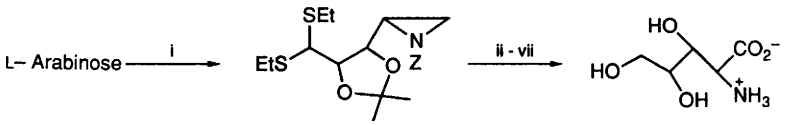
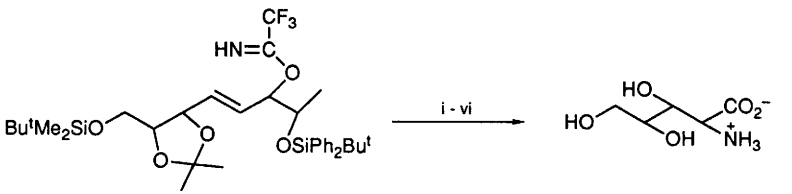
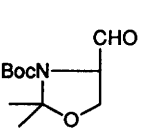


Reagents: i, protection, aziridination *via* azide; ii, PhS^-; iii, $I_2/NaHCO_3$;
iv, PhS— → $PhSO_2$—;$PhSO_2$—CH< → —CO_2H; v, $(EtS)_2C<$ → —$CH_2OTBDPS$; vi, H_2—Pd/C; vii, aq. TFA



Reagents: i, Xylene, heat, 20 h; ii, $NaBH_4$, EtOH; iii, $(Boc)_2O$, Et_3N; iv, O_3, Me_2S/MeOH; v, RuO_4; vi, TFA

Scheme 10



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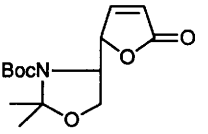

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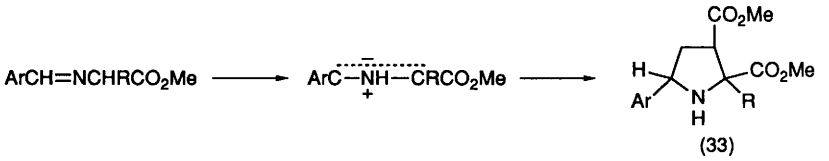

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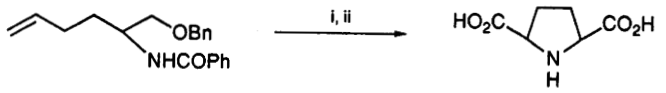
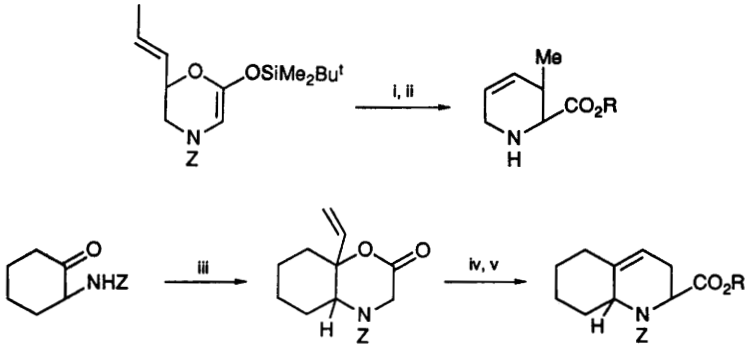


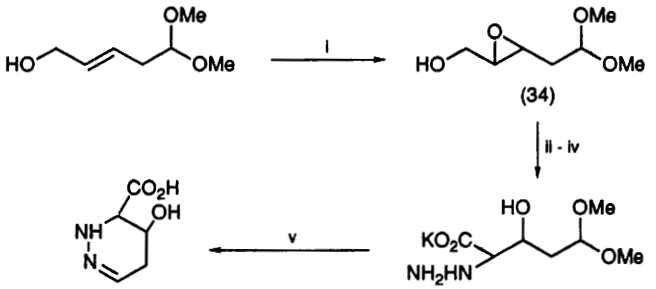
Reagents: i, I_2(3 equivalents), MeCN; ii, routine elaboration $BnOCH_2$– ⟶ HO_2C–

Scheme 11



Reagents: i, Toluene, reflux; ii, H_2/Pd-C; iii, CH_2=CHMgBr, then $BrCH_2CO_2Et$ Et_3N; then ZCl, then catalytic TsOH; iv, $Bu^tMe_2SiOCOCF_3/Et_3N$; v, toluene, reflux

Scheme 12



Reagents: i, Bu^tOOH, $Ti(OPr^i)_4$, L-(+)-diethyl tartrate; ii, RuO_4; iii, CH_2N_2; iv, NH_2NH_2/K_2CO_3; v, TFA

Scheme 13

13), a route similarly demonstrated for a synthesis of trans-3-hydroxy-L-proline.[117]

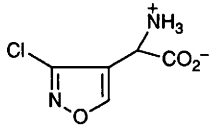
Heterocyclic side-chains are featured in some important non-protein α-amino acids, such as the isoxazole acivicin (alias AT-125) whose methyl analogue (35; Me in place of Cl) has been synthesised (as a stereoisomer mixture) from MeC≡NO and α-vinylglycine as described in a carefully scripted account.[118] A new synthesis of acivicin (AT-125) and its biosynthetic source, N^6-hydroxyornithine, can be operated so as to introduce ^{2}H-labelling at C-3 and C-4.[119] Diethyl aminopropyl acetamidomalonate on condensation with anisaldehyde, gives 4-MeO-C_6H_4-CH=NCH$_2$CH$_2$CH$_2$C(NHAc)(CO$_2$Me)$_2$ from which the nitrone 4-MeO-C_6H_4-CH=N$^+$(O$^-$)CH$_2$CH$_2$CH$_2$C(NHAc)(CO$_2$Me)$_2$ was prepared via the oxaziridine. Established routes lead to the named amino acids. Another acivicin synthesis study[120] applied to the preparation of analogues employs familiar steps exploiting asymmetric induction to achieve modest enantiomeric yields.

Ovothiols (1)[28] have been prepared in various standard ways, including the Schöllkopf method using (14) and the S-protected mercaptoimidazole (36) or from the corresponding aldehyde (Scheme 14).[121]

The only aromatic side-chain to be featured in this section this year appears in iso-dityrosine, a naturally-occurring crosslinking protein amino acid from cell wall glycoproteins. A straightforward synthesis from two appropriately protected L-tyrosines has been achieved through the Ullmann coupling reaction.[122] L,L-Isodityrosine has also been an attractive target for other research groups. It has been prepared through construction of the biphenyl moiety first, then to build the alanine chain on to each aryl ring (Scheme 15).[123] Use of the Schöllkopf piperazine (14) has been illustrated in a further iso-dityrosine synthesis.[124]

4.4 α-Alkyl Analogues of Protein Amino Acids.- These continue to offer useful pharmacological and enzyme inhibitory properties (e.g. α-aminoisobutyric acid offers considerable merit as a wood-rotting fungus control agent). They are accessible either through routine general methods, particularly the hydantoin route starting from a ketone, but also through Schiff bases, such as 4-Cl-C_6H_4-CH=NCHRCO$_2$Me. These undergo efficient α-arylation with (arene)halotricarbonyl chromium(II) complexes.[125] Chiral half-esters (37) of monosubstituted malonic acids yield di-anions with 2 equivalents of lithium di-isopropylamide, reactions with alkyl halides offering a versatile asymmetric synthesis.[126]

An efficient synthesis of α-trifluoromethyl-α-amino acids (see Vol.21, p.20) through Ireland-Steglich rearrangement of allyl or benzyl



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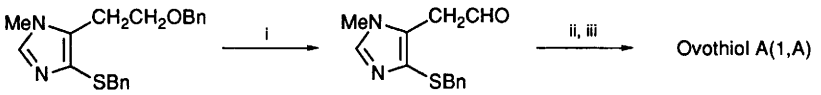

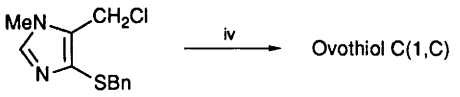

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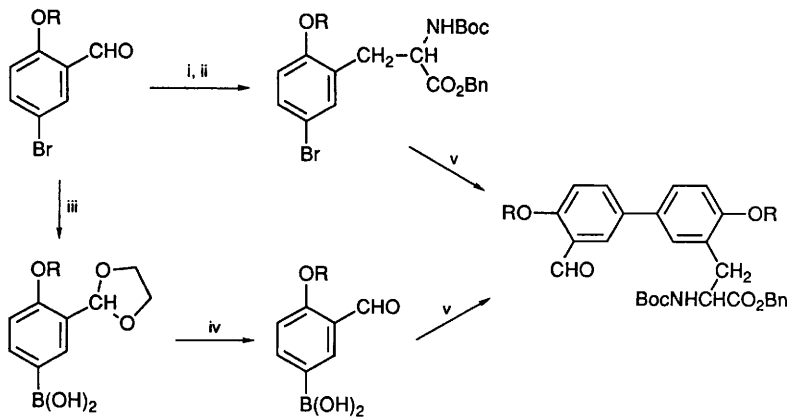
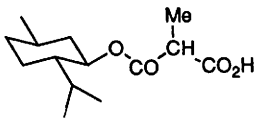
Reagents: i, BF_3/Et_2O, $HSCH_2CH_2SH$; ii, ClCOCOCl, DMSO; iii, NaCN, $Me_2\overset{+}{N}H_2Cl^-$;
iv, Schöllkopf piperazine (14), conventional elaboration

Scheme 14



Reagents: i, $BocNHCH[P(O)(OMe)_2]CO_2Bn/KOBu^t$, –60°C→20°C; ii, H_2-$[Rh(dipamp)]^+$;
iii, propane-1,3-diol, BF_3Et_2O, Mg, then $B(OMe)_3$; iv, H_3O^+;
v, $Pd(Ph_3P)_4$/THF/2M-Na_2CO_3

Scheme 15



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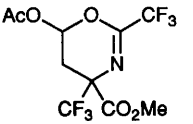

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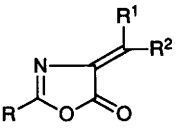

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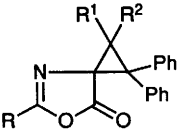

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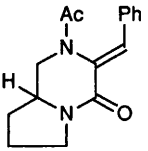

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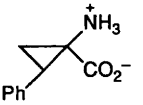
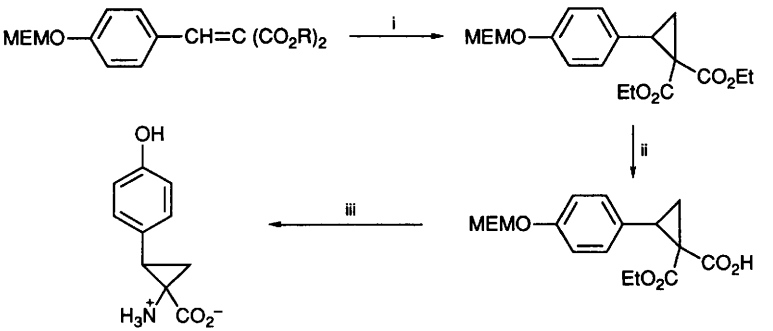


(42)

(2*S*, 3*S*, 4*R*)-isomer
(43)



Reagents; i, Me_3SOI, NaH, DMSO; ii, aq.NaOH; iii, Curtius rearrangement, deprotection

Scheme 16

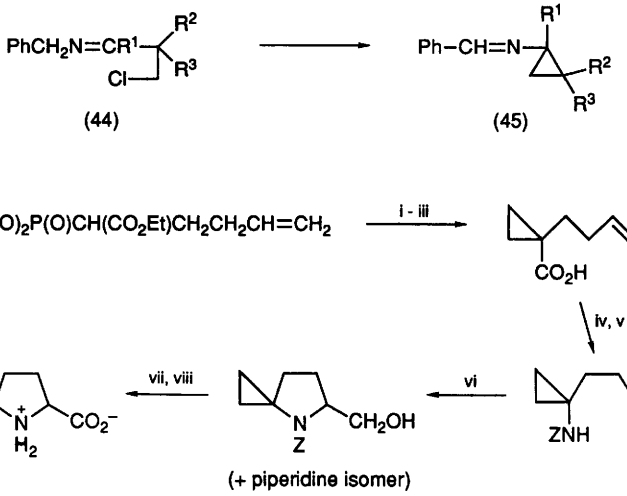
enol ethers of 4-trifluoromethyl-2-phenyloxazol-5(4H)-ones has been fully developed.[127] α-Trifluoromethyl aspartic acid α-methyl ester has been obtained from the oxazine (38), methanolysis with HCl in aq MeOH being followed by $KMnO_4$-H_2SO_4 oxidation.[128]

4.5 Alicyclic α-Amino Acids and Close Relatives.- An increasing number of examples under this heading, some known in natural sources, others found to show useful properties, is being featured in the literature.

Construction of a cyclopropyl ring on to a C = C (39 → 40),[129] (41 → 42)[130] illustrates some standard approaches. (2S,3S,4R)- and (2S,3R,4S)-α-(Carboxycyclopropyl)glycines (43 is the 2S,3S,4R-isomer) have been synthesized,[131] the latter being a potent neuroactive amino acid.[132] The routes involve the palladium(II)-catalyzed stereoselective cyclopropanation of αβ-unsaturated pyrrolidone and γ-amino-δ-lactones respectively, obtained from L-glutamic acid, as key steps, followed by routine development towards the products. The other isomers were reported on recently (see Vol.21, p.24). Another example of this growing family of "2,3-methano-amino acids" is 2,3-methanotyrosine (Scheme 16) formed as racemates of E- and Z-isomers through cyclopropanation of the cinnamate, and classical elaboration of the malonates through Curtius rearrangement.[133]

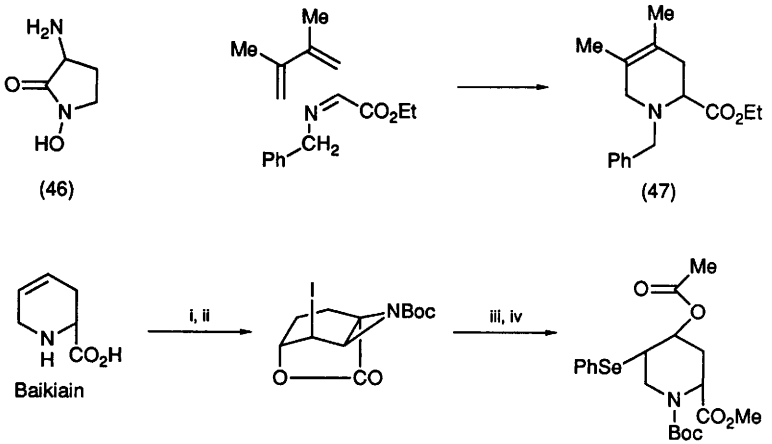
1-Aminocyclopropenecarboxylic acid has been synthesized from dimethyl diazomalonate and bis(trimethylsilyl)acetylene,[134] a previously-published method,[135] and shown to be a poor source of acetylene in plant tissue (the saturated analogue is the natural source of ethylene), but a good inhibitor of ethylene synthesis *in vivo*. Aminocyclopropanecarboxylic acid (ACC) synthase is responsible for converting S-adenosylmethionine into ACC (and is inactivated by S-adenosylmethionine during the process), while L-α-vinylglycine is a competitive inhibitor of the enzyme.[136] The C = N function (44 → 45[137]) is also amenable to cyclopropanation[137,138] (see also Refs. 38 and 109) and aziridine formation, respectively, the former case showing a reaction with chloro-imines, with the interesting feature of electron supply originating at a benzylic carbanion.[137] Aziridine synthesis has already been referred to in Section 4.1, covering "General Methods of Synthesis of α-Amino Acids".

4-Substituted prolines have been synthesized as conformationally-constrained analogues of some common amino acids, through alkylation at C-4 of α-t-butyl γ-methyl Fmoc-L-glutamate after conversion into its enolate anion.[139] A novel analogue of proline with a cyclopropyl ring grafted on at C-5 has been synthesized through the route in Scheme



Reagents: i, NaH, benzene, catalytic EtOH; ii, oxirane; iii, KOH-aq.EtOH; iv, $(PhO)_2P(O)N_3$; v, $PhCH_2OH$; vi, RCO_3H; vii, CrO_3-Me_2CO; viii, Me_3SiI

Scheme 17



Reagents: i, Boc_2O; ii, I_2/KI/H_2O/$NaHCO_3$/r.t.; iii, PhSeCl/Et_3N/CH_2Cl_2; iv, MeOH

Scheme 18

17.[140] The novel glycine antagonist 3-amino-1-hydroxypyrrolidin-2-one (46) is an exciting example of the new generation of neuroprotective agents that is emerging; it was synthesized from D- or L-methionine *via* routine elaboration of the derived S-benzylsulphonium salt.[141]

Pipecolic acid derivatives are accessible (*e.g.* 47) through aza-Diels-Alder reactions of dienes and iminium salts from benzylamine ethyl glyoxylate in DMF in the presence of 1 equiv TFA and catalytic traces of water.[142] Baikiain is a naturally-occurring, unsaturated, pipecolic acid that has been largely ignored as a chiral starting material in imino acid synthesis (and in organic synthesis more generally, perhaps). Iodolactonization (Scheme 18) followed by nucleophilic ring-opening is a good start to an active life for this compound.[143] For other examples of iodolactonization, see Ref.389.

4.6 Models for Prebiotic Synthesis of Amino Acids.- The topic has its fascination for a number of reasons, not only the need to find a credible basis for the genesis of what are now recognized as essential components for life processes, but also for unravelling reactions of amino acids subjected to different energy sources. The topic has been reviewed,[144] and familiar experiments have been repeated, often with novel variations.

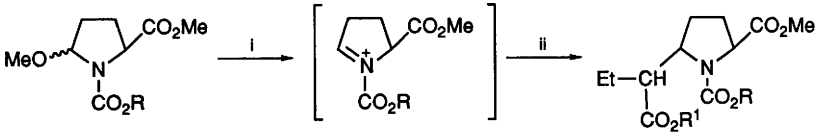
Irradiation during 5 hours, of mixtures of CO, CO_2, N_2, and H_2O by 3.0 MeV protons, simulating cosmic rays and solar flare particles, gives glycine, alanine, aspartic acid, and β-alanine, in yields higher than in all previous experiments with such mixtures (but with different energy sources).[145] Photoreduction (365 nm) of N_2 in H_2O catalyzed by TiO_2 for 1 - 4 hours at 20 - 220° to give ammonia, and the formation of glycine, alanine and serine in this mixture when acetaldehyde is added, has been demonstrated.[146] A system that is unusually sophisticated in physical terms, for this type of study, employs tunnelling spectroscopy to study events at the alumina barrier in Al - AlO_x - Pb tunnelling junctions exposed to aqueous ammonia, wet CO gas, and aqueous HCHO vapour. Spectra very similar to those of authentic glycine are obtained, very different to what is seen in the absence of a nitrogen source. For the CO - HCHO - H_2O system, spectra indicate the formation of a sugar-like polymer.[147] It has long been fashionable, and still is, to consider what precursors there may be for amino acids that are eventually formed in these experiments, and to study their behaviour under the same conditions. Thus, methyleneaminoacetonitrile $CH_2{=}NCH_2CN$ reacts with water to give glycine and simple glycine derivatives.[148]

4.7 α-Alkoxy α-Amino Acids.- Because of their value in some general α-amino acid synthetic methods, and as precursors to reactive intermediates for organic synthesis of other systems, these somewhat transient O,N-acetals (best prepared in the case of protected prolines by anodic methoxylation)[145] lend themselves to nucleophilic substitution, as illustrated in Scheme 19 for a carbapenem synthesis.[150] Phthalimido α-methoxyglycine t-butyl ester derivatives serve as starting materials in a novel Cephalosporin C synthesis (Scheme 20).[151]

4.8 Halogenoalkyl α-Amino Acids.- The simplest possible example, a protected α-fluoroglycine of known configuration (X-ray crystal structure) has been prepared by amination of a chiral fluoro-iodo-acetamide (48 in Scheme 21).[152] The value of halogen analogues of common amino acids in deceiving natural processes by causing hiatuses in metabolic pathways is illustrated by roles for αα-difluoromethyl ornithine, as an effective anti-parasitic agent (trypanosomes from tsetse fly) following apparent success established some time ago for cancer therapy.[153]

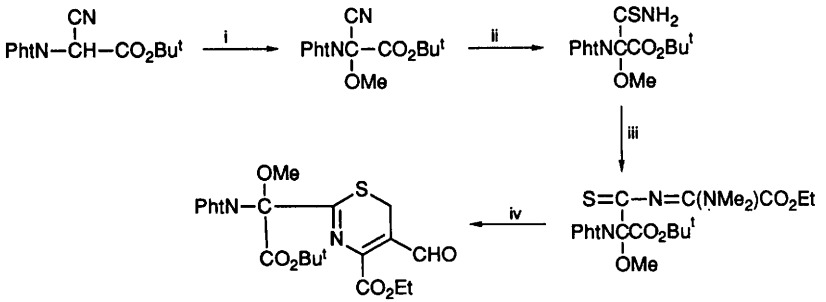
This sort of potential lies behind the synthesis of fluorinated methionines,[154] where the scope for the fluorination of the methyl group of the amino acid itself has been investigated. Methionine sulphoxide reacts with diethylaminosulphur trifluoride to give the easily-hydrolyzed monofluoromethyl analogue, and access to the di- and trifluoromethyl analogues is through a roundabout route: N-acetyl-DL-homocysteine + $ClCHF_2$ gives the hitherto-unknown difluoromethyl analogue, while the trifluoromethyl compound is prepared through the same route in low yield using CF_3I with photochemical activation.[154] ββ-Difluoro-γ-keto-homophenylalanine is a novel kynurenase inhibitor formed from $PhC(OSiMe_3)=CF_2$ and a protected α-chloroglycine.[155] A lengthy route is also required for the provision of (2R,3R)- and (2R,3S)-3-fluoroglutamic acids, the use of glutamate dehydrogenase assuring the delivery of these stereoisomers.[156] Claisen condensation of diethyl oxalate with ethylfluoroacetate gives $EtO_2CCF=C(ONa)CO_2Et$, α-substitution leading to $EtO_2CCH_2CF(CO_2Et)COCO_2Et$ acceptable to the enzyme for reductive amination.[156] A correction has been published[157] for the paper describing the synthesis of 3-fluoroaspartic acid.[158]

A new efficient synthesis of 3,3,3-trifluoro-alanine, permitting easy access to its α-^2H or α-^3H analogues (as do general routes, on which this synthesis is based, leading to unsaturated analogues), involves condensation of CF_3COCO_2Et with $RCONH_2$ followed by trifluoroacetic anhydride - pyridine → $CF_3C(CO_2Et)=NCOR$.[159] New efficient syntheses of 4,4,4-trifluorovaline, 5,5,5-trifluoronorvaline, 5,5,5-



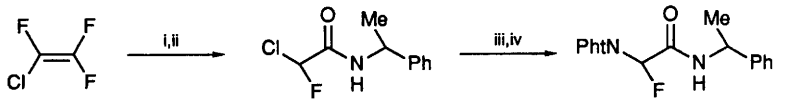
Reagents: i, Lewis acid; ii, $EtCH{=}C(OR^1)OSiMe_3$

Scheme 19



Reagents: i, NBS, $AgBF_4$, MeOH; ii, H_2S; iii, Me_2NCOCO_2Et; iv, $CH_2{=}CHO$

Scheme 20



(48)

Reagents: i; (*S*)-$PhCHMeNH_2$; ii, boiling 10% aq.H_2SO_4; iii, NaI-Me_2CO;
iv, KNPht-DMF with each diastereoisomer

Scheme 21

trifluoroleucine, 6,6,6-trifluoronorleucine, 4,5,6,7-tetrafluorotryptophan, and α-(trifluoromethyl)-β-alanine use amidocarbonylation of 2-(trifluoromethyl)propanal and 3-(trifluoromethyl)propanal for the first two; and the azlactone route for the others.[160] Enzymatic resolution provides D- and L-enantiomers.

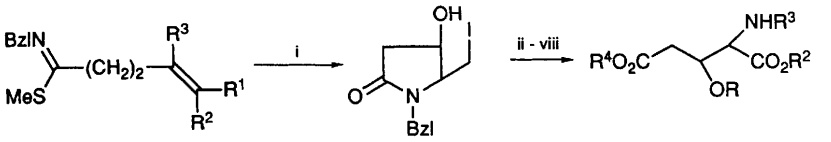
4.9 Hydroxyalkyl α-Amino Acids.- Optically-active epoxy-alkanols prepared by Sharpless kinetic resolution - epoxidation can be aminolysed and elaborated into β-hydroxy-α-amino acids.[161] An unrelated D-chirospecific synthesis of these compounds from N-benzenesulphonyl-L-serine involves addition of vinyl or allylmagnesium bromide, or (methylthio)methyl-lithium, to the carboxy group to form the corresponding ketones, then oxidation of the original serine side-chain to a carboxy group.[162] (2S,3R)-3-Hydroxyglutamic acid can be obtained through iodolactamization of γδ-unsaturated thioimidates (Scheme 22).[163]

Isomers with hydroxy groups elsewhere in the side-chain, as well as these β-hydroxyalkyl-α-amino acids, are mentioned elsewhere in this Chapter, but this section is sustained for discussion of methods capable of variation so as to constitute general syntheses. D-Xylose serves as starting material for Z-2-amino-5-hydroxypent-3-enoic acid lactone (Scheme 23).[164]

Hydroxylated 1-azabicyclo[3.1.0]hexanes feature in a synthesis of (2S,3S,4S)-3-hydroxy-4-methylproline, an echinocandin constituent (Scheme 24).[165]

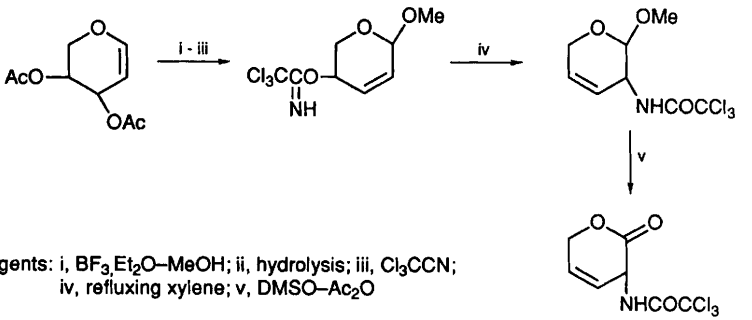
4.10 α-Amino Acids with Unsaturated Side-chains.- The chemistry of αβ-unsaturated α-amino acids ("dehydro-amino acids") has become somewhat routine, and omitted from this section (though mentioned elsewhere in this Chapter: 4.1 General Methods of Synthesis). βγ-Unsaturated analogues are more interesting in their own right, and as potential enzyme inhibitors since L-3,4-dehydro-amino acids mimic many of the conformational features of their saturated analogues. L-Vinylglycine lives up to such promise, and hearsay to the effect that it is unstable has been largely annulled. Enol ether analogues $ROCH{=}CHCH(NHX)CO_2Me$ are prepared through Wittig-type reactions of alkoxy-aldehydes with $(MeO)_2P(O)CH(NHX)CO_2Me$ followed by double bond isomerization to give the trans-isomer, using lithium di-isopropylamide followed by NH_4Cl.[166]

Other familiar functional group transformations to the same end include alkylation by aldehydes (*i.e.* aldol formation) α to the sulphonyl group in (2R)-2-Boc-amino-3-benzenesulphonyl-1-(2-tetrahydropyranyl-oxy)propane derived from L-serine (or its 2S-



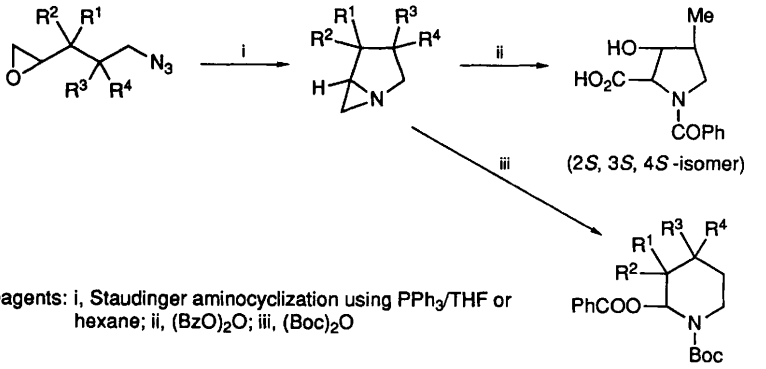
Reagents: i, I_2 –THF; ii, KOH; iii, Ac_2O; iv, TBDMSCl; v, Na/NH_3;
vi, $(Boc)_2O$,NaH, CH_2N_2; vii, $Bu^n{}_4N^+F^-$; viii, O_2–Pt

Scheme 22



Reagents: i, BF_3,Et_2O–MeOH; ii, hydrolysis; iii, Cl_3CCN;
iv, refluxing xylene; v, DMSO–Ac_2O

Scheme 23



Reagents: i, Staudinger aminocyclization using PPh_3/THF or
hexane; ii, $(BzO)_2O$; iii, $(Boc)_2O$

Scheme 24

enantiomer), [BocNHCH(CH$_2$OTHP)CH$_2$SO$_2$Ph → BocNHCH(CH$_2$OTHP)CH(SO$_2$Ph)CH(OH)R → BocNHCH(CH$_2$OTHP)CH=CHR] followed by oxidation of the original serine side-chain to CO$_2$H.[167] Similar elaboration of N-trityl-L-glutamate esters for the same purpose[168] [using Me$_2$NCH(OMe)$_2$ as dehydrating agent, instead of Ac$_2$O as in the preceding citation]. In a paper easily accessible only through its abstract, mention is made of syntheses of L-(Z)-3,4-dehydronorvaline, L-(E)-3,4-dehydro-ornithine, and L-2,6-diamino-4-hexynoic acid.[169]

4.11 α-Amino Acids with Aromatic and Heteroaromatic Side-chains.- β-Iodo-L-alanine reacts as its organozinc derivative, with aryl iodides at 50° in the presence of bis(tri-o-tolylphosphine)palladium chloride, to give variable yields of β-arylalanines.[170] Some heteroaryl examples are included, and a parallel paper from this group[60] describes similar applications of the same derivatives.

4',α-Dimethylhistidine, a new H2 agonist, has been synthesized from 4-ethoxycarbonyl-5-methyl-3-N-benzylimidazole, through development of the ester function → -CHO → -CH=CMeNO$_2$ → -CH$_2$-CMe=NOH → -CH$_2$COMe and Bucherer-Bergs amino acid synthesis.[171]

4-Bromohomo-ibotenic acid has been synthesized in the same general routes (building the amino acid moiety on to the functionalized hydroxyisoxazoles) as used (see Vol.21, p.20) for the closer analogues to the natural product.[172]

4.12 N-Substituted α-Amino Acids.- Excluding protected derivatives for peptide synthesis, this Section is represented by N-hydroxyamino acids,[4] and by α-hydrazino acids, prepared by the base-induced conversion of diacylhydrazides ArCHBrCONHNHCOR into corresponding hydantoins, followed by work-up as for the hydantoin amino acid general synthesis.[173]

4.13 α-Amino Acids Containing Phosphorus Functional Groups.- Enantioselective synthesis of D-(-)-2-amino 5-phosphonopentanoic acid, a potential N-methyl-D-aspartic acid antagonist, illustrates further the enantiospecific alkylation of the chiral oxazinone (16), in this case by BrCH$_2$CH=CHP(O)(OEt)$_2$.[174] A recipe for the preparation of the racemate of this amino acid, using the same alkylating agent but with the Schiff base Ph$_2$C=NCH$_2$CO$_2$Et as substrate, is operated on the 50g scale.[175] The near-homologue, the "bialaphos" constituent L-phosphonotricene H$_3$N$^+$CH(CO$_2^-$)CH$_2$CH$_2$P(O)(OH)Me, has been synthesised through the Ugi route, and resolved using chymotrypsin-catalyzed

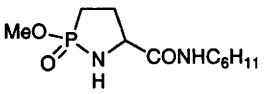
hydrolysis of its di-ethyl ester.[176] The N-hexylamide of its cyclic analogue (49) is also described in this study.

This section continues to exclude all but representative citations of amino acids in which the carboxy group is replaced by a phosphorus oxy-acid moiety. Phosphonate analogues of phenylalanine and lysine ethyl esters have been synthesized as potential serine protease inhibitors.[177]

4.14 Labelled Amino Acids.- As this Chapter unfolds, the standard methods of amino acid synthesis are encountered repeatedly in different contexts. However, these are overlaid with much ingenuity in cases where isotopic labelling must be stereospecific as well as regiospecific, for biosynthetic and other mechanistic studies.

Excellent examples are based on (2S,3S)- and (2R,3R)-[2,3-$^{2}H_{2}$]oxiranes used in a synthesis of chirally-labelled homoserine.[178] The oxiranes are prepared by asymmetric epoxidation of (E)-3-(triphenylsilyl)-2-propenol, itself prepared from 2-propynol.[179] The Schöllkopf bis-lactim ether prepared from (R)-(+)-2-methyl-3-phenylalanine and the homoserine lactone derived from $^{2}H_{2}$ and methyl (Z)-2-acetamido-4-methoxybut-2-enoate is used in a synthesis of (1S,2R)- and (1S,2S)-[2-^{2}H]-1-aminocyclopropane-1-carboxylic acids.[179] A large scale preparation of (2S,3S)-[2,3-$^{2}H_{2}$]- and (2S,3R)-[3-^{2}H]aspartic acid from labelled fumaric acids uses previously-established chemistry but benefits from the use of immobilized aspartase-containing *E.coli* whole cells as catalyst.[180] More routine work is covered in a preparation of DL-[4-^{3}H]glutamic acid for clavulanic acid biosynthesis studies, using the Bucherer-Bergs general α-amino acid synthesis starting from the aldehyde $MeO_{2}CCH^{3}HCH^{3}HCHO$, itself derived from the di-anion of methyl hydrogen succinate and $CF_{3}CO_{2}{}^{3}H$.[181] Preparation of DL- and L-[4,5-$^{3}H_{2}$]leucine by catalyzed $^{3}H_{2}$ addition to the corresponding unsaturated amino acids also represents a standard method for hydrogen isotope-labelling.[182] Methyl-labelled L-methionines (either $C^{3}H_{3}$- or $^{14}CH_{3}$-labelled) prepared from the appropriate iodomethanes and L-homocysteine, were used to demonstrate the presumed precursory *in vivo* role of methionine for S-adenosyl-L-methionine.[183]

Representative of the continuing trickle of papers describing the synthesis of ^{11}C-labelled amino acids is a one-pot preparation of [4-^{11}C]GABA using H^{11}CN (from $^{11}CO_{2}$) entrapped in KOH-THF in the presence of the aminopolyether "Kryptofix 2.2.2" for Michael addition to ethyl acrylate. A special feature, seen in the fuller accounts of this topic given in preceding Volumes of this Specialist Periodical Report, is the need to produce the final product and use it (usually for whole body tomography) within the shortest possible time, bearing in mind the



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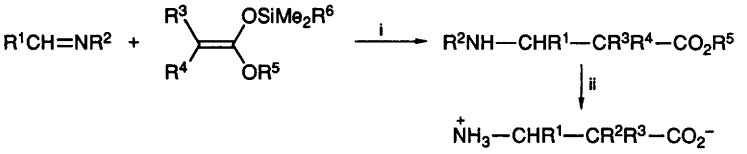
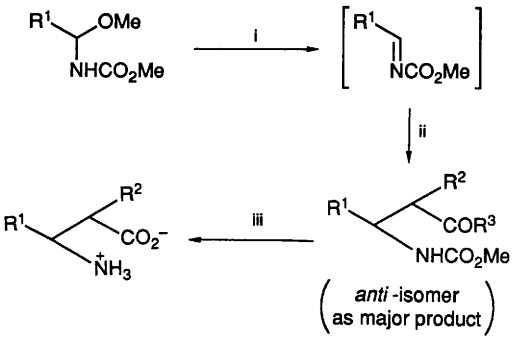


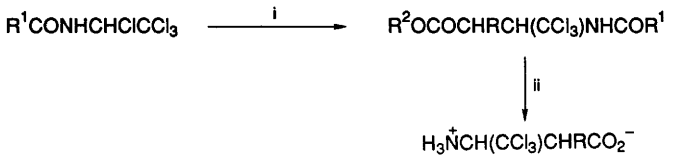
Reagents: i, $[R_3P^+]_2O(CF_3SO_3^-)_2$; ii, deprotection and H_3O^+

Scheme 25



Reagents: i, 2 equiv.LDA/THF/–78°C; ii, $R^2CH{=}CR^3O^-M^+$; iii, as ii, with $TiCl_x(OR)_{4-x}$; (R = Pr^i, $x = 0 \longrightarrow$ *syn*-isomer preferentially)

Scheme 26



Reagents: i, $RCH_2CO_2R^2$, base; ii, hydrolysis

Scheme 27

short half-life of the ^{11}C isotope. At 40 minutes for the Michael addition step in the GABA preparation, to be followed by other steps, the final radiochemical yield is low.[184] δ-Aminolaevulinic acids, $H_3N^+(CH_2)_4CO_2^-$, ^{13}C-labelled at each of the five carbon atoms, have been prepared from ^{13}C-glycine, from ^{13}C-Meldrum's acid, or from ^{13}C-sodium acetate, through standard functional group operations as appropriate for an ω-amino acid.[185]

DL-[1-^{14}C]Penicillamine is accessible from Me_2CHCHO through carboxylation of the derived 2,2,4,4-tetramethylthiazoline.[186]

^{17}O- and ^{18}O-Labelled L-tyrosines and ^{13}C-analogues of some of these, have been prepared from the O-labelled 1- and 4-^{13}C and 2- and 3-^{13}C-labelled phenols metabolized by the bacterium Erwinia herbicola.[187]

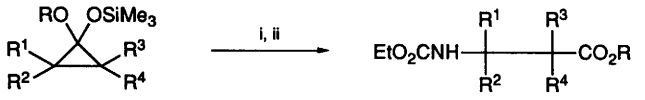
Demethylation of biosynthesized ^{35}S-L-methionine with $Na-NH_3$ gives ^{35}S-L-homocysteine lactone.[188]

Radiobromination of m-tyrosine gives 75% 6-bromo-isomer, the rest being an unidentified isomer.[189]

4.15 β- and Higher Amino Acids.- An increasing number of papers is being published under this heading, a trend that is especially noticeable in the year under review. The surge is not stimulated by any recent novel biological discoveries but more as a display of newly-established synthetic methodology presented as organic chemistry in its own right. Having said that, there are several biologically-important compounds under this heading, many with significant stereochemical challenges.

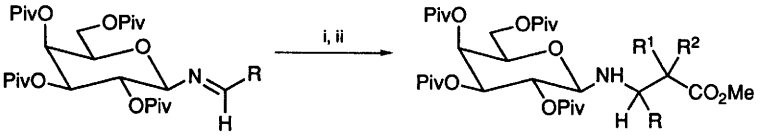
β-Amino acid esters are formed by the addition of imines to ketene silylacetals, catalyzed by diphosphonium salts $R_3P^+-O-^+PR_3$ $(CF_3SO_3^-)_2$ (Scheme 25),[190] or to other Michael receptors of a simpler type (αβ-unsaturated esters, ketones, and aldehydes).[191] N-(α-Methoxyalkyl) carbamates $MeO_2CNHCHR'OMe$ generate N-methoxycarbonylimines in situ, to add to enolate anions giving diastereoisomer mixtures (Scheme 26).[192] Benzyl vinylcarbamate undergoes concurrent alkylation (with benzyl acetoacetate sodium salt) and carboxylation (with CO) catalyzed by $PdCl_2$, to give β-substituted β-amino acid derivatives.[193] Other interesting methods of constructing these compounds have been described, α-amidoalkylation of simple alkanoates (Scheme 27),[194] and ethoxycarbonylamination with ring-opening of cyclopropane acetals (Scheme 28).[195]

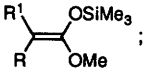
Asymmetric synthesis prospects are as good for this family of amino acids as for any other, and the general imine alkylation route already mentioned[102] (Scheme 8) operates with the increasingly familiar 2,3,4-tri-O-pivalyl-1-amino-α-D-arabinose to favour the (S)-configuration at



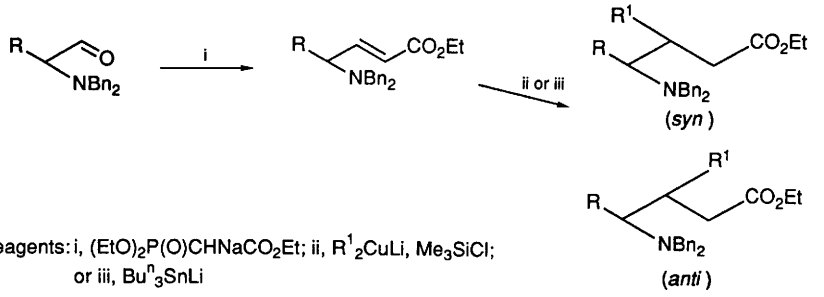
Reagents: i, N_3CO_2Et, *h*ν, MeCN; ii, heat, DMSO

Scheme 28



Reagents: i,  ; ii, $ZnCl_2$, Et_2O, –78 °C → 30 °C

Scheme 29



Reagents: i, $(EtO)_2P(O)CHNaCO_2Et$; ii, $R^1{}_2CuLi$, Me_3SiCl;
or iii, $Bu^n{}_3SnLi$

Scheme 30

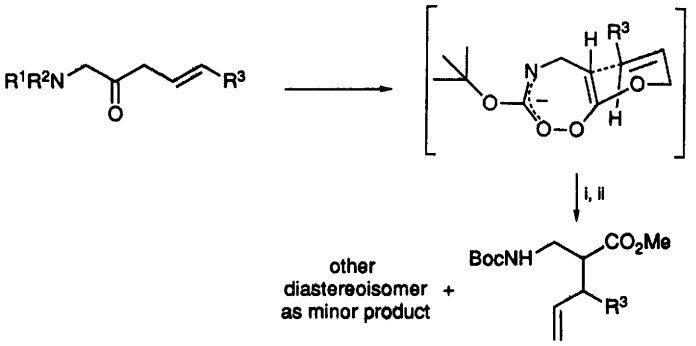
the eventual C-2 chiral centre (Scheme 29).[196] 95:5-Syn/anti-mixtures are formed by β-alkylation of alkenes formed from α-(NN-dibenzylamino)aldehydes and phosphonates $(EtO)_2P(O)CHNaCO_2Et$ (Scheme 30), amounting to a stereoselective synthesis of γ-amino acid enantiomers.[197] Alkylation was effective using n-Bu_3SnLi, but alkylation by a dialkylcuprate was unsuccessful unless Me_3SiCl was present.

Alkylation of protected β-amino acids can be accomplished to extend the available range, e.g. through lithium di-isopropylamide deprotonation followed by an alkyl halide (anti-diastereoselective),[198] or through Ireland-type Claisen rearrangement (Scheme 31) of allyl esters.[199]

Familiar natural examples that have stimulated the interest in synthesis and led to the studies described in the preceding paragraph, continue to receive the attention of those seeking sleeker synthetic routes.

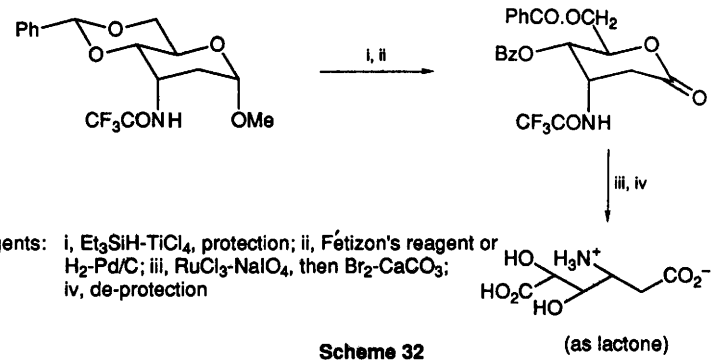
γ-Fluoro-δ-hydroxy-β-amino acids are the end result of a lengthy sequence starting with the acetaldehyde - ethyl 2-fluoropropanoate adduct, via the chain extension product (50).[200] From one point of view, this product is related to the β-amino acid moiety present in AI-77-B, the presence of three chiral centres determining the best starting point to be a protected 3-aminopyranose (Scheme 32).[201] Other naturally-occurring chiral ω-amino acids carry a hydroxylated chiral centre between the amino and carboxy groups, such as the enantiomer of 3-amino-2-hydroxy-4-phenylbutanoic acid (51) that acylates L-leucine to constitute (-)-bestatin. Two syntheses of this enantiomer are displayed in Scheme 33, one route employing the dioxalan-4-one derived from (-)-9-phenylmenthol,[202] the other starting more conventionally from Z-L-phenylalaninal,[203] and adapted so as to produce all four stereoisomers. (2R,3S)-3-Amino-4-cyclohexyl-2-hydroxybutanoic acid ("cyclohexylnorstatine") and its (2S,3S)-diastereoisomer have been synthesized, starting from N-Boc-L- or D-phenylalaninols, respectively, through established functional group transformations.[204]

γ-Amino acids are frequently of synthetic interest as analogues of GABA, the simplest member of the clan. (S)-γ-EthynylGABA and its (S)-γ-trans-butenyl analogue have been prepared by Mitsunobu inversion of $(R)\text{-}Me_3SiC{\equiv}CCH(OH)CH_2CH_2CH_2OSiPh_2Bu^t$ by phthalimide, followed by routine elaboration.[205] Amination of ω-(cycloalkanonyl)alkanoic acids through reaction with an enantiomer of phenylethylamine, followed by hydrogenation cum hydrogenolysis with H_2 and Raney nickel, gives cyclic analogues of ω-amino-alkanoic acids as potential GABA analogues.[206] Statine (52 in Scheme 34) is the other γ-amino acid featured most prominently in the recent literature, and further routes have been



Reagents: i,LDA(3 equiv.)/TMSCl/–78°C ⟶ reflux; ii, CH_2N_2

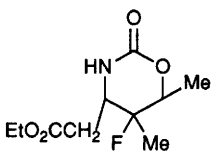
Scheme 31



Reagents: i, Et_3SiH-$TiCl_4$, protection; ii, Fétizon's reagent or H_2-Pd/C; iii, $RuCl_3$-$NaIO_4$, then Br_2-$CaCO_3$; iv, de-protection

(as lactone)

Scheme 32



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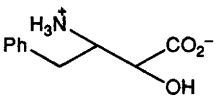

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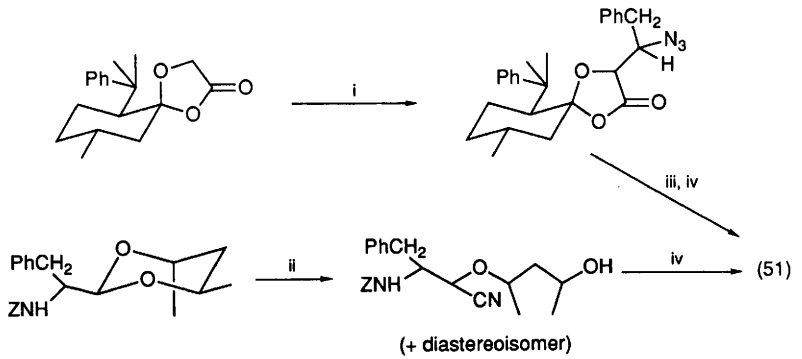
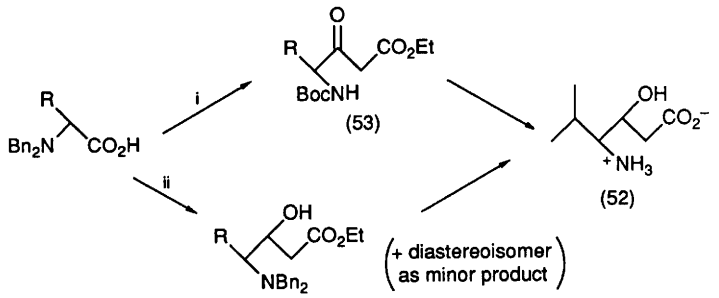


Reagents: i, $PhCH_2CHO$, base, then OH ⟶ N_3; ii, (2*S*, 4*S*)-pentanediol, $TMSCN\text{-}BF_3$, Et_2O; iii, pyridinium chlorochromate and then routine elaboration; iv, Z-removal after hydrolysis

Scheme 33



Reagents: i, *N*,*N*-carbonyldi-imidazole, then $Pr^iMgCl + HO_2CCH_2CO_2Et$; ii, $NaBH_4$-MeOH at –20°C

Scheme 34

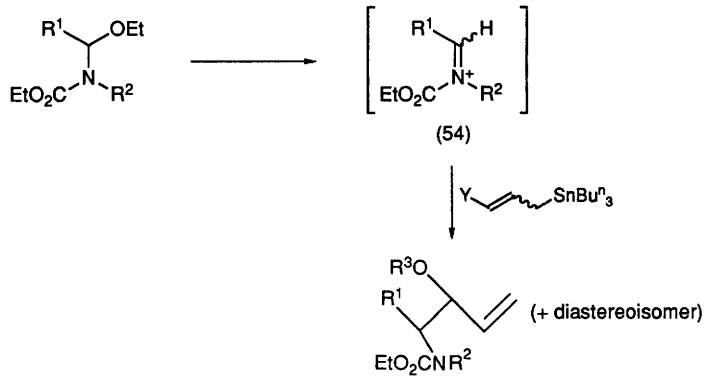
described. One of these routes starts with NN-dibenzyl-L-leucine, converted into the chiral β-keto-ester (53), which gives a 90:10 mixture of statine and its C-3 epimer on $NaBH_4$ reduction.[207] Another route is based on the addition of an allyltin reagent to the iminium ion (54) generated from the corresponding O,N-acetal.[208] Chiral auxiliaries are employed in an interesting route to (3S,4S)-statine starting from N-Boc-L-leucinal,[209] and in the "epoxy-sugar" route shown in Scheme 35.[210] An interesting one-pot general route to 4-amino-3-hydroxyalkanoic acids uses isoxazoles (55) prepared by 1,3-dipolar cycloadditions of alkenes (in the presence of $KHCO_3$) to di-chloro- or -bromo-formaldoximes (prepared from glyoxylic acid aldoxime RO_2C-CH=NOH with N-chloro- or -bromosuccinimide and Bu^tOCl).[211]

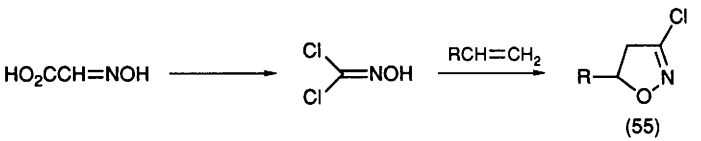
Although lengthy, the route described for the synthesis of (-)-detoxinine starting from N-Boc-D-serine is an improvement over existing routes.[212] Once again, the synthesis of the unusual cyclosporin component "MeBMT" is tackled, but to the point in Wenger's original synthesis (the 28th step!) at which the nitrogen function is to be introduced into the intermediate (56).[213]

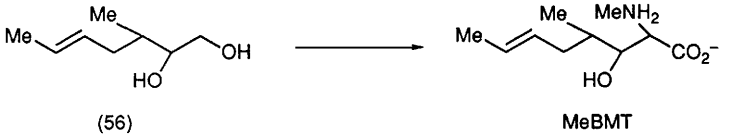
Greater separation of amino and carboxy groups in other ω-amino acids introduces some variety in synthetic approaches, first to 5,6-diaminocaproic acid ("δ-lysine") through Bamberger imidazole cleavage of 4-(β-malonylethyl)imidazole,[214] and to (+)-galantinic acid through an Ohfune-inspired Diels-Alder addition to a protected L-serinal (Scheme 36).[215]

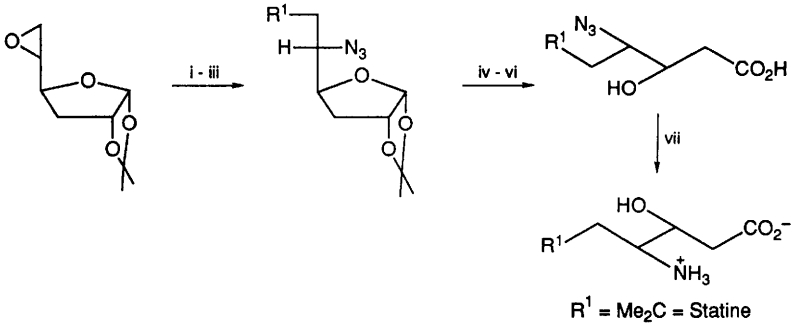
4.16 Resolution of α-Amino Acids.- This topic has an increasing number of facets and in its analytical aspects, too, covered in the later Section (7: Analytical Methods). The major division between enzymatic methods, and chemical or physical methods, determines the way that the literature is covered here.

Enzymatic methods for resolution of α-amino acids continue to be developed on traditional lines, with novel details emerging. Use of reverse micelles may have benefits for yeast-mediated resolution of N-acetylamino acid methyl esters.[216] Another "whole cell" approach describes the resolution of DL-aspartic acid using immobilized *Pseudomonas dacunhae*.[217] N-Boc-DL-α-amino acid methyl esters are substrates for thermitase-catalyzed stereoselective hydrolysis,[218] and analogous carbamate esters serve bacterial L- and D-carbamatases (with notably high selectivity: N-methoxycarbonyl-L-phenylalanine is best obtained from the former, the analogous L-alanine from the latter; NN-bis(methoxycarbonyl)-DL-lysine esters retain the N^{ε}-methoxycarbonyl group).[219] Similarly, both D-hydantoinase and D-N-carbamylase



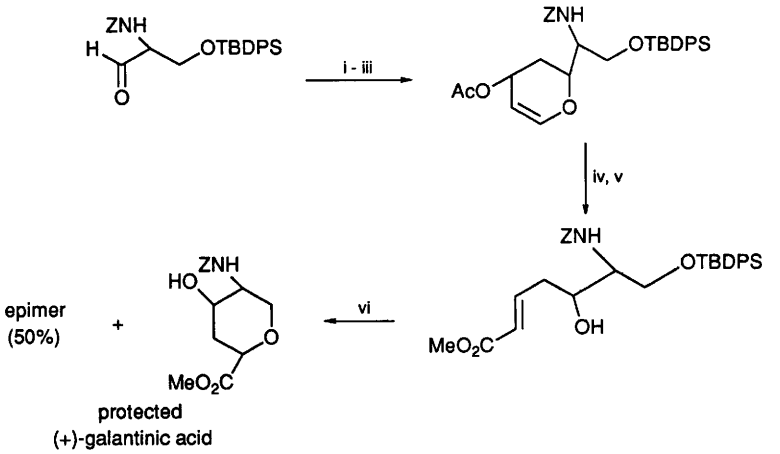






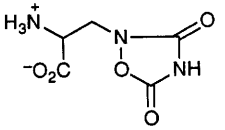
Reagents: i, R^1MgBr, CuI; ii, $MeSO_2Cl$; iii, NaN_3; iv, aq.AcOH; v, $NaIO_4$-$KMnO_4$; vi, 1 mol aq.NaOH; vii, H_2-Pd/C

Scheme 35



Reagents: i, EtOCH=CHC(OTMS)=CH_2/$ZnBr_2$/THF; ii, $NaBH_4$, $CeCl_3$; iii, Ac_2O, Et_3N; iv, $HgSO_4$, 5M aq.H_2SO_4; v, NaCN, *etc* ; vi, K_2CO_3, MeOH

Scheme 36



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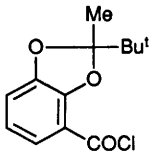

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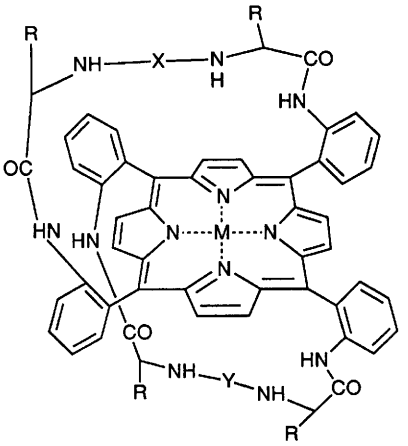


(58) X, Y = —CO—*p*-C_6H_4—CO— and other diacyl groups; M = H_2

(Arthrobacter crystallopoietes) are needed for the preparation of optically-pure L-amino acids from hydantoins (one of the intermediates most favoured in recent studies of large-scale resolution opportunities).[220] Racemases are useful for obtaining maximum amounts of one enantiomer from a racemic amino acid source, and mechanistic studies with pyridoxal phosphate-derived Schiff bases show that deprotonation is the initial step in the process.[221]

Immobilization of representative enzymes on activated ceramic porous alumina for these resolution approaches has been investigated.[222]

Conventional uses of enzymes have been reported for the final stages of synthesis of 2,6-di-aminopimelic acid (papain),[89] fluoro-substituted 2-amino-alkanoic acids,[160] and phosphonotricene (chymotrypsin).[176]

Application of the asymmetric transformation principle allows complete conversion of DL-cysteine into either enantiomer via (RS)-4-thiazolidinecarboxylic acid, using (2R,3R)- or (2S,3S)-tartaric acid and acetic acid to set up the chemical equilibrium in which this heterocycle participates. The R-heterocycle S,S-tartrate salt is least soluble, and the other diastereoisomeric salt is readily epimerized.[223] Several other examples of conventional resolution through diastereoisomeric salt formation appear in this year's literature.

Major advances can be expected in enantiomer separation through exploitation of both established and new physical discrimination mechanisms. A "three contact-point" requirement for chiral recognition is supported by molecular orbital calculations compared with experimental data for enantiomers of N-(2-naphthyl)alanine methyl ester interacting with N-(3,5-dinitrobenzoyl)leucine n-propylamide, the latter representing some currently-used species for creating chiral stationary phases for liquid chromatography (see Section 7.5).[224] Preparative-scale chromatographic resolution opportunities have been considered for β- and γ-cyclodextrins (L-dansyl-leucine complexes 62-78% more readily than its D-enantiomer with β-cyclodextrin, the variability indicating an importance of host:guest ratios).[225] β-Cyclodextrin enantioselectively catalyzes the (2,4-dinitrophenyl)ation of amino acids[226] and this offers a way of "resolving" DL-amino acids based on the separation of the N-substituted enantiomer from the reaction mixture.

Crown ethers are represented by the novel 18-6 crown, 1,2:5,6-di-O-isopropylidene-3,4-O-(1,2-bis[ethoxyethoxyl)-D-mannitol,[227] and by polymeric crown ethers containing moieties derived from L-tartaric acid.[228] (2-Aminomethyl)ated crosslinked poly(styrene) serves as stationary phase to which an L-proline moiety is bonded, which as its copper(II) complex forms another type of novel chiral polymer for

chromatographic resolution of DL-amino acids.[229] These examples tend to retain the L-enantiomer of an amino acid ester more strongly, though "tailor-made" chiral phases of these types for a given large-scale resolution must be some way into the future. This may not be so with "molecular imprints" - familiar polymers such as poly(acrylate)s prepared from the monomer containing L-phenylalanine anilide, the chiral additive being washed out from the polymer, which is then capable of efficient chiral discrimination between enantiomers of phenylalanine-containing dipeptides.[230,231] In one of these studies,[231] using perspex imprinted with L-phenylalanine anilide, enantiomers of a very close structural relative to the imprint, viz. DL-phenylalanine N-methylamide, were shown to be totally unresolved.

Other solution studies drawing on similar principles involve enantioselective transport of amino acid salts through polymeric and liquid crystal films containing chiral crown ethers[232] and ligand-exchange separation using octadecylsilica coated with NN-di-octyl-L-alanine with copper(II) ions in the mobile phase (hydrophobic amino acids retained more strongly)[233] and preparative resolution on this principle of 2-pentafluoroethyl- and trifluoromethyl-DL-alanine using D- or L-phenylalanine - copper(II) complex in the mobile phase.[234]

A small part of this section each year covers the literature on natural prebiotic enantioselection of L-amino acids, a topic that has been reviewed from the point of view of the consequences of the microscopic energy difference between D- and L-components of a racemate due to parity violation.[235] Calculations have been presented, purporting to show that L-amino acids more readily form and condense into peptides on kaolinite in its "direct" structure rather than on its less-abundant "inverse" form, thus re-presenting a familiar type of argument for the predominance of the L-series.[236]

5 Physical Studies of Amino Acids

This Section covers the major spectroscopic techniques, and other physical studies, as applied to amino acids and their derivatives.

5.1 Crystal Structures.- A neutron diffraction structure for DL-aspartic acid has been published.[237] All other studies cited here are X-ray crystal structures, i.e. lacking locations of hydrogen atoms. The structure for DL-quisqualic acid (57) reveals an unusual feature: the nitrogen atom connecting the heterocycle to the β-alanine carbon atom is held in a pyramidal configuration.[238] L-2-oxothiazolidine-4-carboxylic acid[239] and amino acid salts studied include (+)-L-arginine

di-arsenate[240] (in which L-arginine di-cations and $AsO_2(OH)_2^-$ anions form a network of hydrogen bonds), a new crystal form of L-arginine D-glutamate[241] and trimethyltin DL-glutamate.[242] Numerous amino acid derivatives (excluding peptides) have been studied: hydrochlorides of L-cysteine methyl and ethyl esters,[243] N-phosphonomethyl-L-threonine,[244] N-phosphonomethyl-L-proline,[245] N-Boc-L-tyrosine (4-bromophenacyl) ester,[246] N-Boc-alanine o-nitrophenyl ester (shown to be a rigid structure with very restricted rotation about the ester bond because one of the nitro-group oxygen atoms is wedged between the two ester oxygen atoms),[247] N-tritylglycine methylamide,[248] (-)-S-benzyl-α-hydroxymethylcysteine (as its N-benzoyl derivative; shown to possess the S-configuration in confirmation of earlier chemical correlations)[249] N-acetylamino acid amides,[250] and N-(3,5-dinitrobenzoyl)leucine n-propylamide and N-(2-naphthyl)alanine methyl ester.[251] The last-mentioned pair were studied in this way as close models of "chiral selectors" used in preparation of stationary phases for chiral h.p.l.c. (see Section 7.5); each is capable of "recognising" the chirality of the other, and it is postulated that the S-enantiomers of each of these two amino acid derivatives complex together in solution.

A review of crystal structures of amino acids, extracting data on hydrogen bonding distances and bond angles, has been published.[252]

Crystallization behaviour of amino acids has been surveyed, with particular regard to trends in, and effects of trace impurities on polymorphism (e.g. L-glutamic acid exists in α- and β-polymorphic forms).[253]

5.2 Nuclear Magnetic Resonance Spectrometry.- Specialized studies are briefly described here(as opposed to the routine, which is not). Low temperature ^{1}H-n.m.r. spectra of N^{α}-acetyl L-arginine isopropyl ester hydrochloride have been interpreted in terms of the resonances of the four NH_2 protons.[254] The puzzling dependence of the stability of S-adenosyl-L-methionine on the nature of the accompanying anion has been a long-standing problem. The molecular events in solutions at various temperatures have been concluded to involve pyramidal inversion at the sulphonium chiral centre, based on careful n.m.r. studies, and the next problem is to link this with a role for the anion.[255]

Bis-strapped chiral porphyrins (58) involving amino acid bridging units have been shown by ^{1}H-n.m.r. studies to be formed free from racemization. This illustrates in an unusual context a typical role for n.m.r. spectrometry in amino acid reactions in general, not only in the monitoring of the synthesis of small peptides.[256]

The most familiar uses of n.m.r. in the study of amino acids and their derivatives lie in the study of conformational and acid-base equilibria, and in molecular interactions in solution. The first-mentioned topic is usually well-represented here (but not this year), while the other two are represented, respectively, in the pH-dependence of ^{13}C-n.m.r. features of aspartic acid (the addition of two protons to totally-deprotonated solute is to N and O simultaneously rather than sequentially);[257] in the effect of $ClCH_2CH_2OH$ on self-association of N-acetylamino acid dimethylamides in H_2O-^2H_2O;[258] and in establishment of the site of co-ordination of methionine to lanthanide cations (La, Pr, Nd, Dy, Ho, Er, Tm) to be the carboxy group oxygen atoms.[259]

5.3 Optical Rotatory Dispersion and Circular Dichroism.- The topic, which has usually treated amino acids and their derivatives either as simple vehicles for developing general understanding of the techniques or as microcosms of proteins, has not advanced in any substantial way through the period under review. An account has been published of the use of a chiral benzoylating agent ["(+)-TBM"; 59] for derivatizing amino acids and alcohols for configurational assignments,[260] without convincing evidence for any particular advantages over existing chromophoric derivatives.

5.4 Mass Spectrometry.- Like other instrumental and spectrometric techniques discussed in this Chapter, considerable technical advances are being made, though without substantial new principles of chemistry being involved.

Negative ion (Cl^-) chemical ionization spectra of amino acids include prominent molecular ions, $[M + Cl]^-$ in the case of simple hydrophobic amino acids, and $[M - H]^-$ for polar types.[261] Positive and negative ions formed through ^{252}Cf-fission product bombardment of valine have been linked with fragmentation pathways.[262]

At a more familiar level, given the ever-widening use of the technique, fast atom bombardment mass spectra have been listed for N-benzyloxycarbonylamino acids.[263]

5.5 Other Physical Studies.- Studies that are more appropriately placed elsewhere, such as theoretical aspects of analytical techniques dependent on physical phenomena, will be found under other headings.

Topics discussed here fall under three main headings: consideration of intermolecular interactions; measurement of physical data using simple apparatus encountered in the traditional physical chemistry laboratory; more sophisticated physical measurements.

Under the first heading, discussion has been published of the relationships between the polar character and molecular dimensions of simple amino acids, and their selection for coding into proteins,[264] and a dispute has broken out over the related claim that the genetic code preferentially conserves long-range interactions between amino acids that are beneficial in some ill-defined way, between the advocate of this claim[265] and its opponents.[266] A discussion of molecular aggregation of amino acids leading to abiotic condensation and polymerization, with particular reference to the effect of chirality, is another example of armchair chemistry in this area.[267]

Micro-emulsions form with N^{α}-lauroylarginine methyl ester in aqueous media.[268] This is one of a group of papers based on interactions between amino acids and the liquid phase in which they are located; others discuss selective transport of salts of amino acid esters from aqueous media through organic membranes, with antamanide as carrier;[269] an error correction has appeared for the earlier paper from this group.[270] Hydrophobicity and surface activity data for N^{α}-dodecanoylamino acids in water have been published.[271] The effect on the structures of sodium salts of N-acylamino acids on their surface properties in aqueous solutions, has been demonstrated.[272] Interestingly, these salts show higher surface activity in solutions made with hard water, than corresponding concentrations of sodium salts of common fatty acids. A substantial study of the N^{α}-stearoyl-L-serine monolayer has appeared,[273] from the point of view of molecular recognition. Since this is a chiral monolayer, there is scope for association ("complex formation" in all its meanings) between the monolayer and one particular enantiomer from a mixture contained in the underlying solution. A similar objective is the basis of a study of the diffusion of N-(3,5-dinitrobenzoyl)-L-leucine n-butyl ester through a silicone-supported liquid membrane.[274] Oriented crystal growth of glycine has been reported at the water interface with a Langmuir-Blodgett monolayer containing L-amino acids.[275] Amino acid - receptor studies that may depend on distantly-related molecular recognition phenomena are covered in a study of the salt taste properties of amino acids, and their methyl ester hydrochlorides, in company with the dipeptide ornithyl-β-alanine.[276] Basic amino acids and their derivatives enhance the saltiness of sodium chloride but are not themselves salty. However, the human intake of Na^{+} can be cut by 75%, 50% or 25% by substituting the NaCl in a meal by the dipeptide, by glycine methyl ester hydrochloride, or by increasing the level of certain amino acids to maintain the same "saltiness", clearly a useful and presumably safe way forward for those who link sodium intake with various unwelcome consequences. At a more

scientific level, the apparent specific molar volumes of 17 amino acids have been compared with taste data to conclude that steric exclusion from taste receptors operates with certain enantiomers.[277] While on the subject of taste, side-chain mono-esters of aspartic and glutamic acids are repellent to oriental weatherfish (Misyornus anguillicaudatus), but the di-esters are not, indicating a leading taste role for the terminal carboxy group.[278] The marked anti-amnaesic effect of D-pipecolic acid in rats has been reported, a property for which the peptide H-Pro-Leu-Gly-NH_2 is already known.[279] Apparent partial volumes of 11 amino acids in water at 15-55°C[280] and similar properties related to infinitely dilute aqueous amino acid solutions[281] including effects of various salts on structure-making and structure-breaking in aqueous glycine solutions,[282] and adiabatic compressibility of aqueous methanolic solutions of amino acids[283] have all been determined by densitometry.

Amino acid first and second thermodynamic dissociation constants have been given precise values for 0.1 mole fraction 1,2-propanediol - water solutions of glycine, through e.m.f. measurements based on the silver chloride - hydrogen electrodes.[284] Calorimetric measurements of the same data have been extended to third dissociation constants of acidic amino acids (glutamic and aspartic acids, tyrosine),[285] and also encompass other hydroxyl- and fluorine-substituted phenylalanines.[286] This extensive study includes discussion of the effects of amino acid side-chains on the magnitude of dissociation constants.[287] Dissociation constants for DL-alanine, DL-valine, L-valine, and DL-leucine in aqueous dioxan have been determined.[288]

Calorimetry studies of N-acetylamino acid amides yield excess enthalpy data.[289] Volume changes accompanying ultrasound absorption by L-cysteine in aqueous solutions have been interpreted for the first time in terms of proton transfer reactions and individual ionization rate constants.[290] Differential thermal analysis data lead to estimates of kinetic, stability of amino acids (Met < Ser ≃ Arg < Arg,HCl) and corresponding methyl ester hydrochlorides (roughly the same order).[291] The thermal stability of asparagine from these data is greatest in aqueous solutions at pH 5 - 7, but its stability diminishes in the solid state as the proportion of water of crystallization increases.[292] A thermodynamic study of the interaction energy between alkylureas and L-valine, L-valyl-L-valine, L-leucine and L-leucyl-L-leucine respectively, has been reported.[293]

More sophisticated instrumentation provides Raman spectrometric data for tryptophan and valine (D, L-, and DL-),[294] and a series of substituted tryptophans.[295] The newer generation of Raman studies employing polarized radiation has included studies of aliphatic amino

acids in water and in ^2H_2O at pH values appropriate for predominance of zwitterionic and deprotonated forms, respectively.[296] Features of intermolecular vibrational coupling in solid glycine have been identified.[297]

E.s.r. Spectrometry of irradiated amino acids is featured regularly in the literature, this year represented by variations in $^\alpha$H-hyperfine splitting as a function of temperature for X-irradiated solid L-alanine.[298]

Undoubtedly, the star paper this year has to be the account of the visualization of individual amino acid molecules adsorbed on highly orientated pyrolytic graphite, using scanning tunnelling microscopy. The amino acids are "seen" (probably through a charge transfer mechanism) to cluster, in pairs for leucine, methionine, or tryptophan, but in larger clusters for glycine.[299]

5.6 Molecular Orbital Calculations.- These methods have a role to play in conformational studies in particular, and have been applied in this context to N-acetyl-L-serine methylamide,[300] serine phosphate,[301] and the cyclosporin constituent "MeBMT", the threonine derivative (2S,3R,4R,6E)-3-hydroxy-4-methyl-2-methylamino-6-octenoic acid depicted beside formula (56).[302] The particular context for the last-mentioned study is the elucidation of the effects of change of chirality and of further substitution on the side-chain conformation of this important amino acid.

6 Chemical Studies of Amino Acids

The organisation of this section follows the pattern of preceding Volumes, with an account of racemization separated out as the starting section, due to the importance claimed for it, for assignments of age to fossils.

6.1 Racemization.- There is more of a spread in this, than in any other Section of this Chapter, from thoughtful science to somewhat whimsical applications based on suspect principles. At the former extreme, rates of racemization at pH 8, 140°C, of 13 α-amino acids with functional groups in their side-chains have been compared, with some simple amino acids with alkyl side-chains included as standards. The hydroxyalkyl amino acids showed most racemization, with a decreasing order methionine, alanine, aspartic acid and glycine, aminoadipic acid, and pyroglutamic acid for certain others.[303] Racemization of amino acid residues during alkaline hydrolysis of casein, oat protein, or rapeseed

protein gives a somewhat similar order of decreasing level of racemization: serine, aspartic acid, phenylalanine, glutamic acid, and valine.[304] However, such data do not separate the propensity for racemization of a residue when part of a protein, from that of the free amino acid, a factor that is important as shown in spectacular fashion by the generation of nearly 70% of the D-enantiomer of proline from the tetrapeptide L-prolyl-L-leucylglycylglycine heated at 148.5°C at pH 6.8 during 90 min. This must mean that some cleavage into dioxopiperazines was occurring and that the neighbouring amino acid(s) residue affect(s) the racemization rate at a particular chiral centre.[305] A similar result was found for the racemization of aspartic acid and asparagine residues in human myelin basic protein, where highest rates were seen for these residues when adjoining arginine.[306]

These reasons for being suspicious of age determinations based on amino acids enantiomer ratios have been aired in preceding Volumes of this Specialist Periodical Report. Another obvious problem is lack of knowledge of the catalytic effect on racemization rates, of any or all the compounds in the immediate vicinity of the racemizing centre. But none of this has dissuaded continuing applications. Teeth dentin from sound teeth can be dated to within four years either way from the known age, based on the D:L-aspartic acid ratio (but not for "abnormal" specimens);[307] darkened teeth from "burned bodies" show a more advanced age.[308] Good scientific practice is shown in a study in which bones from three 12th Century German burials were used to calibrate results for the corpse of Emperor Lothar I, to determine that it was boiled in water for about 6 hours before burial. Evidently, boiling was decided upon to avoid post-mortem decay since the body was to be buried at the Imperial castle 500 Km away from the place of death.[309]

A classical technique for amino acid racemization employs Schiff base formation and is based on the enhanced prototropy in such derivatives. This is particularly rapid using an ordinary domestic microwave oven (≈ 2 min) in a Teflon vial reactor (50 mg amino acid, 1 cm^3 AcOH, 50 μL PhCHO under N_2; the method is even more effective using trifluoroacetic acid).[310]

6.2 General Reactions of Amino Acids.- This section covers reactions that apply to amino and carboxy groups of amino acids, either reacting separately or simultaneously, and therefore are general in character (even though they may be exemplified only for representative amino acids).

Pyrolysis of 18 amino acids in the inlet port of a gas chromatograph, has been carefully standardized for the purposes of pyrolysis-g.l.c.

analysis, but the results are of general relevance in many contexts; from the proper handling of analytical samples to food chemistry, among others. The volatile products identified for protein amino acids are: alanine (acetaldehyde), leucine (3-methylbutyronitrile and 3-methylbutanal), isoleucine (2-methylbutyronitrile and 3-methylbutanal); valine (2-methylpropionitrile); phenylalanine (toluene); proline and hydroxyproline (pyrrole); tyrosine (phenol and p-cresol); tryptophan (indole and skatole); glutamic acid, glutamine, aspartic acid, asparagine (unknown).[311] There is much variation in results from experiments such as these,[312] as shown by a study of proline pyrolysis, forming "the aroma substance" 2-acetyl-1-pyrroline under "bread-making conditions".[313] An area of chemistry that is opening up for amino acids, as for other classes of compound, is the consequences of sonolysis. Radicals are generated in concentrated aqueous solutions due to peroxide formation, and additional radicals are formed from amino acids due to high temperatures created around collapsing cavitation bubbles. The resulting chemical changes have as yet received little attention.[314]

Routine studies of oxidative degradation of amino acids by familiar oxidants (Chloramine-T,[315] acid $KMnO_4$,[316] cerium(IV) sulphate,[317] and potassium periodate[318]) can be indicated by a few representative citations, but more interesting results with an analytical context are described for electrochemical oxidation of N-toluene-p-sulphonylglycine and dansylglycine.[319] During Fenton oxidation (Fe^{2+} - H_2O_2) of amino acids, leucine gives 3-methylbutanal (isovaleraldehyde) and α-ketocaproic acid; CO_2 evolution is stimulated by Fe^{2+} and ADP and unusual Fe - amino acid complexes seem to be involved. Oxidation is maximal when the iron-chelated amino acid is in the presence of free Fe^{2+} ions or a second type of iron complex.[320]

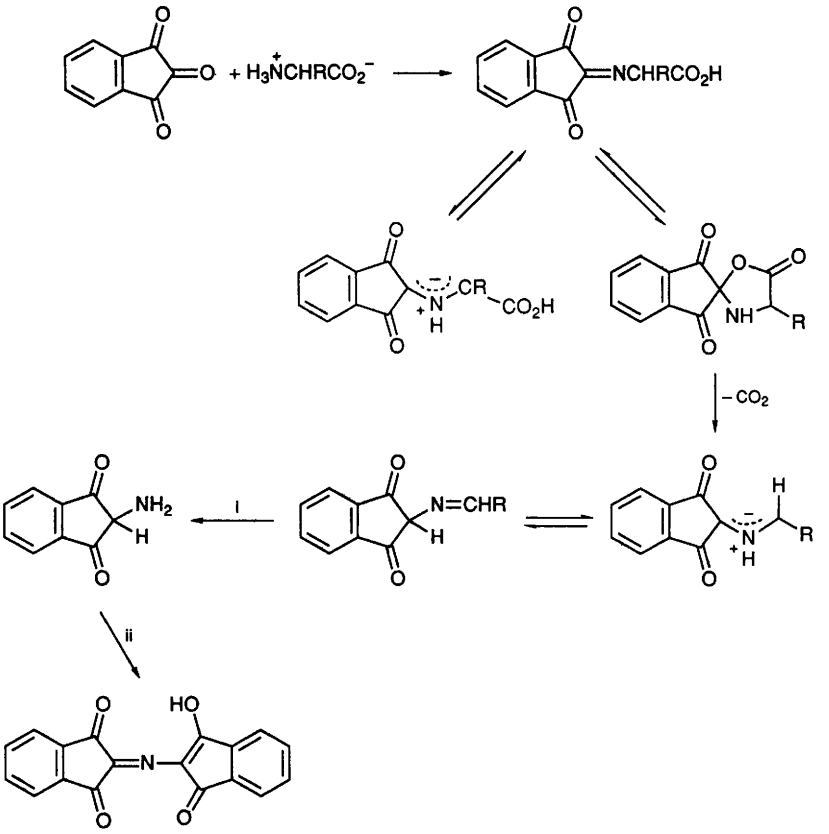
Amino acids react with carbonyl compounds to give Schiff bases, but what is known as the Maillard reaction is the consequence of a number of subsequent reactions made more complex by the fact that the carbonyl compound is a monosaccharide. A deceptively simple model, the reaction between glycine and glyoxal (a 2:1-molar mixture heated in boiling water during 24 hours), leads to products formed by the involvement of formaldehyde (the Strecker degradation product of glycine) and of methanol (the product of the Cannizzaro reaction with formaldehyde). Thus, 2,4,6-trioxaheptane and 2,4,6,8-tetraoxanonane are formed.[321] This reaction is evidently not a suitable model for the Maillard reaction, through which pyrrolecarbaldehydes, pyridines and pyridazines are the most significant products. Four pyrazines have been detected in the reaction mixture formed between glycine ethyl ester and

glyceraldehyde,[322] and 2,3,5-trimethylpyrazine is the most abundant product from glycine, and 2-methylpyrazine from arginine, when these amino acids are reacted with glucose.[323]

Reviews of the Maillard reaction[324] include its course under physiological conditions.[325]

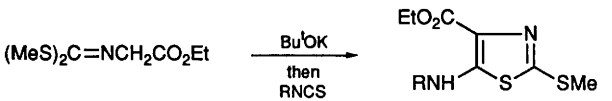
Imine formation, as briefly referred to in preceding paragraphs (racemization; Maillard reactions) is continuing to develop into other profitable mechanistic and synthetic areas of study, mainly due to their prototropic rearrangement into azomethine ylides. A lengthy review has been pubished on the author's work on the formation of these ylides and their cycloaddition reactions,[326] but substantial contributions continue to be made also by Grigg's group. A convenient alternative route to these Schiff bases is to react a primary amine with an α-keto-acid, and Strecker decarboxylation (in boiling benzene for example)[327] gives the azomethine ylide that can be trapped by sulphur (to give a secondary thioamide), or by alkenes through cycloaddition.[328] One area of interest generated by the intermediacy of these dipoles is in properties of pyridoxal imines in relation to metabolic transamination processes. Also, dipolar intermediates have been implicated in the Strecker degradation, as represented by ninhydrin oxidation (Scheme 37), and the textbook mechanism has needed revision,[329] since azomethine ylides of two different types can be trapped by cycloaddition to maleimide. Imines formed in hot DMF between α-amino acids and pyruvic acid, ethyl pyruvate, or pyruvaldehyde undergo decarboxylation, ylide formation and stereospecific cycloaddition to an alkene present in the reaction mixture.[330] It is suggested that pyruvate-dependent carboxylases may react <u>in vivo</u> through azomethine ylide intermediates. Four new classes of tandem Michael addition and 1,3-dipolar cycloaddition have been identified for imines derived from glycine ethyl ester and aminoacetonitrile in the presence of lithium salts (favouring the Michael addition route) and triethylamine.[331] Similar results have been published for N-benzylidenealanine methyl ester.[332] Acyliminium salts formed from N-alkylpyroglutamic acids through decarboxylation using P_2O_5 and methanesulphonic acid can be arylated to give 5-aryl-N-alkylpyrrolidin-2-ones.[333]

Other uses for amino acid Schiff bases also illustrate consequences of α-carbanion formation (on which their use in a general method for α-amino acid synthesis depends; see Section 3.1); in an example (60) given here, carbanion formation is exploited in synthesis of five-membered heterocycles.[334] Support for the oxazolidin-5-one intermediate proposed by Grigg (Scheme 37) is mentioned in the account of



Reagents: i, H_3O^+; ii, indan-1,2,3-trione

Scheme 37



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pyrrolidine synthesis from formaldehyde, an amino acid, and an electron-deficient alkene (dimethyl fumarate give trans-3,4-dimethoxycarbonylpyrrolidine).[336] The azamethine ylide is involved in a synthesis of optically-pure cis- and trans-2,5-disubstituted pyrrolidines from N-benzylidene-amino acid esters by cyclisation of the corresponding alkanols formed by borohydride reduction,[336] and is an intermediate in the cycloaddition of N-arylidene-amino acid esters to nitrosobenzene to give a diaryl nitrone and an α-imino-N-(1-alkoxycarbonylarylidene)arylacetamide.[337] Pyrroloquinolinequinone (PQQ) reacts with amino acids with uptake of oxygen, to yield a yellow compound, considered to be an oxazole formed from a Schiff base via an oxazoline,[338] and explaining the inactivation of "quinoproteins" (PQQ-enzymes) by ammonium salts.

Simple reactions at the amino group include its substitution as a complete unit by chloride by diazotisation. This occurs with retention of configuration with 95-98% e.e. for alanine, valine, leucine, isoleucine, but less hindered side-chains lead to more racemization).[339] Reactions in side-chains that accompany diazotization have been investigated, ornithine, citrulline and arginine giving 5-membered heterocyclic products, cysteine giving thiiranecarboxylic acid and lactic acid sulphate, and cystine undergoing disulphide cleavage.[340] Bromine water and glycine are presumed to yield an equilibrium mixture of N-bromo- and NN-dibromo-glycine, these reacting with hydrogen peroxide so as to generate O_2 and, again presumably, to return the amino acid. These inferences were drawn so as to support a proposed reaction of peroxidase with $Br^- \rightarrow Br_2$ and a roundabout route to the production of oxygen through intervention of amino acids.[341] Selective N-phenylation of α-amino acid esters with triphenylbismuth di-acetate catalyzed by Cu^o or a copper(II) alkanoate gives mono-N-phenylamino acid derivatives in all cases except glycine ethyl ester, which gives a mixture of N-monophenyl- and NN-diphenyl derivatives.[342] Histidine and arginine derivatives do not react.

A simple improved preparation of N-tritylamino acids is somewhat wasteful of trityl bromide, since N-tritylamino acid trityl esters are formed first and cleaved in situ with MeOH at 50°C.[343] Other N-protecting groups studied include 2-[4-(methylsulphonyl)phenylsulphonyl]-methoxycarbonylamino acids.[344] These are alkali-labile on the β-elimination principle, but resist catalytic hydrogenolysis and are somewhat more stable than Fmoc-analogues. Enamines formed by reaction of amino acids with 9-(hydroxymethylene)fluorene (in equilibrium with 9-formylfluorene) are claimed on the basis of representative examples to be more protective towards racemization than well-known urethane

protecting groups.[345] Routine but useful preparative work covers the preparation of pure Fmoc-amino acids and other urethanes using O,N-bis-trimethylsilyl amino acids.[346] "Pure" in this context means free from Fmoc-oligopeptides, formed in a well-known side-reaction that accompanies Schotten-Baumann acylation at the amino group when the carboxy group is unprotected.

The formation of substituted iso-indoles from the reaction of amino acids with o-phthaldialdehyde and a thiol has been confirmed by n.m.r. studies with glutamic acid, glutamine, and aspartic acid, for the case where the thiol is glutathione.[347] Though there may appear to be no special reason why a complicated thiol should be used when a simple one will do, isoindoles formed using N-acetyl-L-cysteine are much more stable than corresponding compounds prepared using mercaptoethanol.[348]

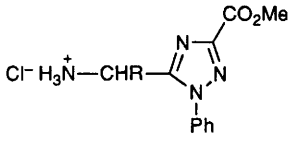
As already indicated, some reactions at the amino group are followed by further processes involving other sites in the molecule. N-Phenylthiocarbamoylamino acids are believed to require fairly drastic treatment in order to undergo cyclization and rearrangement to N-phenylthiohydantoins (PTHs; the basis of the booming analytical process involving these "PTC-amino acids"), in spite of the inability to isolate PTC-amino acids on the part of Edman himself[349] and others (but see Section 7.5), because these compounds readily cyclized to PTHs. It is now found that sulphur-containing cyclic amino acids form PTHs spontaneously through reaction with phenyl isothiocyanate.[350] Reference to the corresponding reaction with pipecolic acids[351] shows that the course of this reaction is not so simple with cyclic imino acids as might be thought.

The PTC-amino acid, in fact,cyclizes in acid media to form a 2-anilinothiazol-5(4H)-one, and this is usually required to rearrange to the PTH in the normal operation of the Edman sequence analysis of peptides. However, ring-opening to form a PTC-amino acid ester through use of an alkanol avoids the stringent need to remove traces of acids for clean PTH formation, and gives an easily analyzed derivative.[352] N-(N-Benzyloxycarbonylaminosulphonyl)amino acids $ZNHSO_2NHCHRCO_2H$ formed through reaction of chlorosulphonyl isocyanate, benzyl alcohol and an amino acid hydrochloride, cyclize to 1,1-di-oxo-1,2,5-thiadiazolidin-3-ones after Z-cleavage, cyclic sulphonamides that were found after tasting (through accident or misunderstood instruction?) to be not sweet.[353] Boroxazolidinones, cyclic mixed anhydrides formed between 1,1-diphenylborinic acid and an amino acid or an N-monoalkylamino acid, are specifically suggested to offer a means of analyzing amino acids in admixture with peptides and proteins.[354] 2-Oxazolidinones are formed from amino acids and $Cl_3COCOCl$ ("diphosgene") after BH_3-SMe_2-BF_3

reduction of the carboxy group to give 2-amino alkanols without racemization,[355] and the carboxy group is also involved in a preparation of chiral triazoles (61) by reaction of Z-amino acid mixed anhydrides with methyl phenylhydrazono esters, followed by Z-removal.[356] More continuing interest is likely to be found in the formation of the Meisenheimer adduct (62) from N-methyl-N-(2,4,6-trinitrophenyl)glycinate anion, the first example of participation by the carboxylate anion in such systems,[357] and for the formation of lactams from β- and higher ω-amino acids using triethylgallium in benzene.[358] A cycloaddition route to 3-(N,N-disubstituted)amino-β-lactams uses imines RN=CHR' with zinc enolates of N,N-disubstituted amino acid esters formed by deprotonation with lithium di-isopropylamide and treatment with $ZnCl_2$.[359] Optically-active oxazol-5(4H)-ones are formed from N-acylamino acids and EEDQ or IIDQ;[360] and from L-tryptophan the optically active 2-trifluoromethyloxazol-5(4H)-one is formed by dissolution in trifluoroacetic anhydride.[361] Aminolysis of racemic 4-t-butyl 2-phenyloxazol-5(4H)-ones by L-proline . methyl ester leads to almost 100% N-benzoyl-D-t-leucyl-L-proline methyl ester.[362] This is an amazing result, achieved for reactions in aromatic hydrocarbon solvents (results are worse in other solvents and worst in DMF), based on previously-established modest levels of asymmetric induction in this reaction with other hindered oxazolones.

N-Protected α-amino-aldehydes (for a review see ref. 363) are becoming increasingly important among carboxyl-modified amino acids. Their uses in synthesis of ω-amino acids have been discussed earlier (Section 4.15) and further applications in asymmetric synthesis include diastereoselective (syn) addition of lithiated heterocycles (LiR + $(PhCH_2)_2NCHR'CHO \rightarrow (PhCH_2)_2NCHR'CH(OH)R$),[364] of allylsilanes catalyzed by $TiCl_4$ ($ZNHCHRCHO \rightarrow ZNHCHRCH(OH)CH_2CH=CH_2$) with diastereoselectivity dramatically dependent on the relative amount of catalyst,[365] and hetero-Diels-Alder addition to Brassard's diene [$CH_2=C(OMe)CH=C(OMe)OTMS$] to give chiral δ-lactones.[366]

Literature of α-amino acid esters continues to provide a range of routine and novel chemistry. (2-Phenyl-3-butenyl) esters, prepared by coupling the alcohol to the N-protected amino acids using phosgene, are cleaved by ozonolysis. It is difficult to see benefits, and disadvantages appear to be non-crystallinity in representative cases where familiar simple esters are crystalline, as well as the restriction placed on side-chains by the harshness of ester cleavage.[367] Cyanoethyl esters, used for phosphate protection in oligonucleotide synthesis (deblocking involves K_2CO_3 in aq MeOH), have been prepared in the amino acid series using 3-hydroxypropionitrile.[368] 6-Chloro-2-



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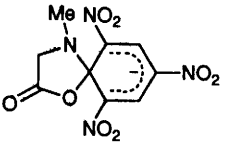

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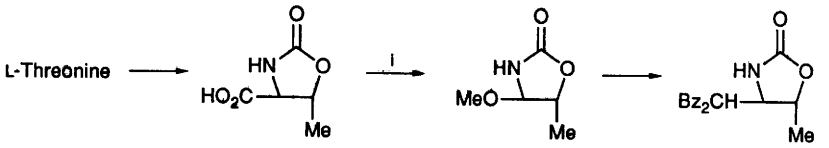
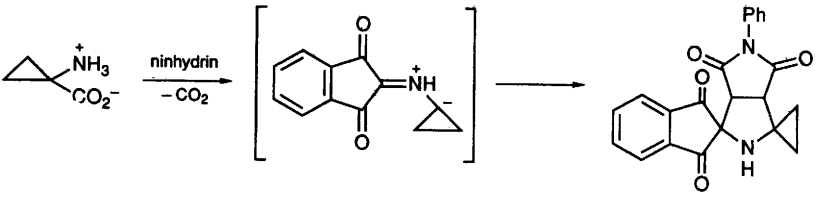


(+ 23% epimer)

Reagents: i, e^-, MeOH-NaOAc; ii, Bz_2CH_2-TFA

Scheme 38



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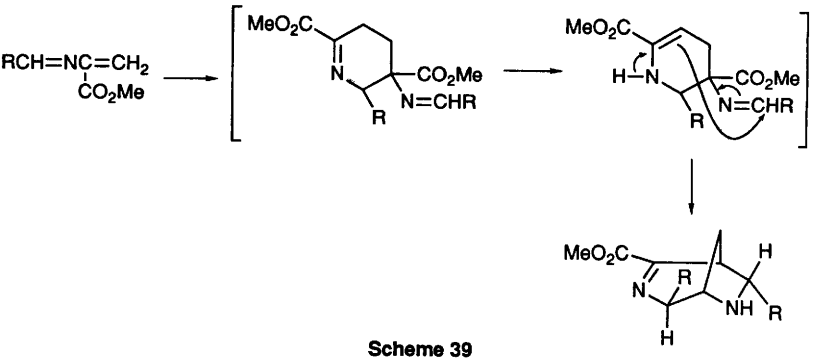


Scheme 39

pyridyl esters show higher reactivity as "active esters" in peptide synthesis than 2-pyridyl esters themselves, and higher than p-nitrophenyl esters, particularly in coupling of Z-Asp(BZL)OR with amino acid esters, there is no detectable aspartimide side-product formed.[369]

p-Chlorotetrafluorophenyl esters, also advocated as "active esters", crystallise better than 2,3,5,6-tetrafluorophenyl analogues, and have higher m.p.s than pentafluorophenyl esters,[370] for which a synthesis using di(pentafluorophenyl)carbonate has been proposed.[371] 9-Fluorenyl esters, formed using diazofluorene[372] and removable by mild acidolysis or hydrogenolysis, are becoming popular for C-protection of amino acids. t-Butyl esters are conveniently prepared using t-butyl fluorocarbonate (Boc-F),[373] better yields being obtainable with this reagent than with $(Boc)_2O$, proposed earlier.[374] A satisfactory protocol for side-chain esterification of Boc-L-aspartic and glutamic α-benzyl esters has been described.[375] Preparations of Z-tyrosine methyl and ethyl esters and hydrazides have been optimized,[376] More controversial is the claim for the formation of Z-amino acid N-acyl-NN-dicyclohexylureas[377] but with physical characteristics different from those recorded by some of us[378] that agree with data described in the literature.[379] Other simple results involving the carboxy group alone (preparation of Boc-L-alanine and glycine N-methylthioamides;[380] and formation of amino acid tetra-n-butylammonium salts,[381]) include in the last-mentioned item a very useful, stable, highly nucleophilic form of amino acids (but not for aspartic and glutamic side-chain esters).

The burgeoning field of "peptide surrogates" requires α-diketone and α-keto-ester homologues of N-protected amino acids, and these are prepared from N-protected α-amino acids *via* N-methoxy-N-methylamides ($RNHCHR'CO_2H \rightarrow RNHCHR'CON(OMe)Me \rightarrow RNHCHR'COC(=CH_2)OEt$ using $CH_2=CHOEt$ with tBuLi, followed by $O_3 \rightarrow RNHCHR'COCO_2Et$ or HCl → RNHCHR'COCOMe).[382]

Substitution at the α-carbon chiral centre in α-amino acids continues to be represented by two main strands: chloride ion-mediated electrochemical methoxylation (see also Section 4.7)[383] and illustrated in Scheme 38 for carboxy-group substitution,[384] and free-radical t-butoxylation (using di-t-butyl peroxide) or halogenation (using N-bromosuccinimide; $PhCONHCHRCO_2Me \rightarrow PhCONHCHRBrCO_2Me$).[385]

A vast volume of data is the only phrase suitable for describing what has accumulated in recent years from studies of enantioselectively-catalyzed hydrolysis of esters of N-acylamino acids. The enzyme-catalyzed aspect of this has been a subject of interest for many years, and recent accounts deal with papain immobilized on Sephadex G-50,[386] or α-chymotrypsin immobilized on poly(vinyl alcohol) by absorption from aqueous solution.[387] These enzymes, and porcine pancreatic lipase, can

cleave allyl esters, even though these are not natural substrates; and there is therefore an alternative to the palladium(0)-catalyzed hydrolysis of these compounds.[388] Acylase I catalyzes the enantioselective hydrolysis of esters of unnatural or rarely-occurring amino acids in the form of their N-acyl derivatives.[389] The potential of this discovery has been realized in the resolution of several examples, including α-methyl-α-amino acids, on a 2 - 29 g scale. Uses for homochiral unnatural amino acids are demonstrated in this study, for example, α-aminobutyric acid enantiomers used for the preparation of (R)- and (S)-1-butene oxide [AcNH- → -Cl → β-chloroalkanols that yield epoxides with KOH], and enantiomers of 2-acetylamino-4-alkenoic acids undergoing iodolactonization to give homochiral 2-acetylamino-4-substituted γ-butyrolactones. The reverse process, use of papain for enantioselective esterification of Boc-amino acids by alkanols and diols, also illustrates the benefits of immobilization; in this report, entrapment in XAD-7 or Sepharose was employed.[390] The other approach, use of synthetic peptides as catalysts for enantioselective ester hydrolysis, also contributes increasingly to the literature, and has been reviewed.[391] N-Acetyl-DL-amino acid p-nitrophenyl esters undergo highly efficient enantioselective hydrolysis (L:D = 167 ± 21) in NN-ditetradecyl-NN-dimethylammonium hydroxide bi-layers through catalysis by N-benzyloxycarbonyl-L-leucyl-L-histidine.[392] Chirality in the quaternary alkyl ammonium hydroxide seems to enhance the stereoselectivity, judging by results for the same system but using NN-ditetradecyl-N-methyl-N-[$CH(OH)_4]CH_2OH^+$ Br- derived from N-methyl D-glucosamine.[393] Copolymers of N-methacryloyl-L-histidine methyl ester accomplish stereoselective hydrolysis of Z-DL-amino acid p-nitrophenyl esters in the presence of a quaternary alkylammonium hydroxide,[394] and also the analogous methanolysis.[395] To put matters in perspective, a figure should be quoted: 60% optical purity is claimed for Z-L-phenylalanine methylamide formed through hydrolysis of the corresponding DL-p-nitrophenyl ester in the presence of a cationic surfactant (Span 60) and the chiral dioxopiperazine cyclo(L-phenylalanyl-L-histidyl), implying that the D-enantiomer is preferentially hydrolyzed.[396] All permutations of the ingredients just described, are completed with incorporation of the hydrophobic moiety in the substrate, as in N-dodecanoyl-DL-phenylalanine p-nitrophenyl ester + Z-L-Phe-L-His-OMe,[397] or Z-L-Phe-L-His-L-Leu-OH,[398] or in the chiral catalyst, as in N-dodecanoyl-L-histidine, for example,[399] or N-dodecanoyl-L-cysteinamide and the L-histidine and L-histidyl-L-cysteine analogues.[400] The ligand exchange principle has been exploited in this topic area, as exemplified by enantioselective hydrolysis, followed by

potentiometric and spectrophotometric methods, of α-amino acid esters catalyzed by the glycyl-L-tyrosine - copper(II) complex.[401]

6.3 Specific Reactions of Amino Acids.- This section covers papers that describe reactions of side-chains first and foremost, though the amino, carboxy, and α-chiral centre combination (that forms the raison d'etre of the preceding sub-section of this Chapter) may also be involved.

Nearly all the papers covered here, deal with protein amino acids and other familiar biologically-important α-amino acids. Free-radical carboxylation of N^{α}-acetylglycine ethylamide has been studied as a route to aminomalonic acid, a recently-discovered protein amino acid whose presence in proteins may be implicated in pathological calcification of arterial proteins in atherosclerosis.[402] A prior study of the decarboxylation of N^{α}-acetylamino malonic acid ethylamide was carried out to establish the behaviour of the product intended to be formed by carboxylation of glycine derivatives.[403] N-Bromosuccinimide bromination of N-phthaloyl valine and N-phthaloyl phenylalanine occurs at the β-carbon atom, reflecting a steric influence on the course of the reaction, since α-halogenation is more usual in this process (e.g. Ref. 45).[404] Decarboxylation of 1-aminocyclopropanecarboxylic acid and derivatives by ninhydrin provides a special test case for the azomethine ylide intermediate that would be expected (based on recent work discussed in Section 6.2), and indeed found since the cycloadduct (63) is formed with N-phenyl maleimide.[405] Perhaps surprisingly, no ring-opening rearrangement was observed in the preceding example, in spite of the vigorous reaction conditions, but was observed in homolytic side-chain decarboxylation of α-methyl N-Boc-3-cyclopropylglutamate [→ BocNHCH(CO2Me)CEt=CH2].[406] The 3-fluoroglutamic acid analogue underwent clean decarboxylation under the same conditions.

A 93.1% yield of crystalline L-glutamic acid is claimed for the Zn-mediated hydrolytic ring-opening of L-pyroglutamic acid.[407] The precipitated chelate is dissolved in mineral acid, and the solution is brought to the isoelectric point, to complete the surprisingly easy reaction. Selective reduction of Z-aspartic and Z-glutamic anhydrides has been observed with sodium borohydride, leading to α-alkanols.[408]

Reactions of α-hydroxyalkyl-α-amino acids are represented by a preparation of O-benzyl-L-serine that is effective when transient N-(4-methoxybenzyl)oxycarbonylation is employed.[409] A mixed O-seryl O-threonyl O-benzyl phosphate has been synthesized through routine reactions, as a model for the phosphodi-ester linkage in Azotobacter

flavodoxins.[410] Like many other examples of permanganate oxidation, autocatalysis by Mn^{2+} has been established for the oxidation of L-serine.[411] A full paper on the synthesis and nucleophilic ring-opening reactivity of β-lactones from L-threonine and related β-hydroxy α-amino acids has been published.[412] For the preparation of the threonine β-lactone, N-benzenesulphonylthreonine was treated with p-bromobenzenesulphonyl chloride and pyridine, since Mitsunobu conditions that were successful with serine gave only the products of decarboxylative anti-elimination with threonine derivatives. There is a tendency for nucleophiles to attack at the carbonyl group rather than at the β-carbon atom (the latter process would be much more useful for general organic synthesis). L-Homoserine can be cyclized via its O-trimethylsilyl ester enolate PhthNC(CH$_2$CH$_2$OMEM)=C(OBzl)OTMS through catalysis by $TiCl_4$, presumably involving an oxonium chloride intermediate, to give 3-aminotetrahydrofuran-3-carboxylic acid in a novel Mukaiyama aldol condensation.[413]

Formation of (Z)-β-arylamino dehydroalanines PhCONHC(CO$_2$Me)=CHNHAr from (Z)-dimethylamino- analogues has been demonstrated.[414] The propensity for amino acids with a good leaving group in the γ-position has been considered to be a possible source of toxic vinyl glyoxylates (2-oxo-3-butenoates) via unsaturated amino acids.[415] Siting of another unsaturated feature in conjugation with that of the side-chain, as in dehydroalanine Schiff bases RCH=NC(CO$_2$Me)=CH$_2$, opens up the possibility of Diels-Alder dimerization to 3,4,5,6-tetrahydropyridines. These undergo intramolecular cyclization after tautomerization (Scheme 39).[416]

Lysine reacts with formaldehyde and H_2O_2 to give N-formyl derivatives rather than methylation that is the well-known result of the reaction in the absence of peroxide. The formation of a formaldehyde radical and singlet oxygen is suggested.[417] Monoglycosylated lysine undergoes Amadori rearrangement to give eventually, 4-, 5- and 6-membered nitrogen heterocycles, while diglycosylated lysines lead to pyrans and sugar degradation products.[418] Lysine plays a role in creating crosslinking amino acid residues, e.g. allysine, in proteins in vivo, another manifestation of the amino group - carbonyl group repertoire of reactions that underlie the two preceding citations. Phthaloyl allysine p-nitrobenzyl ester has been synthesized and its reactions with nucleophiles studied to clarify the potential of crosslinks to undergo subsequent changes.[419]

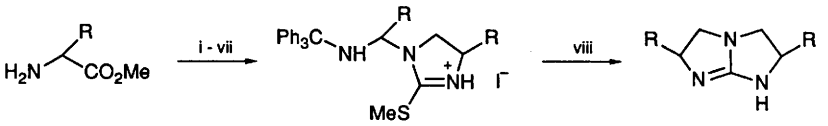
Cysteine is also a versatile performer when presented with other reactive species, and more than 45 compounds are formed with 2,4-decadienal (the major degradation product of linoleic acid) in water during 1 hour at 180°C, 2,4,6-trimethylperhydro-1,3,5-dithiazine being

the major product.[420] Even under controlled conditions, the kinetics of copper- or iron-catalyzed oxidation of cysteine by O_2 are impossible to relate to a mechanistic scheme.[421] More straightforward is the acylation - cyclization of cysteine with 1,1'-carbonyldi-imidazole to give L-2-oxothiazoline-4-carboxylic acid,[422] and S-nitrosation by alkyl nitrites[423] or by N-methyl-N-nitrosotoluene-p-sulphonamide has been clarified to reveal direct nitroso-group transfer.[423] Cysteine or methionine inhibit the nitrosation by nitrous acid, of N-methylaniliine or morpholine under physiological conditions at pH 2.[424] As with other reactive side-chain functionalities, SH-protection is essential for most synthetic applications of cysteine, and reactions of N^{α}-Boc-S-[(N'-methyl-N'-phenylcarbamoyl)sulphenyl]-L-cysteine have been investigated from this point of view.[425] Careful study has been made of iodoacetic acid-mediated hydrolysis of L-methionine to L-homoserine.[426]

The use of abundant chiral α-amino acids in the asymmetric synthesis of target molecules, many of which are of structural types totally unrelated to the amino acids, continues to expand. Stereoselective synthesis of syn-β-amino alkanols from natural amino acids by Reetz and co-workers has been reviewed.[427] D-(-)-Phenylglycine methyl ester has served in a synthesis of chiral guanidines (Scheme 40).[428] (S)-Pyroglutamic acid has been modified (through reduction of its unsaturated functional groups and Mitsunobu substitution) for syntheses in the pyrrolidine series.[429]

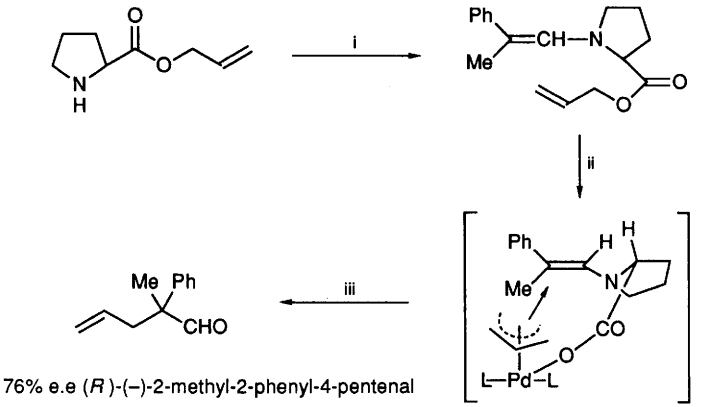
Asymmetric allylation of aldehydes has been elegantly acheived (Scheme 41) using L-proline as chiral determinant.[430] The same imino acid has been used for construction of chiral fused isoxazines.[431] Reactions at aromatic sites in side-chains continiue to be studied from the points of view, of preparative opportunities leading to analogues of common amino acids, and of mechanistic interest. Rate constants for sequential mono- and di-iodination of tyrosine have been elucidated with the help of ^{3}H- and ^{14}C-labelling.[432] Hydroxylation of phenylalanyl and tyrosyl side-chains is the predominant structural change accompanying γ-irradiation of aqueous solutions,[433] and the products of further oxidation, e.g. dopaquinone, can undergo condensation, though the cyclization of this particular compound is inhibited by copper(II) ions.[434] A novel violet colour reaction (λ_{max} 560 nm), shown for tyrosine methyl ester with iron(III) at pH 8, may be useful in analysis. It is suggested to be a 2:1-complex.[435]

Heteroaromatic residues reported to be substituted, include protected tryptophans with benzenesulphenyl or benzeneselenenyl chloride, the final destination of the substituent being the indole 2-position (possibly via rearrangement of an initial 3-substituted isomer).[436]



Reagents: i, Ph_3CCl, NEt_3; ii, $-CO_2Me \longrightarrow CH_2NH_2$; iii, (*R*)-ZNHCHRCO$_2$H, DCC-HOBt; iv, H_2-Pd/C; v, DIBAL; vi, $Cl_2C{=}S$; vii, MeI; viii, DMF/120°C, 1 hour

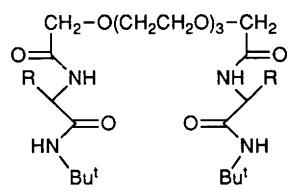
Scheme 40



76% e.e (*R*)-(−)-2-methyl-2-phenyl-4-pentenal

Reagents: i, PhCHMeCHO; ii, $Pd(PPh_3)_4$, PPh_3, reflux THF; iii, 10% aq.HCl, reflux 4h

Scheme 41



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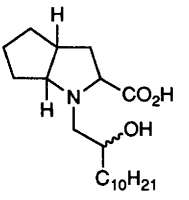

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Selective protection problems for piperazine 2-carboxylic acid have been solved by establishing a method for placing a Boc group on one nitrogen function and a Z group on the other.[437] A review of the chemistry of the histidine side-chain has appeared.[438]

Ovothiol A, a natural α-amino acid carrying the mercaptoimidazole functional group in its side-chain, is a more efficient tyrosyl radical scavenger than cysteine or glutathione.[439]

6.4 Effects of Electromagnetic Radiation on Amino Acids.- A number of papers discussed here are concerned with chemical structural changes undergone by photo-excited aromatic and heteroaromatic amino acids, extending studies that have been pursued over many years. Other papers deal with the fundamental physical processes that precede these chemical changes.

Photolysis of aqueous L-tryptophan in the well-known way, to give kynurenine and its N-formyl derivative, occurs without notable extra assistance from uranyl sulphate, compared with other previously-studied photosensitising species.[440]

Photo-CIDNP analysis of those amino acids that are photopolarizable in the presence of a flavin dye (i.e., tyrosine, tryptophan, histidine, N-methylated lysines and methionine) reveal a hydrogen-atom abstraction mechanism for generating radical pairs in the cases of tyrosine and histidine, but an electron transfer mechanism for the other amino acids.[441] A pulsed radiolysis study yields redox midpoint potentials for a neutral radical created in the indole moiety of the tryptophan side-chain.[442]

Effects of solvent on fluorescence characteristics of tyrosine and its derivatives have been elucidated.[443] Single-photon timing data have been extracted from fluorescence decay curves measured for tryptophan solutions at pH 6 for various emission wavelengths.[444] Solvent complexation of tryptophan has been studied by laser-induced fluorescence excitation spectroscopy of solutions subjected to supersonic free-jet expansion.[445]

7 Analytical Methods

7.1 General.- Early Chapters in a recent book on protein analysis, review current methods for amino acid analysis.[446] The general situation amounts to the consolidation of existing methods, with some success at raising sensitivity levels, and substantial progress with determinations of enantiomeric composition.

7.2 Gas-Liquid Chromatography.- Derivatization protocols continue to favour N-pentafluoropropionyl[447] and heptafluorobutyroyl butyl esters,[448,449] though N,O(S)-t-butyldimethylsilyl derivatives continue to hold their initial promise.[450] These studies, representative of a larger routine literature, have been selected to illustrate different detection methods (FTIR; ECD; CIMS), and other aspects: "losses" that occur during derivatization, and other problems of interference; trace analysis (amino acids in 600My sedimentary rocks; GABA and Glu in rat brain striatal microdialyzate at 6 pg sensitivity, 20μL fluid containing 105.5 pg GABA and 9.4ng Glu).

A stationary phase carrying the N,N'-3,6,9-oxadecanoyl-bridged L-phenylalanine t-butylamides (64) has been proposed for analytical resolution of amino acids by capillary g.l.c. of the N-trifluoroacetyl derivatives of their alkyl esters.[451] An n.m.r.study of the mechanism of chiral recognition by this host in solutions containing amino acid derivatives as guest, has been included in this paper. An application of this system for analysis of the D-amino acid composition of foods has been published.[452] A Chirasil-type chiral polysiloxane di-amide has been investigated for enantiomeric analysis by g.l.c.[453]

7.3 Ion-Exchange Chromatography.- There is some overlap with the later h.p.l.c. section, as far as trace analysis is concerned. Non-routine papers include amino acids in fossil bones (samples available only at the milligram level),[454] a study of equilibria and rates of uptake of phenylalanine and tyrosine by the strong acid cation exchange resin, Amberlite 252,[455] and estimation of total cysteine + cystine in proteins after reduction and derivatization with 3,3'-dithiopropionic acid,[456] and of homocysteine in plasma (elution with dithreitol-containing buffers).[457]

Sorption of amino acids on to a cellulose-based ampholyte represents an ongoing study of potential new ion-exchange media.[458]

7.4 Thin Layer Chromatography.- As with a number of other topic areas, techniques that have become thoroughly routine and therefore generate literature that is largely excluded from this Chapter are given an apparently uneven airing for this reason.

In the "overpressured" mode (one mobile phase moving over another immiscible liquid) the simultaneous t.l.c. of 100 or more plates has been demonstrated for PTHs.[459] Further data enabling the parametrization of 55 amino acids for peptide QSAR (see Vol.21, p.55) include t.l.c. in several systems.[460] Several papers from one research group constitute a concise picture of current uses of t.l.c. for enantiomeric analysis.

The topic has been reviewed in general terms[461] and specifically (for post-1972 literature) for dansyl and 2,4-dinitrophenylamino acids.[462] The copper(II) complex of the D-proline-derived species (65) has been used to impregnate reversed-phase octadecylsilica t.l.c. plates for the purpose of enantiomer separation of imino acids.[463]

7.5 High Performance Liquid Chromatography.- In the context of this Chapter, the literature on this technique cannot yet be said to be recycling established results (as it appears to be in some other analytical areas). H.p.l.c. offers a less expensive amino acid analysis facility than any other instrumental technique, with the opportunity to use the h.p.l.c. instrumentation for other analytical purposes. What is needed - and not exactly lacking - is a clear statement of what is the best chemistry for the purpose, since there are many derivatization protocols available. Appraisal of four reagents, o-phthaldialdehyde, phenyl isothiocyanate, Fmoc chloride, and dansyl chloride,[464] for the analysis of 24 - 26 of the major amino acids in 20 - 30 minutes leads to the brief judgments, that phenyl isothiocyanate is the least sensitive, and its reproducibility and linearity are poor (and identification of cysteine is not possible), but the other three methods are reliable. A similar review of fluorescence-based methods concludes that the o-phthaldialdehyde method is most suitable compared with fluoresceamine and 7-chloro-4-nitrobenzofurazan (but the latter method is resorted to for proline and other imino acids since the o-phthaldialdehyde method is not applicable to these).[465] A comparison of h.p.l.c. with the revived methods of electrophoresis has been reviewed.[466]

A number of fundamental physical studies are included in the recent literature, including the influence of salts on the retention of amino acid derivatives on reversed phase columns,[467] adsorption of amino acids on to spherical titania [amorphous titanium(IV) hydrous oxide],[468] and on to synthetic hydroxylapatite.[469] Microbore h.p.l.c. has been authoritatively reviewed.[470]

Use of h.p.l.c. for the detection of N-nitroso-imino acids has been described.[471] Another class of "naturally-derivatized amino acids", as they might be called, that is represented in the h.p.l.c. literature, is the unusual but not uncommon α-amino acid amide group. Many important peptides possess an amidated C-terminus, which might be released intact in biological breakdown processes. Thermospray mass spectrometry detection in biological samples, down to 1 pmol levels, with ammonium formate added post-column to generate the necessary ions in the m.s. ion source, has been explored.[472]

Phenyl isothiocyanate dervatization has a major role now, in h.p.l.c. analysis of amino acids. Papers offering creative contributions include thermospray m.s. detection,[473] application to identification of amino acid hydrazide mixtures formed by hydrazinolysis of peptides, and to identify the C-terminal amino acid - the only residue not to appear as an amino acid hydrazide in the hydrazinolysate,[474] attention to composition of solvents for gradient elution,[475,476] and sources of contamination.[477] Several articles on PTC-amino acid technique, appear in this source,[477] *e.g.* discussing the Applied Biosystems automated system.[478] Two new isothiocyanate reagents have been described, that incorporate a ferrocenyl moiety as "electrophore" - *i.e.* sensitive to electrochemical detection.[479]

4-Substituted N-phenylthiohydantoins (PTHs) continue to be reported upon for their h.p.l.c. properties. The purpose of these studies is to improve the analytical aspect that is on the end of the Edman peptide sequencing chemistry; an auto-injection system has been described, that is not dependent on critical timing for efficient transfer of PTHs from the conversion flsk to the sample loop of the injection valve.[480] Picomole analysis of PTHs, using the BAS 200A instrument,[481] and sub-picomole analysis,[482] and other details in particular applications,[483] have been described.

The *o*-phthaldialdehyde - thiol reagent system continues to be the mainstay for h.p.l.c. amino acid analysis, though awareness of its potential for erratic results must be well known to all users (*cf.* Refs.347 and 348). An elegant study using ^{14}C-labelled amino acids shows that the 2-alkythio-isoindoles created by the OPA - thiol reagent are unstable during h.p.l.c. on a reverse phase C-18 column.[484] Comparison of the emergence profile as determined by radioactivity, with the fluorescence detection profile, shows that there is excessive "^{14}C-fronting" of the peaks. This is accounted for on the basis of faster-running ^{14}C-containing degradation products. Column half-lives of glutamic acid, arginine, and ornithine iso-indoles are 16, 40, and 54 minutes respectively, explaining the importance emphasised in several papers, of following a strict protocol so that reliable results might be obtained using the OPA method. The same ^{14}C-monitoring method applied to PTC-amino acids showed superimposed ^{14}C- and light absorption profiles, indicating no degradation of PTC-derivatives on the h.p.l.c. timescale. Not only is the iso-indole product unstable, but the premixed reagent system would also be expected to be unstable (as a result of thiol oxidation), and this has now been shown to be so,[485] but this uncertainty factor, once appreciated, can be eliminated by proper attention to operating technique.

As part of a study of the proteolytic activity of Lactobacillus bulgaricus, a comparison of the OPA technique with classical ion-exchange analysis puts them at equal ranking, but notes that the ion-exchange methods provide additional analytical information.[486] A number of papers have appeared employing the OPA technique in different contexts: neurotransmitter amino acids (glutamic acid at 0.5 pmol level, and aspartic acid, in rat striatum using electrochemical detection,[487] corresponding results for the same method, using 2-methylpropane-2-thiol but detecting at 50-100 fmol levels,[488] and using a broader range (18 represented) of amino acids).[489] OPA - mercaptoethanol has been used for the analysis of amino acids in tea,[490] in blood samples,[491] and in exploratory studies of automated operation.[492] One bonus of the OPA - thiol reagent system is the opportunity to use a chiral thiol so that proportions of enantiomers in derivatized amino acids can be determined since they lead to diastereoisomer mixtures. OPA - N-acetyl-L-cysteine continues to be used reliably in this context. This study includes a comparison of this method with the "chiral mobile phase" approach using copper(II) acetate and L-proline in isocratic h.p.l.c. analysis of representative α-amino acids and their α-alkyl analogues.[493]

A new variant is the use of naphthalenedialdehyde with CN^-, yielding fluorescent cyano[f]benzoiso-indoles with amino acids, that are more stable than the OPA - thiol adducts, and suitable for electrochemical detection.[494] The potential of the method is illustrated with assays of desmosine and isodesmosine protein cross-linking amino acid residues in proteins.

9-Fluorenylmethoxycarbonyl chloride (Fmoc-Cl) is making headway in competition with the other fluorescent-derivatization protocols, and its complementary nature with the OPA method has been pointed out;[495] a commercial robotic autosampler can effect OPA adduct formation (amino acids) followed by Fmoc-Cl derivatization (imino acids), and then h.p.l.c. The accuracy of the Fmoc protocol has been compared favourably with that of the other standard methods,[496] and shown to be routinely convenient.[497] The supercritical fluid chromatography of Fmoc-amino acids and of the diastereoisomer mixtures formed with the chiral analogue (+)-1-(9-fluorenyl)ethyloxycarbonyl chloride ("Flec-Cl") have been emphatically established, opening up exciting new possibilities.[498]

Several other fluorescence-generating derivatization reactions have been developed, monobromobimane for homocysteine h.p.l.c.,[499] naphthyl isocyanate,[500] and 4-(N-1-dimethylaminonaphthalene-5-sulphonylamino)phenyl isothiocyanate, an ingenious combination of the dansyl and PTC-derivatives.[501] Preparation of DABS-amino acids using 4'-

(NN-dimethylaminoazobenzene)sulphonyl chloride (dansyl chloride) and their derived DABTH's for h.p.l.c. analysis of amino acids[502] is continuing to gain advocates.[503] Better recovery of serine and threonine derivatives is claimed for preparations of DABTH's using BF_3 etherate in place of trifluoroacetic acid for the conversion step for substituted anilinothiazol-5(4H)-ones created in amino acid sequence analysis. These derivatives allow detection down to 100 fmol levels.[504] An ingenious alterative to converting such thiazolones into thiohydantoins is to aminolyse them with 4-aminofluorescein, to give PTC-amino acid fluoresceinamides; these can be detected down to 100 amol levels.[505] Similar h.p.l.c. studies of fluorescent 4-(NN-dimethylamino)-1-naphthyl thiohydantoins[506] has been described.

Miscellaneous h.p.l.c. studies extending the specific examples described above, include the six putative neurotransmitter amino acids (h.p.l.c. analysis after derivatization with electrochemical detection),[507] amino acids in cerebrospinal fluid,[508] and six coded amino acids in dried blood samples.[509]

Chiral h.p.l.c. of amino acid derivatives using either chiral stationary phases based on Boc-D-valine bonded to silica[510] or Pirkle-type phases with (R)-N-(3,5-dinitrobenzoyl)phenylglycine bonded to γ-aminopropyl-silica[511] (as used for the resolution of N^{α}-substituted DL-amino acid anilides).[512] α-Chymotrypsin bonded to silica gel gives a chiral phase that has been used for the resolution of amino acid esters - though not only through the familiar enantioselectivity of catalyzed hydrolysis but using the enzyme as a chiral surface, since the inhibited enzyme was also effective in h.p.l.c. resolution.[513] Chiral mobile phases incorporating copper(II) complexes of α-t-butyl L-aspartate,[514] proline,[515] and ligands of the di-amino di-amido-type containing L-amino acid moieties[516] have been used for amino acid analytical resolution (for dansyl DL-amino acids in the last-mentioned example).

7.6 Fluorescence Analysis. - This section is reserved for methods that appear promising or have become established, for fluorescence-generating reactions that do not depend on subsequent h.p.l.c. procedures. Thus, post-column OPA - 3-mercaptopropanoic acid fluorimetry allows estimation of amino acids separated by ion exchange chromatography with sensitivity at low picomole levels,[517] and N-(2-pyridyl)amino acids formed by condensation of amino acids with 2-aminopyridine, might show similar potential.[518]

The authors' 1979 method for tryptophan estimation in food, based on measuring the fluorescence increase following N^{in}-hydroxymethylation

caused by addition of formaldehyde at pH 10, has been given further attention through assessing the pH-dependence of the fluorescence.[819]

7.7 Other Analytical Methods.- Capillary zone electrophoresis seems destined to take a larger role, with further impressive results such as sub-attomole detection of fluorescein isothiocyanate adducts using laser-induced fluorescence measurements.[820] Following polyacrylamide-gel electrophoresis of amino acids and peptides in an acetic acid - formic acid pH 2 buffer, using fabric-reinforced gels, the samples were fixed by freeze-drying of excised zones. The purpose was to be able to perfuse the zones uniformly with ninhydrin solution, so as to give lowered detection limits, and 0.1 - 0.25 μg levels were achieved.[821] Micellar electrokinetic chromatography on chiral media has been demonstrated as capable of the resolution of dansyl-DL-amino acids.[822] Specific chemiluminescence of excited NO_2, created using a commercial nitrogen analyzer in which amino acids are combusted at 1000 - 1100°C to give NO, converted by ozone oxidation into the excited NO_2, can be detected with 200 times greater sensitivity than existing (Lowry) nitrogen oxides analysis and therefore operates at sub-microgram sample levels.[823]

7.8 Determination of Specific Amino Acids.- Molecular structural factors from which specific assays can be derived occur in histidine and in tyrosine. Tyrosine PTH, or the amino acid itself, or histidine - copper(II) complex coupled with diazotized sulphanilic acid, can be assayed by adsorptive stripping voltammetry at low levels.[824] Characteristic polarographic profiles have been determined for histidine.[825]

This section continues to report enzyme electrodes coping with tyrosine, DOPA, and α-methylDOPA, based on tyrosinase immobilized on a pH electrode,[826] and an electrode for L-asparagine using a sensor employing L-aspartase immobilized on an ammonia-sensing probe (capable of 1.6×10^{-5} - 1.5×10^{-3} molar sensitivity, and stable for more than 30 days),[827] and a more horticultural version (presumably with a shorter lifespan!) of the same system using minced parsley leaves on a potentiometric ammonia gas sensor, for L-asparagine and L-glutamine assays.[828]

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2
Peptide Synthesis

BY D. T. ELMORE

1. Introduction

The format of this report is almost identical to that used last year.[1] An extra section has been added to the main body of the report to include some miscellaneous papers that do not fit easily into other sections. An extra section has also been introduced in the Appendix in which peptides containing phosphate or phosphonate groups are included. Meetings abstracts and patents are not included. There is an increased tendency for the synthesis of peptides by solid phase methodology to be reported without adequate details of coupling techniques, protecting groups used for side chains, deprotection methods and characterization of products. With the warning, *lector caveat*, references to papers of this type are included in the Appendix. Nevertheless, the number of references is lower than reported last year and this may be due in part to the unavailability of *Peptide Information* to the Reporter.

Pertinent reviews[2-35] abound, some readily accessible while others are less so. A wide variety of topics are reviewed including solid phase peptide synthesis[2-8], coupling methods[9-11], semisynthesis[12,13], enzyme-catalysed synthesis[14,15], peptide drugs and vaccines[16-20] and pseudopeptides[21-23].

2. Methods

The arrangement of the main body of the text is identical to that used last year.

2.1.1 Amino-group protection

Chiral α-azido acids, synthesized by the method of Evans and Britton[36], can be converted into pentafluorophenyl esters for the synthesis of peptides with no detectable racemization[37]. The azido group can be reduced to an amino group by hydrogenation over 10% Pd on charcoal in 5% CH_3CO_2H/EtOH. This is clearly an

excellent route to peptides of unnatural amino acids that have to be synthesized and resolved.

Reaction of 4-hydroxyphenyldimethylsulphonium methyl sulphate (1; R = H) with benzyl chloroformate (ZCl), di-t-butyl dicarbonate [$(Boc)_2O$] or 9-fluorenylmethyloxycarbonyl chloride (FmocCl) gave stable, water-soluble reagents (1; R = Z, Boc, Fmoc) for preparing the corresponding *N*-protected amino acids and dipeptides.[38] Of particular interest is the direct preparation of H-Lys(Z)-OH and H-Orn(Z)-OH in good yield at pH 11.3. A common problem in the preparation of Fmoc amino acids and similar urethane derivatives is the formation of oligomers. It is now reported[39] that this side reaction can be avoided by forming *O,N*-bis-trimethylsilyl derivatives of amino acids by reaction with Me_3SiCl and base in an aprotic solvent then treating with the appropriate acylating agent such as a chloroformate.

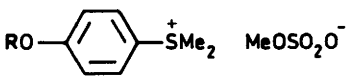
Treatment of 9-(hydroxymethyl)fluorene/9-formylfluorene with amino acids or their esters gives enamines (2; R = H).[40] Although attempted coupling reactions of these failed because the basicity of the *N*-atom was too high, introduction of electron-withdrawing groups (2; R = Cl) corrected this sufficiently for peptide synthesis to be feasible. The protecting group could be removed by catalytic transfer hydrogenolysis or by mild acid hydrolysis. Further development will be awaited with interest because racemization is reported to be low.

Although the 4-methylthiobenzyloxycarbonyl (Mtz) group has nothing special to recommend it,[41] the related 4-methylsulphinylbenzyloxycarbonyl (Msz) group (3) is of interest because it is stable to both acids and bases. It is readily removed, however, by reductive acidolysis using $SiCl_4/CF_3.CO_2H/C_6H_5.OMe$ at 0° C for 60 min. The Msz group is useful for protecting the ϵ-amino group of Lys and, as a test of its utility, scyliorhinin I:

H-Ala-Lys-Phe-Asp-Lys-Phe-Tyr-Gly-Leu-Met-NH_2

was synthesized by a solution method.

New base-labile protecting groups[42] (4; R = NO_2-, $MeSO_2$-) have been developed from earlier models. They are reported to



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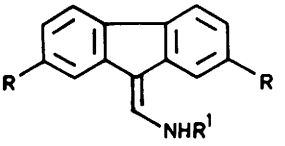

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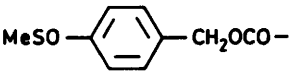

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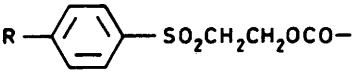

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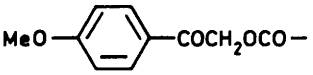

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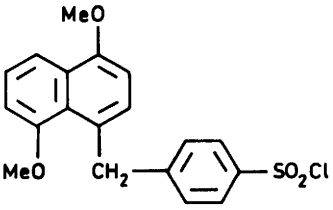


(6)

Scheme 1

be more stable to base than are Fmoc derivatives.

Photolabile groups for amino-group protection continue to be developed. The 4-methoxyphenacyloxycarbonyl (Phenoc) group[43] (5) has been used to synthesize a simple dipeptide derivative (Scheme 1). Although this approach may find some application in carrying out fragment condensations, it is unlikely to achieve wide popularity because of the tedious preparation of Phenoc derivatives of amino acids. 4-[(4',8'-dimethoxy)naphthylmethyl-benzenesulphonyl chloride (6)[44] and several related compounds have been used for amino-group protection. The group is readily cleaved by exposure to light of wavelength 333 nm without affecting a range of other commonly used protecting groups.

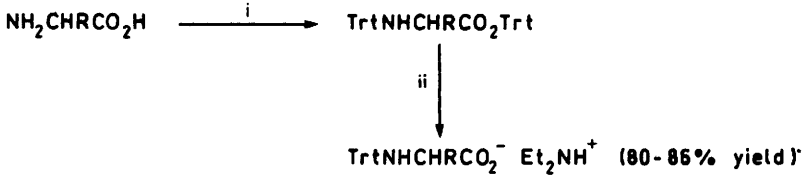
The preparation of *N*-trityl (Trt) amino acids has been improved[45]. The trityl ester of an *N*-trityl amino acid is deliberately formed as an intermediate and then hydrolysed under closely defined conditions (Scheme 2); only Pro gave poor yields. α-Trityl derivatives of H-Lys(Boc)-OH and H-Lys(Fmoc)-OH can be made satisfactorily by this method.

For the synthesis of block polymers segments, derivatives such as (7; Teoc = $Me_3SiCH_2CH_2OCO$-) are recommended.[46]

The isolation of α-*N*-protected derivatives of His is troublesome because of their solubility in water and the consequent difficulty of removing inorganic salts. A general method for purifying these compounds by ion-exchange chromatography using a pH-gradient has been described.[47]

2.1.2 Carboxyl-group protection

In a simple and convenient procedure,[48] t-butyl esters of *N*-protected amino acids are prepared in high yield from the commercially available t-butyl fluoroformate in the presence of Et_3N and 4-dimethylaminopyridine (DMAP) in CH_2Cl_2/Bu^tOH at room temperature. Z-Asp(OBu^t)-OH and Z-Glu(OBu^t)-OH are easily available, the latter through the oxazolidinone. A new type of tertiary ester has been synthesized[49] by treatment of a Z-amino acid with 2-(1'-adamantyl)propene in presence of H_2SO_4 to give 2-(1'-adamantyl)-2-propyl esters.

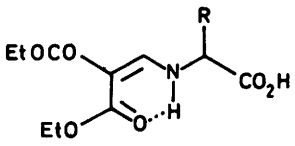


Reagents: i. TrtBr in $CHCl_3$/$HCONMe_2$ at room temperature, 20-60 min. then Et_3N at room temperature, 50 min.;
ii. MeOH/$CHCl_3$/$HCONMe_2$ (3:2:1) at 50°C. 20-150 min. then Et_2NH/Et_2O

Scheme 2

Fmoc—Lys(Z)—[Lys(Teoc)]$_n$—

(7)



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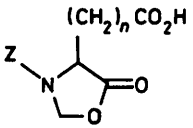

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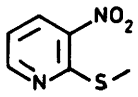


(10)

Another new method for blocking carboxyl groups has been described.[50] 9-Diazofluorene, which is easily prepared in a stable, crystalline form and has been known for nearly 80 years, reacts readily with *N*-protected amino acids in CH_2Cl_2 or with free amino acids as their tosyl salts in CH_2Cl_2/Pr^iOH. The resultant 9-fluorenyl esters can be cleaved by CF_3CO_2H/CH_2Cl_2 (1:1) in presence of anisole during 30 min. or by hydrogenolysis in MeOH over 10% Pd-charcoal catalyst.

For those who require esters that are cleaved under very mild alkaline conditions, 3-hydroxypropionitrile may be a useful reagent. *N*-Protected amino acids are esterified with 3-hydroxypropionitrile using *NN'*-dicyclohexylcarbodi-imide (DCCI) and DMAP.[51] The resulting esters are hydrolysed by 10% K_2CO_3 at room temperature during a few minutes.

A variety of primary and secondary alcohols in the presence of DMAP will cleave the unsymmetrical anhydride generated from isopropenyl chloroformate and an *N*-protected amino acid to give the corresponding esters.[52] Even Bu^tOH gives satisfactory yields under more drastic conditions. Competing formation of isopropenyl esters is negligible under the conditions used.

Allyl esters have found further application in the synthesis of *O*-glycopeptides[53] mainly because they can be deprotected for peptide extension at the *C*-terminus under neutral conditions by Pd-catalysed allyl transfer to morpholine.

Amino acids can be esterified as their *N*-2,2-bis(ethoxycarbonylvinyl derivatives (8), which are obtained by reaction of the potassium salts of amino acids with diethyl (ethoxymethylene)malonate, by reaction with an alkyl bromide in presence of Et_3N in acetone.[54] The *N*-protecting group is removed with bromine in $CHCl_3$ at room temperature to give the amino ester hydrobromide.

The β-1- and β-2-adamantyl esters of Asp reported last year[55] have been subjected to searching examination[56] by synthesizing the *C*-terminal octapeptide of the β-subunit of human chorionic gonadotropin by a conventional solution method and a hexacosapeptide of the α-subunit of the insulin receptor by solid

phase peptide synthesis. The security offered against aspartimide formation already reported[55] seems assured by the latest research. A different philosophy has emerged from Bartlett's laboratory.[57] Instead of selectively protecting the side chain carboxyl groups of Asp and Glu, they are selectively exposed by forming the 5-oxazolidinones (9) from the reaction of the *N*-Z-amino acids with paraformaldehyde in the presence of a catalytic amount of *p*-toluenesulphonic acid, a reaction discovered long ago by Ben-Ishai. The free carboxyl group of (9) can be coupled to amino acid esters using a carbodi-imide or it can be subjected to a modified Curtius-type degradation using diphenyl phosphoroazidate. When the Z-group is removed by hydrogenolysis, the oxazolidinone decomposes liberating the α-CO_2H group as well as the α-NH_2 group.

2.1.3 Side-chain protection

A new synthesis[58] of *O*-benzylserine involves *N*-protection with the 4-methoxybenzyloxycarbonyl group followed by *O*-benzylation with NaH in $HCO.NH_2$ and benzyl bromide then selective deprotection with 10% $CF_3.CO_2H$ in CH_2Cl_2.

The *S*-Bu^t, *S*-Acm and *S*-Trt derivatives of Fmoc-Cys-OH have all been used[59] in the solid phase synthesis of somatostatin (1-14). The *S*-trimethylacetamidomethyl (Tacm) group is introduced[60] and removed by methods that are very similar to those used with the *S*-Acm group. The Tacm group is less prone to oxidation in air or by other mild oxidising agents. It was used in the synthesis of a new natriuretic peptide isolated from porcine brain. The hydrochlorides of *S*-methylcarbamoyl-, *S*-ethylcarbamoyl- and *S*-dimethylcarbamoyl-cysteine as well as *N*-acetylcysteine and *N*-acetylcysteinylglycine have been prepared.[61] It was found that deprotection with mercury (II) acetate caused some cleavage of Boc groups. Protection of the thiol group of cysteine with the 3-nitro-2-pyridylsulphenyl group (10) has been examined[62] in solid phase peptide synthesis. Results were satisfactory when it was used in conjunction with the Boc and Bzl groups but it was useless when Fmoc groups were used. In

addition, it cannot be used in the "low-high" method of deprotection with HF. Two further thiol-protecting groups (Snm, Scm) have been used in Barany's laboratory (Scheme 3).[63] The former has been employed before by *inter alia* Kemp's laboratory in the method of peptide synthesis by prior thiol capture (see below). Both groups are stable to strong acids and are removed by treatment with dithiothreitol in presence of morpholine.

A new method for reducing methionine sulphoxide residues to Met involves reduction with SO_3 - $HCO.NMe_2$ complex and thiols.[64] For example, reduction of Z(OMe)-Phe-Met(O)-OMe with SO_3 - $HCO.NMe_2$ and $HSCH_2.CH_2SH$ gave 85% of Z(OMe)-Phe-Met-OMe.

Dynorphin(1-24) and peptide E, containing respectively one and two Trp residues, have been synthesized by solution methods using a new protecting group, 2,4,6-tri-isopropylbenzenesulphonyl (Tps), for the indole nitrogen atom.[65] The Tps group is removed by $CF_3.SO_3H$/PhSMe in $CF_3.CO_2H$.

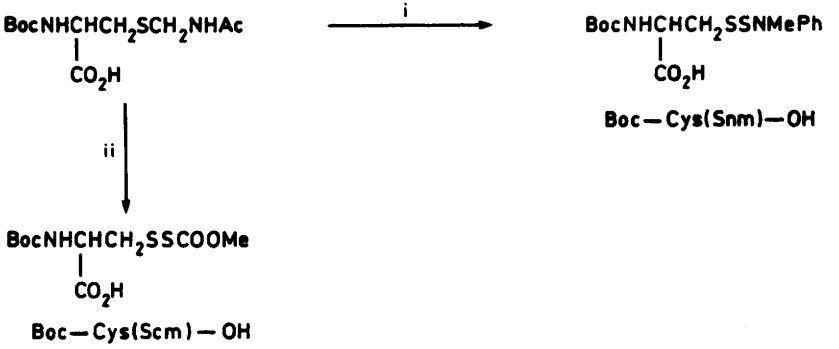
An account of the problems associated with the protection of the guanidino group of Arg has appeared.[66]

2.2 General deprotection

During the removal of Boc, Z and OBzl groups from protected somatostatin analogues by hydrogenolysis and acidolysis, the extent of deprotection was monitored by FAB mass spectrometry,[67] a technique that will surely be used more widely.

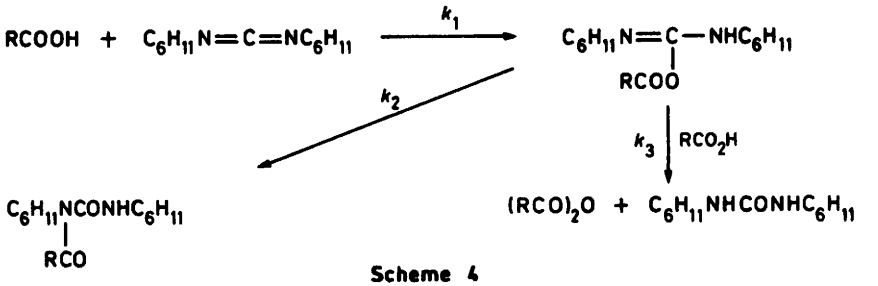
Although tetrafluoroboric acid in $CF_3.CO_2H$ is a weaker acid than HBr in $CF_3.CO_2H$, it is useful for the deprotection of blocked peptides.[68] 1 M HBF_4 in $CF_3.CO_2H$ containing PhSMe removes Boc, OBzl and Z groups at 4° C, but Mts and OC_6H_{11} groups require treatment at 25° C. Met(O) was reduced to Met. This reagent is also able to cleave peptide amides from a 4-methylbenzhydrylamine resin after solid phase synthesis. The technique was tested by synthesizing lamprey gonadotropin-releasing hormone by both solution and solid phase methods.

Trialkylsilanes, which are hydride donors, have been tested as scavengers for carbocations during acidolytic deprotection.[69] Unfortunately, Et_3SiH rapidly reduces the indole ring of Trp

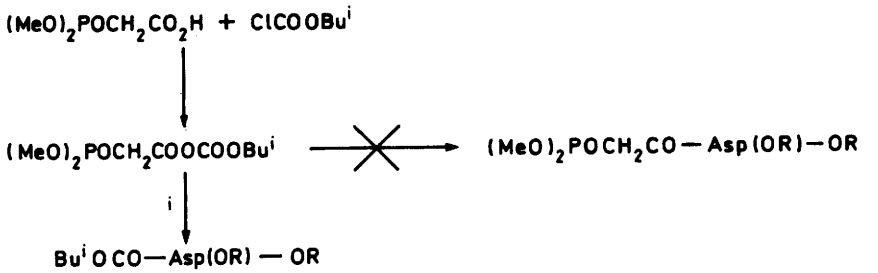


Reagents: i. $ClCOSCl/CHCl_3$ then PhNHMe;
ii. $MeOCOSCl/CHCl_3$

Scheme 3



Scheme 4



R = 4—$NO_2C_6H_4CH_2O$—

Reagent: i. H—Asp(OR)—OR

Scheme 5

under acidic conditions, but Pr^i_3SiH proved to be quite satisfactory. In a searching test, the tridecapeptide, CGECGEGGCGECG, was synthesized by a solid phase method on a Wang resin using Fmoc protection of α-amino groups, and t-Bu ester and *S*-Tr protection of the side chains of Glu and Cys respectively. Cleavage and deprotection was carried out in $CF_3.CO_2H$ with Et_3SiH and a high recovery of pure peptide was obtained.

Silver trifluoromethanesulphonate is a valuable tool for removing some *S*-protecting groups such as Mbzl and Acm, but not 1-adamantyl, Bu^t or 4-methylbenzyl.[70] This method should be useful for the selective formation of disulphide bonds in peptides containing >2 cysteinyl residues.

The solid phase synthesis of bradykinin potentiating peptide 5a, Pyr-Lys-Trp-Ala-Pro-OH, provided a good example of the use of orthogonal protecting groups and the use of F^- ion for deprotection of the peptide and cleavage from the resin.[71] α-Amino groups were protected with Boc, ε-amino group with Fmoc, and the diphenylphosphinothioyl (Ppt) group was used for the indole nitrogen atom. The last group was removed and the phenacyl linkage to the resin was cleaved by $Bu_4N^+F^-$.

2.3 Peptide bond formation

It has long been known that carbodi-imides react with carboxylic acids to give an unstable *O*-acylisourea (Scheme 4) and this either rearranges to the stable *N*-acylurea or reacts with another molecule of carboxylic acid to give the acid anhydride and the urea corresponding to the carbodi-imide. It is also well established in practice that the formation of the unwanted *N*-acylurea is disfavoured *inter alia* by using a nonpolar solvent. While not dealing specifically with peptide synthesis, the kinetics of the reactions (Scheme 4) between carbodi-imides and carboxylic acids have been examined.[72] The rate constants, k_1 and k_3, both depend on the interaction between acid and solvent and correlate well with the hydrogen-bond accepting ability of the solvent. Thus, a plot of *ln* k_1 versus the Shorter B hydrogen-bonding parameter or Taft's hydrogen-bond acceptor

basicity, β, is linear with negative slope. In contrast, k_2 is independent of the nature of the solvent. The formation of acid dimer is not important at low concentration but favours anhydride formation whenever the retardation of the k_1 and k_3 steps is minimized. This occurs in solvents in which the acid is least soluble. To summarize a substantial paper in the form of a rule of thumb, for a given concentration of carbodi-imide and acid, reaction is faster and anhydride yield is better in a solvent in which the carboxylic acid is less soluble. It should be pointed out that this paper does not examine the case of water-soluble carbodi-imides, an important point for peptide chemists.

Amides of *N*-protected amino acids and peptides have been prepared by using DCCI and 1-hydroxybenzotriazole (HOBt) to make 1-acyloxybenzotriazoles and then treating these with 25% aqueous NH_3.[73] The formation of peptides under the influence of hydrophobic carbodi-imides at the interface between the hydrophobic membrane and the aqueous pool inside reversed micelles has been studied.[74]

Isopropenyl chloroformate can be used to form unsymmetrical anhydrides at ambient temperature for peptide synthesis.[75] Di-t-butyl pyrocarbonate has been used as a condensing agent to synthesize 6-quinolylamides from *N*-protected amino acids.[76] Unsymmetrical anhydrides generated from EtO.COCl have been used to couple *N*-protected amino acids and α-aminoalkylphosphonates.[77] An unusual course for an aminolysis reaction of a carboxylic - carbonic acid anhydride has been reported (Scheme 5).[78]

In the coupling of a Z-dipeptide and an amino acid ester by an unsymmetrical carbonic anhydride, it has been reported that addition of a molar equivalent of $CuCl_2$ suppresses racemization to <0.1%.[79] Similar results were obtained with the EEDQ and BOP coupling methods. The addition of $CuCl_2$ is claimed to be more effective than addition of HOBt. Racemization is also reported to be decreased if the unsymmetrical anhydride coupling procedure is carried out in the presence of crown ethers.[80]

Asymmetric induction can occur in the aminolysis of oxazolones from *N*-benzoyl derivatives of amino acids with bulky

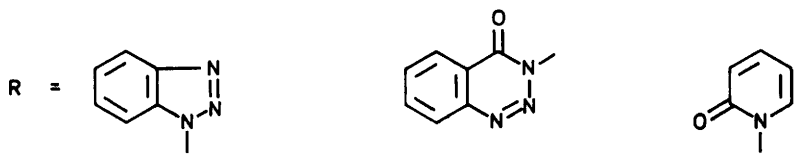
side chains. For example, reaction between 4-t-butyl-2-phenyl-oxazol-5-one and H-Pro-OMe afforded Bz-D-t-Leu-L-Pro-OMe almost specifically under suitable conditions.[81]

4-Chloro-2,3,5,6-tetrafluorophenyl esters of *N*-protected amino acids have been proposed for peptide synthesis,[82] but the advantages claimed over pentafluorophenyl esters are minimal. The same group used dipentafluorophenyl carbonate to prepare pentafluorophenyl esters[83] perhaps underlining the last point. Pentafluorophenyl esters of *N*-protected amino acids have also been made from bis-pentafluorophenyl sulphite.[84] The preparation of 2-nitrophenyl esters from 2-nitrophenyl trifluoroacetate[85] is also not world-shaking. There are two reports[86,87] of the synthesis of insoluble activated esters of *N*-protected amino acids and their use in the preparation of dipeptide derivatives.

Esters of *N*-protected amino acids and 2-hydroxyimino-2-phenylacetonitrile have been prepared by both the carbodi-imide and unsymmetrical anhydride routes.[88] These reactive esters were used to synthesize the *C*-terminal pentapeptide of substance P, the coupling reactions being carried out in aqueous dioxan in the presence of *N*-methylmorpholine. Unfortunately, no systematic tests for racemization were carried out. 6-Chloro-2-pyridyl esters (OPyCl) of Z and Boc amino acids are much more reactive than 2-pyridyl or 4-nitrophenyl esters in aminolysis reactions in dioxan and $HCO.NMe_2$.[89] Two additional advantages are the lack of interference by unprotected hydroxyl groups and the absence of aspartimide derivatives when Z-Asp(OBzl)-OPyCl was allowed to react with amino acid esters in dioxan. Another way to accelerate aminolysis reactions of active esters is to add the potassium salt of 1-hydroxybenzotriazole in the presence of dicyclohexyl-18-crown-6.[90]

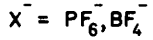
Several other new coupling methods have been reported. These include the use of esters of *N*-hydroxy-2,3-pyridine-dicarboximide,[91] several reagents (*a* - *e*) of type (11),[92] *NN'*-bismorpholinophosphinic chloride,[93] 1-β-naphthalenesulphonyl-oxybenzotriazole,[94] 1,1'-carbonylbis(3-methyl-imidazolium) triflate (12).[95] This last reaction is carried out without

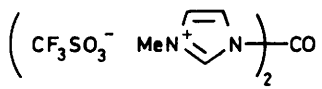
(11)



(a) (b) (c)

(d) (e)





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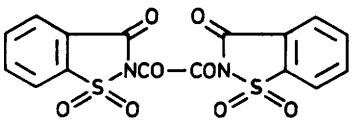

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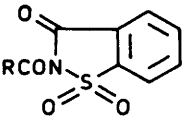
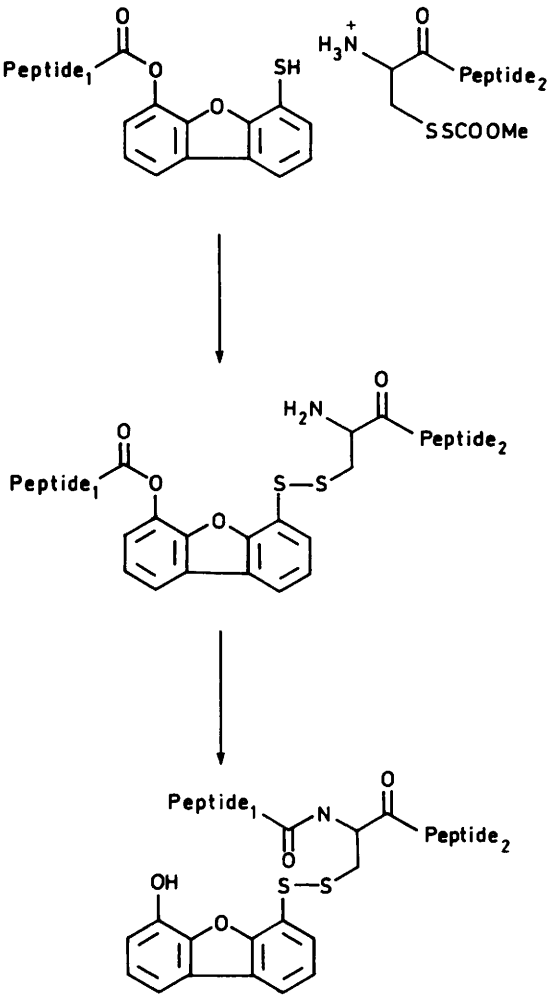


(14)

adding base and so racemization does not occur. A new reagent (13), prepared from saccharin and oxalyl chloride, gives 2-acyl-2,3-dihydro-3-oxobenzisosulphonazoles (14) with potassium salts of various carboxylic acids.[96] Aminolysis and alcoholysis reactions of (14) have provided amides, esters and thioesters but surprisingly no peptides were prepared and there is no mention of possible racemization. Triphenylstibine oxide condenses with *N*-protected amino acids to give $Ph_3Sb(O.CO.CHR.NH.CO.R')_2$ which undergoes aminolysis with amino acid esters to give protected dipeptides.[97] The method is improved[98] by using a combination of triphenylstibine oxide and phosphorus pentasulphide.

The interesting biomimetic procedure described in last year's Report has been amplified.[99] Considerable progress has also been made on the development of the method of peptide synthesis by prior thiol capture (Scheme 6).[100-103] The method is intended for fragment coupling where the *N*-terminal residue of the fragment, which is intended to be the *C*-terminal moiety is cysteine. This residue can be incorporated as the *N*-Boc derivative of 2,2-dimethylthiazolidine-4-carboxylic acid and this can be converted into -Cys(Scm)-. The design of the template for the intramolecular *O,N*-acyl transfer has been optimized.

A number of papers have concentrated on the problem of racemization. The use of alcoholates or pentachlorophenolate instead of Et_3N during peptide synthesis to capture protons has been suggested.[104] An additional benefit is claimed in coupling reactions that use carbodi-imides since the amount of *N*-acylurea formed was reduced. The effect of tertiary amine on the BOP-mediated coupling of Z-Gly-Xxx-OH with H-Val-OEt was studied using hplc to separate and quantify diastereoisomers.[105] Least racemization was observed with Pr^i_2NEt and most with *N*-methylmorpholine. Racemization was increased in the presence of excess tertiary amine and diminished but not eliminated by HOBt. The products of coupling of Boc-D-Phe-D-Phe-OSu and H-Gly-OH under various conditions of solvent composition, substrate concentration and temperature were also examined by hplc.[106] The Young test for racemization has been modified by determining the


Peptide1
O
O
SH
O
H3N+
O
Peptide2
SSCOOMe
H2N
O
Peptide2
Peptide1
O
O
S—S
O
Peptide1
N
O
O
Peptide2
OH
S—S
O

Scheme 6

degree of racemization by nmr spectroscopy using the chiral shift reagent $Eu(hfc)_3$.

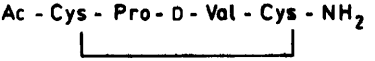
Finally, a mass spectrometric technique for detecting and identifying byproducts in peptide synthesis has been described.[108] There are three steps. Plasma desorption mass spectrometry of monolayer amounts of peptide bound to a thin layer of nitrocellulose is the first step. This detects the presence of and determines the molecular weight of byproducts formed by failure to obtain complete coupling at some stage(s). Secondly, the peptide is partially hydrolysed by proteolytic enzymes and finally the hydrolysis products of this process are subjected to plasma desorption mass spectrometry. These last two stages provide information on the location where errors have occurred. The method was applied to the analysis of synthetic peptides containing Trp such as melittin.

2.4 Disulphide bond formation

There is little to report that is new in this area, although a new review has appeared.[109] A cyclic peptide disulphide (15) has been synthesized by a solid phase method as a model of a type II ß-turn.[110] The synthesis is notable because the disulphide bond was formed while the peptide was still attached to the resin. The thiol groups were initially protected as Acm derivatives and deprotection of these and formation of the disulphide was effected with $Tl(O.CO.CF_3)_3$. Doubtless this method will find considerable application in the future.

2.5 Solid phase peptide synthesis

The claims by manufacturers of peptide synthesizers and the less chemically informed of the users of such hardware continue to assure us that peptide synthesis is now a push button affair. This hardly accords with the flood of research papers, many of which emanate from the laboratories that manufacture peptide synthesizers. Even the design of insoluble matrices for solid phase peptide synthesis is still a lively area of research involving the chemistry and physics of polymers and grafted



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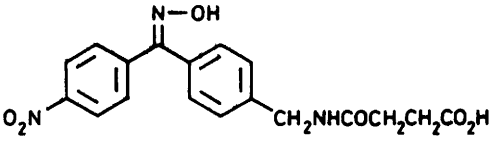

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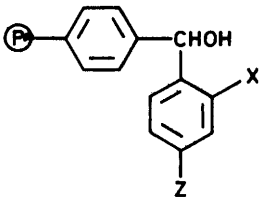

(X,Z = H, Me, Cl)

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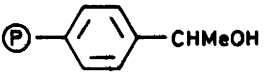

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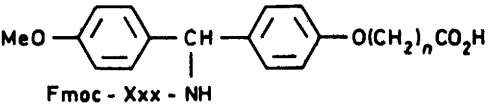

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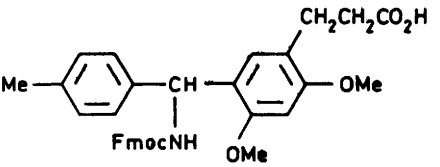
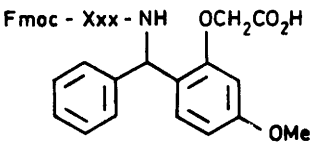


(20)

polymers.[111-123] A few salient points will be made here. A new high capacity composite support, Polyhipe™, has been described;[111] it consists of an extremely porous rigid matrix that acts as a scaffold to hold a soft polyacrylamide-based gel. It can be used in the low-pressure continuous-flow mode that is now so popular. A long chain polystyrene support has been grafted on to a polyethylene film matrix.[112] The polystyrene grafts have M_r *ca.* 10^6 and are probably more accessible than polystyrene chains embedded in beads. Similarly, polypropylene membranes have been coated with cross-linked polyhydroxypropyl-acrylate.[113] This support is intended for Fmoc chemistry with a suitable acid-labile linker. A comparative study has been carried out with five supports,[114] three based on polystyrene and one each on polyacrylamide and controlled-pore glass. The last and a macroporous polystyrene were unsatisfactory in a fragment condensation exercise; the other three were suitable. The nitrobenzophenone oxime (16) was attached to an aminoethyl-polyamide resin.[115] A tritylated polystyrene has been used to synthesize a number of small peptides in good yield.[116,117] Supports with a secondary alcohol linkage point have also been made (17,18) and esterified with *N*-protected amino acids using the Mitsunobu method.[124]

A variety of linkers based on the benzhydrylamine or benzylamine structure have been synthesized. Linkers of type (19) with the *C*-terminal amino acid attached have been coupled to aminomethylpolystyrene.[125] Cleavage of the peptide amide can be achieved with CF_3CO_2H/CH_2Cl_2 in presence of scavengers. The similar linker (20) was coupled to aminomethylpolystyrene and the peptide amide was cleaved readily with acid.[126] Several linkers (21-24) have been made for coupling to a polyamide-kieselguhr support.[127] Fmoc-Val-OH was coupled to each and the kinetics of cleavage of Fmoc-Val-NH_2 using CF_3CO_2H containing 5% phenol were determined. The $t_{\frac{1}{2}}$ values formed a series, (21)>(22)>(23)>(24), in which the largest was 84 times the smallest.

New methods continue to appear for the critical step of attaching the first amino acid in good yield without significant



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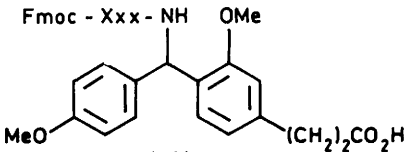

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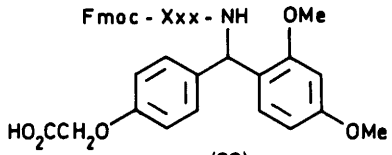

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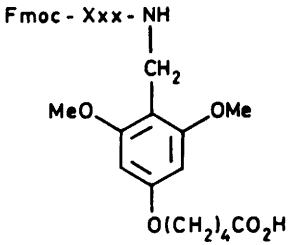

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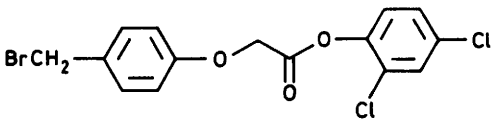

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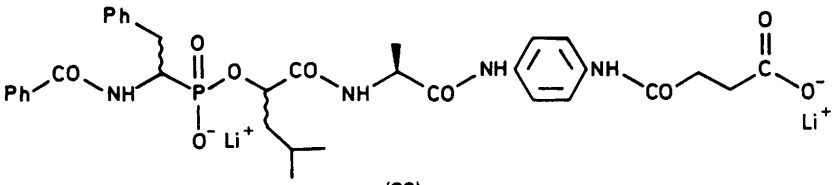

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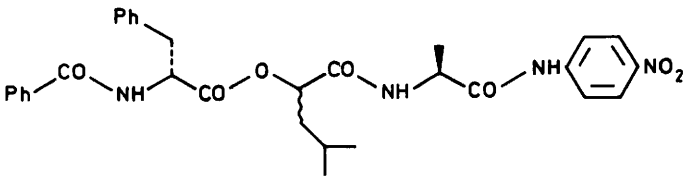


(27)

racemization. Fmoc-amino acids can be attached to hydroxymethyl resins using DCCI and HOBt in the absence of DMAP.[128] Under optimal conditions, 66% coupling was obtained without racemization or dipeptide formation. The yield can be improved at the expense of a small amount of racemization and dipeptide formation by addition of *N*-methylmorpholine. These workers obtained poor attachment using Fmoc amino acid chlorides. Most other methods involve reaction of a salt of an *N*-protected amino acid with a reactive halogenated linker. Coupling of Boc amino acids as their K or Cs salts is improved by the addition of quaternary ammonium salts such as tetra-n-butylammonium bromide.[129] Another classical variation is to couple Cs salts of Fmoc amino acids with chloromethyl resins in $CH_3CO.NMe_2$ in presence of sodium iodide at room temperature.[130] Although coupling is rather slow, racemization is less than when coupling is effected with *NN'*-dicyclohexylcarbodi-imide. Another efficient method is to attach the Fmoc amino acid to the linker precursor (25) in the presence of Pr^i_2NEt in $CHO.NMe_2$. The linker can then be coupled to amino-resins by virtue of the reactive 2,4-dichlorophenyl ester group.[131] It is reported that 4-(methylthio)phenyl esters of *N*-protected amino acids couple rapidly to 4-nitrobenzophenone oxime resin and it is suggested that coupling is favoured by the formation of a charge-transfer complex between the phenyl groups of the resin and the aryl ester.[132]

There is little new to report on coupling methods once the *C*-terminal amino acid has been attached to the linker/resin. 2-Bromo-*N*-methylpyridinium iodide in the presence of HOBt has been reported to lead to rapid coupling.[133] In a very labour-intensive study,[134] five coupling methods were used in polar solvents such as $CHO.NMe_2$ and *N*-methylpyrrolidine since these are claimed to interfere with the formation of β-sheets, a common cause of coupling difficulties in solid phase peptide synthesis. Summarizing, it emerges that symmetrical anhydrides in either MeSOMe containing a little $CHO.NMe_2$ or in CHO.NHMe containing 4% LiCl give good results as does the use of diethyl

phosphorocyanidate with Et_3N in MeSOMe. The BOP reagent, which has already achieved considerable popularity, is now claimed to have additional assets.[135] A CCK7 analogue was successfully synthesized without protecting the hydroxyl group of Thr and Tyr and the *N*-acetyl group was introduced with $CH_3.CO_2H$ and BOP. 2-Nitro-4-sulphophenyl esters of Boc amino acids have been used for solid phase peptide synthesis in aqueous solution. Melanostatin was synthesized on Sephadex LH20 using 1% sodium dodecyl sulphate in 2 M aqueous pyridine as the solvent for the aminolysis steps.[136]

A manually operated peptide synthesizer, which can produce up to 10 analogous peptides concurrently, has been constructed.[137] It operates in the continuous-flow mode. A decapeptide containing the antigenic determinant of the p31 product of the *pol* gene of HIV and 9 mono-omission peptides were synthesized. Some teams pay particular attention to monitoring solid phase peptide synthesis while others leave all to the synthesizer and its software and then rely on hplc to produce a clean product at the completion of all synthetic stages. Using a combination of nmr spectroscopy, FAB mass spectrometry, uv and tryptic mapping to monitor the synthesis of an analogue of parathyroid hormone, an unexpected byproduct was detected.[138] Traces of $CH_3.CO_2H$ in Boc-Nle-OH gave a prematurely terminated *N*-acetyl peptide. A microscale synthesizer has been described which uses a 96-well microtitre plate as a multicompartment reaction vessel and a robotic sample processor to control addition of reagents and solvents.[139] About 5 μmol of peptide is produced in each well used. Solid phase peptide synthesis using pentafluorophenyl (Pfp) and 3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl (Dhbt) esters or symmetrical anhydrides can be monitored by continuous measurement of electrical conductivity which is fed back to the computer controlling the synthesis.[140] A continuous flow system is obviously required together with a polar solvent.

Two side reactions in solid phase synthesis have been reported. One is an old problem; coupling of Fmoc-Asn-OH with DCCI and HOBt or with BOP or by using the Pfp ester gave some

byproducts containing β-cyanoalanine.[141] The difficulty was obviated by side-chain protection using either the 4,4'-dimethoxybenzhydryl or 2,4,6-trimethoxybenzyl groups. The other problem arose from an unwise choice of method for protecting the imidazole ring of His.[142] When His was protected as the π-tosyl derivative, the protecting group was steadily removed by HOBt during coupling steps. Capping with acetic anhydride caused π-acetylation and the acetyl group could later be transferred from His to the current free amino group during coupling leading to chain termination.

A few miscellaneous papers on solid phase peptide synthesis complete this section. Two groups have successfully synthesized peptides with a *C*-terminal β-amino alcohol residue.[143,144] In both cases, the β-amino alcohol was converted into the hemisuccinate which was then coupled to the resin. The peptide derivative could be cleaved from the resin by mild basic hydrolysis. A method has been reported for introducing the fluorescent dansyl group at the *C*-terminus of peptides synthesized as proteinase substrates.[145] Resin-bound peptides constructed on the Kaiser-type oxime resin can be cleaved therefrom and extended at the *C*-terminus by reaction with the tri-n-butylammonium salts of amino acids.[146] Any functional groups in the latter should be protected. An important paper[147] describes the synthesis by solid phase methodology of 8 fragments of the toxin II of *Andrococtus australis Hector*, a North African scorpion. The protein contains 64 amino acids and most fragments apart from the *C*-terminus were arranged to end in Gly or Pro as usual to minimize the risk of racemization. The fragments were then coupled in turn by solid phase methodology. A few difficulties were encountered and the original paper should be consulted for details and methods of overcoming the problems. Phosphopeptides have been synthesized by phosphorylating a Ser peptide while still on the resin using dibenzyl phosphite and *N*-chloro-succinimide in dry toluene.[148] The benzyl protecting groups were removed with other protecting groups when the peptide was cleaved from the resin using $CF_3.CO_2H$.

2.6 Enzyme-mediated synthesis and semi-synthesis

In contrast to the previous report, research on enzyme-mediated peptide synthesis has been more closely focused in 1989. The use of immobilized or micellar proteinases in organic solvents has been the dominant theme and chymotrypsin and papain have been the favourite enzymes. Nevertheless, it is not easy to discuss general principles from such a varied input. Indeed, it is becoming clear that the properties of proteinases (and presumably of other enzymes) are dependent on numerous factors such as the nature of the microenvironment (solvent, presence of inhibitors and or other kinetic modifiers, nature of support if present), structure of substrate and kinetic factors such as the identity of the rate-determining step if such exists.

The reaction between Ac-Tyr-OEt and H-Gly-NH_2 catalysed by chymotrypsin or proteinase K in aqueous alcohol has been examined in some detail.[149,150] About 2% water is optimal for both enzymes. Lower concentrations of water reaction inhibit whereas higher concentrations favour hydrolysis of Ac-Tyr-OEt or other ester substrates. Similar results were obtained[151] in CH_2Cl_2 containing 0.25% of water. Chymotrypsin displayed very high stereospecificity for the P_1 residue. For example, Ac-L-Tyr-OEt and H-L-Phe-NH_2 gave 96% of Ac-L-Tyr-L-Phe-NH_2 in 6 h but no product was isolated when Ac-D-Tyr-OEt was used. There was total relaxation of stereospecificity for the P_1' component. Thus, Ac-L-Tyr-OEt and H-D-Phe-NH_2 yielded 94% of Ac-L-Tyr-D-Phe-NH_2. The use of a P_1' component that can also function as a kinetically favourable P_1 component (e.g. H-L-Leu-OMe) gave none of the desired product with Ac-L-Tyr-OEt. In contrast, H-L-Lys-OBu^i gave 86% of Ac-L-Tyr-L-Lys-OBu^i, presumably because chymotrypsin does not catalyse reactions in which Lys occupies the P_1 site. Chymotrypsin, which had been immobilized on crosslinked polystyrene resins containing various basic substituents (-NHBu, $-NMe_2$, $-NEt_2$), catalysed transesterification reactions of Ac-L-Phe-OEt in toluene.[152] Conjugation of chymotrypsin and polyethylene glycol have been used effectively in organic solvents containing <0.5% of water.[153] Competing hydrolysis of

the acyl donor is nearly eliminated by avoiding an excess of substrate. Significantly, this protocol permitted the coupling of Boc-Tyr-Gly-Gly-Phe-O.CH_2.CO.NH_2 and H-Leu-NH_2 without the competing hydrolysis of the Tyr peptide bond. Addition of a crown ether considerably accelerates transesterification reactions catalysed by chymotrypsin in n-octane.[154] Subtilisin-catalysed transesterifications are less dramatically accelerated. Hammett analysis of subtilisin-catalysed hydrolytic reactions have very similar slopes for plots of $\log(k_{cat}/K_m)$ versus σ^- in a range of solvents which suggests that the microenvironment for the transition state is independent of solvent.[155] It would be interesting to know if this situation holds for enzymes with smaller and more exposed binding sites.

Papain when attached to polyethylene glycol retains its specificity for the P_2 component of the substrate in benzene.[156] A new D-stereospecific aminopeptidase has been isolated from *Ochrobactrum anthropi* (formerly *Achromobacter sp.*) and purified.[157,158] It has been immobilized on a urethane polymer and used in water-saturated organic solvents such as benzene and has a k_{cat} for synthesis of peptide approaching 8000 min^{-1} under these conditions. Addition of *C*-terminal H-Tyr-NH_2 to [des-Tyr^{36}]hNPY has been accomplished very efficiently using *Pseudomonas aeruginosa* elastase in DMF/EtOH (1:1).[159] Formation of a -D-Ala-D-Ala- peptide bond has been effected in an aqueous system starting from Ac-L-Lys(Ac)-D-Ala-D-Lac-OH and H-D-Ala-OMe in the presence of D-Ala carboxypeptidase.[160] Ac-L-Lys-D-Ala-D-Ala-OMe was obtained in 40% yield.

Three methods have been described for immobilizing chymotrypsin on monomethyl polyethylene glycol.[153] Papain has been immobilized both on a polymethylacrylate resin and on Sephadex 4B.[161] Significant differences were observed in the esterification of Boc derivatives of amino acids using the two immobilized and the free forms of the enzyme. Papain has also been immobilized on porous silica using 3-aminopropyltriethoxysilane followed by cyanuric chloride; it was then used to couple Boc-Tyr-Gly-O.CH_2.CO.NH.CH_2.CO_2H and H-Gly-Phe-Leu-OH to give the

Boc derivative of Leu-enkephalin in 60% yield.[162] Thermolysin immobilized on silica works satisfactorily to catalyse peptide bond formation in two-phase systems containing a low water content, but similar insolubilized forms of pepsin, chymotrypsin and subtilisin gave poor results.[163] α-Chymotrypsin can be immobilized on polyvinyl alcohol by adsorption from aqueous solution.[164] It was found that the yields of esters and peptides formed by catalysis with the immobilized chymotrypsin were strongly dependent on the ratio polymer:proteinase.

In addition to the papers on papain cited above, some others deserve mention. When esters of Z-Ala-OH were coupled to H-Arg-OPr^{i}, a high yield of dipeptide derivative was obtained. When Arg esters of primary alcohols were used, however, several products were formed.[165] For example, with Z-Ala-O.CH_2.CO.NH_2 and H-Arg-OMe as substrates, Z-Ala-Arg-OMe, Z-Ala-Arg-Arg-OMe, Z-Ala-Arg-Arg-OH and Z-Ala-Arg-Arg-Arg-OMe were formed. Dehydropeptide derivatives can be synthesized using papain with, for example, Z-Glu(OMe)-OMe and H-Leu-NH_2.[166] The former compound is regiospecifically hydrolysed at the α-ester group.[167] Similarly, the bis-allyl ester of Z-Asp-OH can be regiospecifically hydrolysed to the β-ester using papain.[168] Ficin has been much less studied but the synthesis of Z-Ala-Val-Gly-OH from Z-Ala-O.CH_2.CO.NH.CH_2.CO_2H and H-Val-Gly-OH[169] suggests a strong resemblance to papain-catalysed syntheses.

Depending on the alcohol moiety of diesters of H-L-Asp-OH, chymotrypsin can regioselectively hydrolyse either the α- or β-ester group.[170] Some other applications of chymotrypsin call for no special comment.[171-173]

Several examples of the stereospecificity of proteinases have been cited above. There are further examples of enantioselective hydrolyses leading to the resolution of racemic amino acid derivatives. For example, methyl esters of Boc amino acids are readily resolved with thermitase.[174] A more direct route involves the hydrolysis of *N*-acylamino acids by acylase I from porcine kidney or *Aspergillus* sp.[175] The reaction is almost stereospecific for acylated L-amino acids, but the enzyme will

operate on a wide range of structures including *N*-acylated α-amino-α-methyl acids, compounds that are important in the design of potential peptide-based drugs. Two other papers are more novel. Although enzymes have been used in micellar systems, the use of whole yeast cells in a system containing reverse micelles is a new technique. Yeast cells that had been cross-linked with glutaraldehyde were used in a system containing reverse micelles of the commercially available bis(2-ethylhexyl)-sulphosuccinate in $CHCl_3$/isooctane (1:9 v/v).[176] Methyl esters of *N*-acetyl-DL-α-amino acids were stereospecifically hydrolysed to the corresponding *N*-acetyl-L-amino acids. It was shown that the reaction was faster in reverse micelles than in aqueous solution. Antibodies raised to the hapten (26) presented as a mixture of diastereoisomers hydrolysed the esters (27)[177] since the hapten is a transition-state analogue of the substrates (27). The antibodies were enantioselective towards the D-Phe derivative (27; R_1 = H, R_2 = $PhCH_2$).

The incorporation into the reaction medium of a solvent that possesses hydrogen-bonding properties similar to those of water has been recommended for fragment coupling by proteinases.[178] Formamide is a suitable surrogate solvent for water. Using a solvent system comprising 2-methyl-2-butanol, water and ethylene glycol (90:1:9), thermolysin catalysed the coupling of Z-Gly-Pro-Gly-Gly-Pro-Ala-OH and H-Leu-Leu-Phe-NH_2 to give 67% of the nonapeptide derivative. Yields of peptides formed by proteinase catalysis can be improved by using very high pressures.[179] This observation is directed towards the synthesis of 'Aspartame' derivatives. Several other papers concentrate on the enzyme-catalysed synthesis of this commercially important peptide.[180-182]

2.7 Miscellaneous reactions related to peptide synthesis

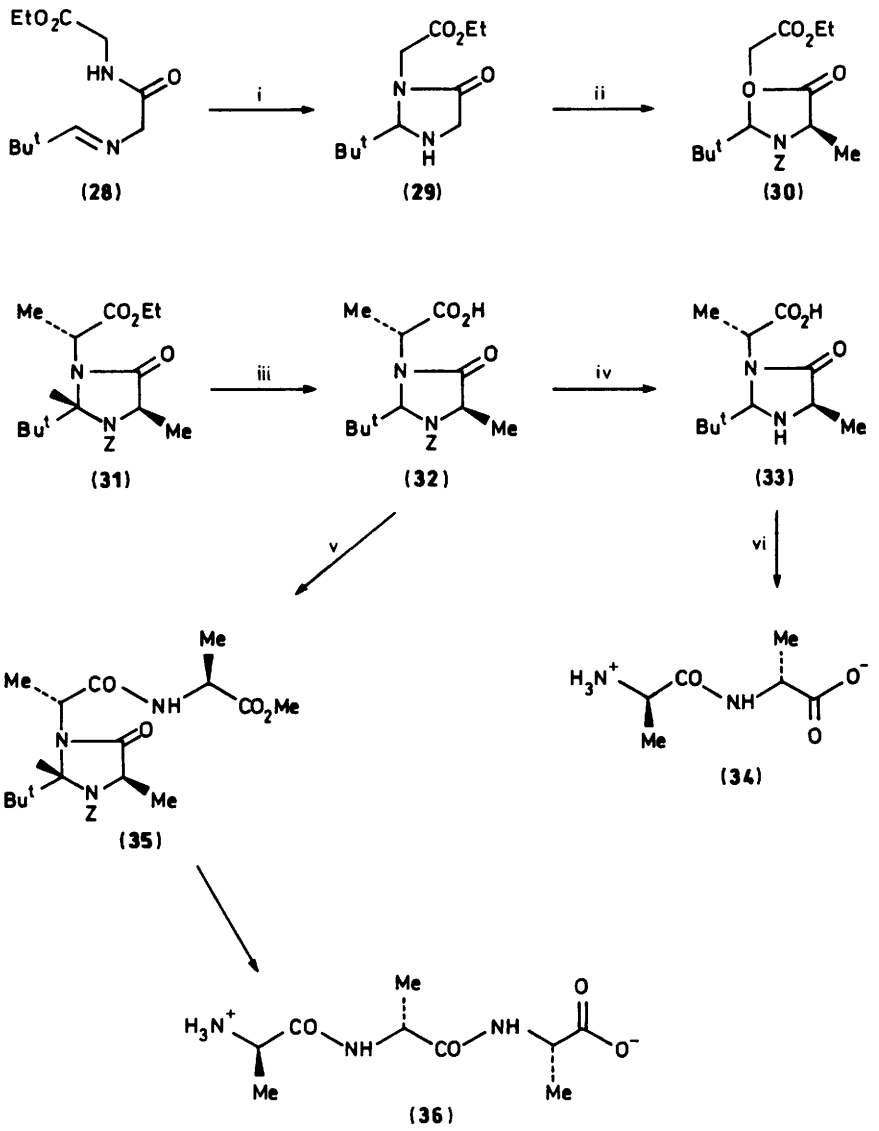
Problems continue to arise from time to time in the synthesis of peptides containing the dibasic amino acids and their amides. Ammonolysis of peptides containing -Asp(OBu^t)-residues has been shown to give a complex mixture of products formed by αβ-rearrangement and epimerization.[183] Succinimide

intermediates were involved in these reactions. Further instances of the cyclization of Asp/Asn peptide derivatives have been reported. This is particularly likely to occur when Asp or Asn is followed by Gly and to a lesser extent when followed by Ser or Ala.[184,185] In addition, peptides containing Asn are more prone to this side reaction than are peptides containing Asp.[185] The use of piperidine to remove Fmoc groups was also found to favour this cyclization process. All of these observations strengthen the case for using safer protecting groups for the β-CO_2H of Asp.[55,56] In the synthesis of analogues of thyrotropin - releasing hormones containing homoglutamine, it was shown that δ-lactam formation proceeded more quickly than γ-lactam formation from Gln derivatives in water or dioxan containing CF_3CO_2H.[186]

A substantial paper[187] describes the stereoselective alkylation of Gly residues in dipeptide derivatives (Scheme 7). For example, the enamine (28) derived from the condensation of trimethylacetaldehyde and H-Gly-Gly-OEt can be cyclized to the imidazolidin-4-one (29). After protection of N_3, these derivatives can be stereoselectively alkylated (e.g. 29 ⟶ 30). Dialkylderivatives (e.g. 31) are also readily accessible. Deprotection (31 ⟶ 32 ⟶ 33) and hydrolysis affords a dipeptide. Alternatively, the acid (32) can be coupled to an amino acid ester to give (35) and thence by deprotection a tripeptide (36). One wonders if this last process could be incorporated into a solid-phase protocol in order to permit the assembly of a peptide two residues at a time. Finally, mention must be made of a stereoselective synthesis[188] of 'Aspartame'and a diastereoisomer by a nitrone cycloaddition reaction (Scheme 8).

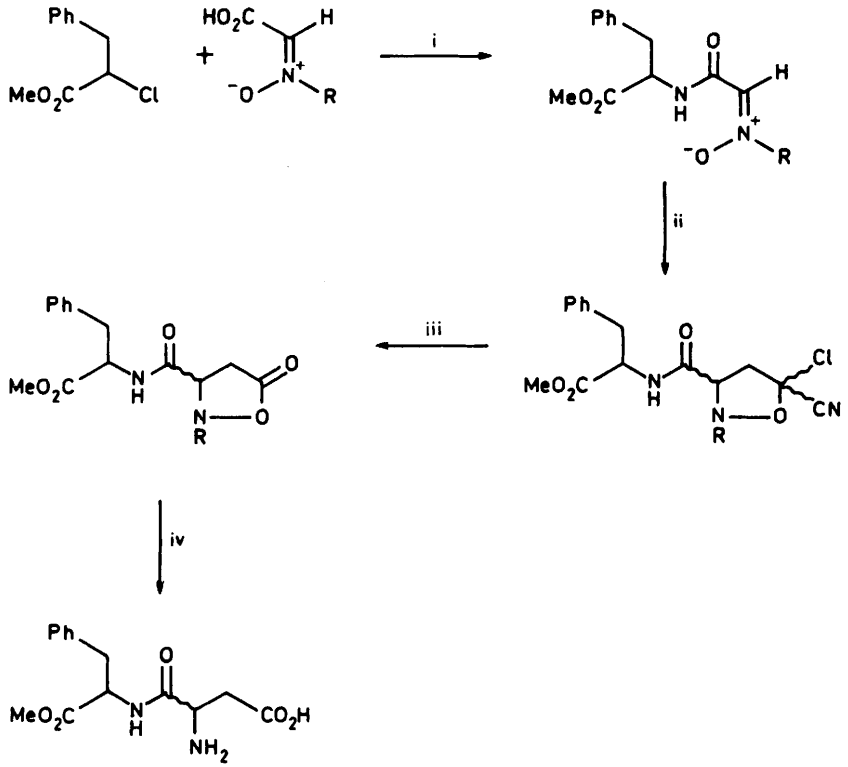
3. Selected examples of peptide syntheses

Since the chemical synthesis of long peptides no longer raises eyebrows, the *raison d'être* for this section is less compelling than hitherto. Nevertheless, a few examples of modern synthetic achievements merit special mention. The linear dimer of a peptide corresponding to the *C*-terminal 37 amino acid residues of human β-chorionic gonadotropin has been synthesized by the continuous-flow variation of solid-phase peptide



Reagents: i, MeCOCl/EtOH; ii, ZCl/DMAP/Et_3N/CH_2Cl_2 then $Pr^i_2N^-$ Li^+; iii, $KOSiMe_3$; iv, H_2/Pd-C; v, H-Ala-OEt/DCCI/HOBt; iv, H_2O/80°C

Scheme 7



Reagents: i, 2-Chloro-*N*-methylpyridinium iodide, Et_3N;
ii, $CH_2{=}CHClCN$; iii, H_3O^+; iv, H_2/Pd

Scheme 8

synthesis.[189] Ubiquitin has been synthesized by solid-phase methodology using Fmoc protection of α-amino groups and Pmc-protection of Arg side chains.[190] Human transforming growth factor-alpha containing 50 amino acid residues and 3 disulphide bonds has been synthesized in less than 7 days.[191] It seems a long time since du Vigneaud was awarded a Nobel prize for the synthesis of oxytocin and vasopressin. The synthesis of cecropin and some analogues suggested that the strongly basic *N*-terminal sequence was required for the manifestation of antibacterial activity.[192] The synthesis of mast cell degranulating peptide from bee venom[193] is notable for the unambiguous method of installing the pair of disulphide bonds. During the assembly of the sequence on a benzhydrylamine resin, Cys^3 and Cys^{15} were protected by *S*-4-methylbenzyl groups while Cys^5 and Cys^{19} were protected by the Acm group. After assembly, cleavage from the resin and deprotection by the low/high HF method, the unprotected thiol groups on Cys^3 and Cys^{15} were oxidized with $K_3Fe(CN)_6$ before removal of the Acm groups. The second -S-S- bond was formed by aerial oxidation. Other noteworthy syntheses are the preparation of urogastrone (hEGF)[194] and bovine pancreatic trypsin inhibitor.[195]

4. Appendix. A List of Syntheses Reported in 1989

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

Peptide/protein	Ref.
4.1 Natural peptides, proteins and partial sequences	
β-Adrenergic receptor	
Fragments of protein from turkey erythrocytes	196
Anaphylatoxin C3a	
18 Analogues	197
Angiogenin	
Inhibitory *C*-terminal peptides	198
Angiotensin(ogen)	

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

BY J. S. DAVIES

1. Introduction

The range of subject matter scanned for this Chapter remains the same as for previous years, but there has not been the usual profusion of papers in all areas of the coverage. The year 1989 was a leaner year for reports on amide bond isosteres and for insertions into peptide backbones, but conformational restriction through the use of lactam and disulfide bridges has remained buoyant. Interest in α,α-dialkylated residues has increased although the thrust of many of the papers in this area was towards a better understanding of the conformational effect of such residues, rather than successful incorporation into biologically active analogues. Interest in hydroxy-ethylene isosteres has become the domain of researchers in protease inhibitors, and have therefore been included under that sub-heading rather than as a separate example of peptide bond surrogacy.

The periodic 'quantum leap in discovery' for certain areas of the subject are usually premiered at international symposia and receive preliminary exposure in Symposia Proceedings. Although tempted to include the significant leaps from such Proceedings to add impact to the Chapter, the reviewer has once again concentrated on the matured and refereed brand of publication, mainly as a control against duplication. The very comprehensive account of the Proceedings of the 11th American Peptide Symposium[1] at La Jolla, California, came to hand while this Chapter was in embryo, but papers within it have not been reviewed. However, the proceedings of the 1st Naples Workshop on 'Bioactive Peptides' (May 1988), published as the first issue of Biopolymers[2] in 1989, have been reviewed as they represent extended papers, edited and refereed as part of the usual practice of that Journal.

Mainstream primary journals in the Bio-Organic area and Chemical Abstracts (up to June 1990, Issue No 21) were again the source of this Chapter's coverage.

2. Peptide-backbone Modifications

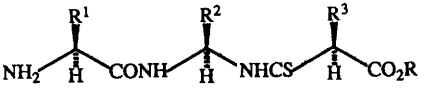
2.1 ψ[CSNH]-Analogues (and a retro-inverso version) - The effects of ψ[CSNH] substitution into the enkephalin sequence has been assessed by X-ray crystallography[3]. Two antiparallel molecules related by a pseudo 2-fold symmetry

stabilised to each other by four intermolecular H-bonds were identified in a crystal of Boc-Tyr-Gly-Gly-Pheψ[CSNH]Leu-OBzl. The molecule as a whole formed an L-shaped conformation, with the Tyr-Gly-Gly fragment perpendicular to that of the Phe-Leu moiety. In this work and in another study[4] on the X-ray crystal structures of Boc-Glyψ[CSNH]Me and Boc-Alaψ[CSNH]Me, the C=S bond length constituted the major difference when compared to the oxo-analogues. Belleau's phenoxy-substituted form of the Lawesson's reagent was used for the synthesis of the amino acid derivatives in the latter work and was also the reagent of choice (because of its improved solubility) in the synthesis[5] of the retroinverso endothiopeptides (1) and (2). Retro-inverso oxygen analogues were first synthesised prior to endothionation, and in this work the authors report problems with preparing geminal diamines (a method of choice for larger peptides) protected in the carbamate form. A preferred route turned out to be based on the Curtius reaction of an isocyanate (Scheme 1). Thionation of pyroglutamic acid with Lawesson's reagent provided[6] the 5-thioxo-L-proline (Top) as the starting material for Boc-Phe-Top-OMe, but improved yields (93%) in the thionation of Boc-Gly-Gly-OMe was obtained[7] using reagent (3) in THF rather than Lawesson's reagent (23% yield).

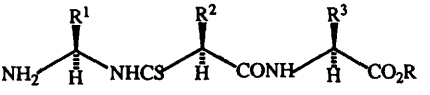
2.2 ψ[NHCO]-Retro-Inverso Analogues - A range of cholecystokinin (CCK) analogues, such as, Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-X (X=m(R,S)Phe-NH_2 or $COCH_2CH_2Ph$), or Boc-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gPhe-CHO (X = Trp, D-Trp), have been prepared[8] by solution phase methods. All the compounds inhibited binding of labelled CCK-8 to various receptors, but did so less efficiently than the unmodified forms. Some of the structure-activity relationships could not be readily explained but in general it is felt that the integrity of the C-terminal tetrapeptide of CCK is essential. Diastereoisomeric mixtures of the enkephalin analogues[9], H-Tyr-D-Ala-gGly-(RS)mPhe-LeuNH_2 and H-Tyr-Gly-gGly-(RS)mPhe-Leu-NH_2 have been assessed in the guinea pig ileum and in antinociceptive tests, and both gave naloxone-reversible responses, but no analgesia was observed *in vivo*. As reported last year, inhibitors of enkephalin-degrading enzymes can be obtained from appropriate retro-inverso analogues but in order to increase the affinity for dipeptidylamino-peptidase and aminopeptidase N without loss of inhibitory potency on neutral endopeptidase, compounds of the general formula $HONHCOCH_2CH(CH_2Ph)NHCOCH(R^1)CH(R^2)COOH$ have been synthesised[10]. The most active inhibitors turned out to be:

R^1 = CH_2Ph, R^2 = H (racemate)	ED_{50} =	71 μg
R^1 = CH_2Ph, R^2 = H (RR form)	ED_{50} =	100 ug
R^1-R^2 = $-CH_2CH_2CH_2CH_2-$(RRS or RSR)	ED_{50} =	>100 ug

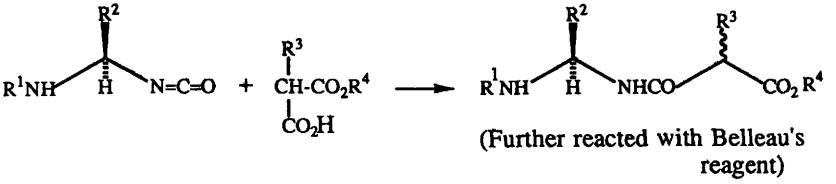
^1H N.m.r. studies have revealed[11] within a series of 14-membered cyclic peptides, with some containing retro-inverso amide bonds, that a significant



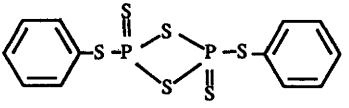
(1)



(2)



Scheme 1



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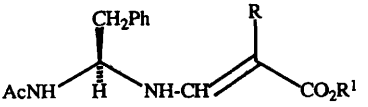
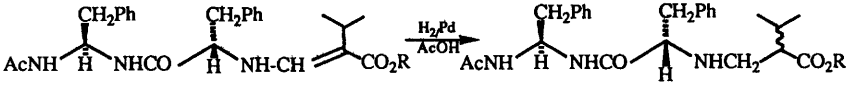


(4)



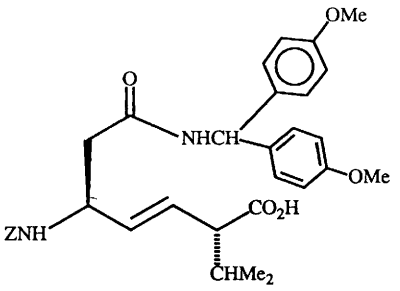
Scheme 2

proportion of the conformational isomers possess a *cisoid* amide bond although they do not contain N-Me or proline residues in their sequence. Thus H-Tyr-*cyclo*-[D-Glu-Phe-gPhe-D-mLeu] in solution exists 28% in the all-*trans* structure with two *cis*-containing isomers accounting for 51% and 21%. No *cis* forms were detected when Gly appeared at position 3. Although not representing individual retro-inverso insertion, it is worth noting that a 31-residue peptide has been synthesised[12] as a Gramicidin A mimic where half the molecule has been designed to include the retro-sequence. Some adaptations, e.g., a D-Ala at position 16 and a compensatory residue instead of the two formyl groups, were needed to maintain similarity with the parent D-L alternating sequence in gramicidin A. The resulting peptide did form long-living channels in a lipid bilayer, but it does not seem to adopt the expected $\pi_{DL}^{6.3}$ helical conformation.

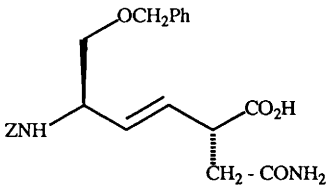
2.3 ψ[CH_2NH] Amino Methylene Analogues (and Retro-Forms) - An X-ray diffraction study[13] on Boc-Proψ[CH_2NH]Leu-Gly-NH_2 representing the C-terminal residues of oxytocin, confirmed a C_7 structure at the Pro-residue which is novel for a linear peptide. Attempts to introduce a retro-reduced unit, i.e,-ψ[$NHCH_2$] were initially hampered[14] by the elimination of an acetamido unit on hydrogenation of a precursor molecule (4) but in combination with a second amide replacement the transformation in Scheme 2 was successful. Structures such as (4) show barriers to rotation about the eneamine N-C bond due to delocalisation of π bonding through the conjugated eneamine ester unit.

2.4 ψ[CH=CH] - Ethylenic Isosteres - Attempts to mimic the amide group using a *trans* alkene isostere can now be studied further as a result of the synthesis[15] of the protected highly functionalised units such as (5) and (6), which are suitable for incorporation into peptides using standard coupling methods. Sodium amalgam reduction of a precursor hydroxy sulfone was the key reaction used in the synthesis. Claisen rearrangements of suitably protected allyl esters have resulted[16] in stereocontrolled synthesis of D,D- and D,L-(E)-$RO_2CNHCH(Me)CH{=}CH\text{-}CH(Me)CO_2Me$, isosteres of the Ala-Ala unit.

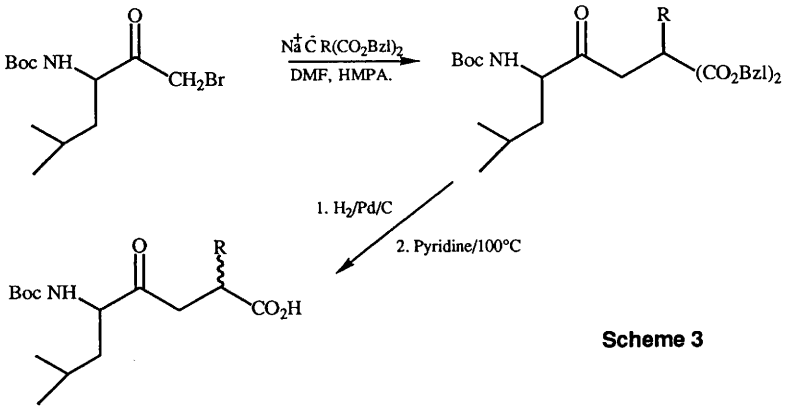
2.5 ψ[$COCH_2$] - Ketomethylene Surrogates - Two synthetic routes have been compared[17] in a study on inhibitors of aminopeptidases. The route outlined in Scheme 3 proved to be the most successful although it suffers from a low-yielding alkylation step and the product is still a diastereoisomeric mixture. All the isosteres examined were weak inhibitors of leucine aminopeptidase and aminopeptidase M, with the isostere H-Lysψ[$COCH_2$](RS)Phe-OH with a K_i = 4nM having potency comparable to the natural product arphamenine A, H-Argψ[$COCH_2$]Phe-OH, K_i = 2.5nM. After carboxyl group activation, N-protected amino-acids take part[18]



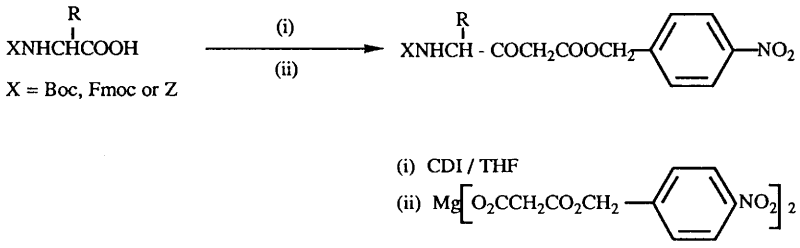
(5)



(6)



Scheme 3



Scheme 4

in a carbon acylation reaction with a magnesium salt to give ketomethylene surrogates of the type shown in Scheme 4.

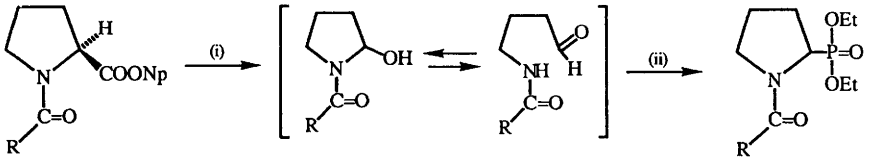
2.6 Phosphono-Peptides - An efficient direct method[19] summarised in Scheme 5 for the preparation of C-terminal proline phosphonates relies on an oxidative decarboxylation step and on h.p.l.c. techniques for the purification of diastereoisomers. Phosphonic analogues of glutathione, in which the N-terminal Glu and C-terminal Gly residues were both modified to include phosphonic acid groups, have been obtained[20] by standard mixed anhydride activation in solution, with simultaneous deprotection of the phosphonic, carboxylic and amino groups with Me_3SiI.

2.7 ψ[SO_2NH] - Sulphonamide Isosteres - The prospect that this isostere would improve stability to proteolytic enzyme degradation, and its possible use as a mimic structure of the tetrahedral adduct during enzyme hydrolysis, underpins an X-ray-crystallographic study[21] of $CH_3CONHCH_2CH_2SO_2NHCH(CH_2Ph)OMe$ (Ac-Tau-Phe-OMe). In the crystal, the sulphonamide junction held the backbone in a folded structure with the Tau and Phe C^{α} atoms in a cisoidal arrangement favouring $d\pi$-$p\pi$ interactions between nitrogen and sulfur, thus giving the S-N bond significant π bond character. The amide bond was planar and in the *trans* form.

2.8 ψ[CN_4] - Tetrazole Surrogates - Isomers (7) and (8) have been synthesised by multistep procedures[22] and their X-ray structures compared with that of H-Pro-Leu-Gly-NH_2. This surrogate, as reported last year, has again been confirmed as a good mimic for a *cis*-peptide bond. The tetrazole analogues did not seem to form -NH----OC H-bonds characteristic of a β-bend and did not enhance the binding of dopamine receptor agonists to the dopamine receptor.

2.9 ψ[CH(Alkyl)NH] - Surrogates - A demand for an isostere in which one of the diastereotopic protons of a reduced amide group could be replaced by an alkyl group has been solved[23] according to the synthetic Scheme 6. The stereochemistry of the inserted alkyl group is defined by the Grignard reaction which gives the diamino alcohol from the oxazolidine.

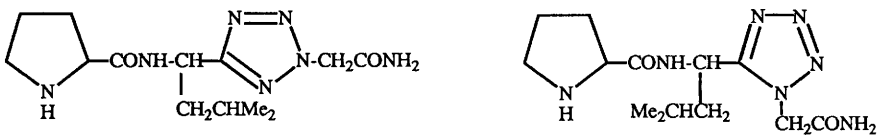
2.10 ψ[CON(Me)] - N-Methylated Analogues - The introduction of N-methylated amino acid residues can now be considered routine, and can be carried out by insertion of the appropriate N-methyl residue in a solid phase protocol[24]. Substitution at four different positions in neurotransmitter neurokinin A (4-10), H-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2, thus giving [$MeSer^5$]NKA(4-10), [$MePhe^6$]NKA(4-10), [$MeVal^7$]NKA(4-10), [$MeGly^8$]NKA(4-10), gave more or less decreased responses at the NK-2 receptor. The constraints of N-methylation



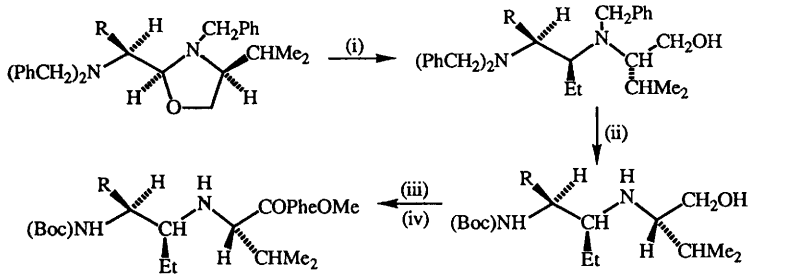
(i) H_2O/OH^- *meta*-chloroperbenzoic acid. (ii) $P(OEt)_3/BF_3$

R=BzlO or CH-CH_2 $CHMe_2$ (NHZ) Np = p-nitrophenyl.

Scheme 5



(7) (8)



(i) EtMgBr (ii) $H_2/Pd/C + Boc_2O$ (iii) $COCl_2/NEt_3$ (iv) Et_2AlCN (v) H-Phe-OMe : HCl.

Scheme 6

therefore does not favour interaction with the NK-2 receptor in these analogues, which differentiates the receptor from the NK-1 and NK-3 sub-types.

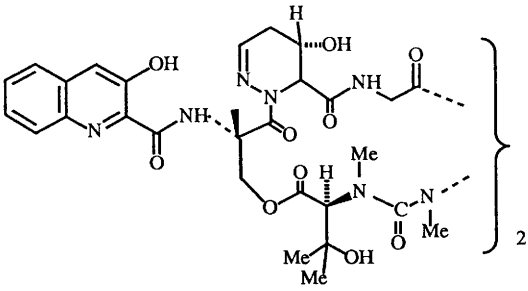
The conformational constraint offered by the insertion of an N-methyl group has the possibility of selectively reducing the number of β-helical structures theoretically possible for D,L-alternating oligopeptides. In a series of N-methyl substitutions at the (n-3) position of oligonorleucines and in position (n-4) in an oligoleucine, n.m.r. data[25] reveal quite different conformational behaviours from non-methylated counterparts. In chloroform the substituted norleucines form predominantly $\uparrow\downarrow\beta^{7.2}$ helices while the substituted oligoleucines form almost exclusively $\uparrow\downarrow\beta^{5.6}$ or $\uparrow\downarrow\beta^{7.2}$ helices.

2.11 Replacement of L- by D-Residues - The solitary paper reviewed under this heading in no way reflects the wide usage of these substitutions. Paucity is really the consequence of this popular routine being subsumed into papers which highlight other modifications. D-Residue insertions into various positions of the 36-residue porcine neuropeptide Y(NPY), *via* routine synthetic strategies have been monitored[26] in terms of the ability of the analogues to alter mean arterial pressure and heart rate in rats. Potencies (relative to NPY) for D-substitution at positions 2-6 and 8-13 inclusive ranged from 0.1 to 1.0. Analogues with D-residues at positions 33-35 were inactive at 40 μg/Kg and [D-Tyr^{36}]NPY was 10-fold less potent, which confirmed the need for a non-altered C-terminal region to maintain hypertensive activity.

2.12 ψ[COO]-Depsipeptides - Examples of this isostere often occur in natural heterodetic peptides and remain the province of Chapter 4 of these Reports. However, synthetic aids for its insertion are always welcomed under the present heading, so a synthesis[27] of a model depsipeptide segment of the luzopeptins (BBM928), potent antitumour and antiretroviral antibiotics (9) using N,N′-dicyclohexylcarbodiimide for the COO link is noteworthy. An N,O-acyl shift has been successfully used[28] in a novel synthesis of the peptide lactone moiety in the actinomycins, using p-toluene sulfonic acid at 80°C to catalyse the conversion of (10) to (11).

2.13 Hydrazinopeptides - In order to gain information on the effect of introducing ψ[CONHNH] as a bond replacement, the proline analogue (12) has been studied[29] by X-ray crystallography, and when compared to its amide counterpart, there is a distinct conformational change in (12) as a consequence of the right angle turn of the hydrazino bond.

2.14 Aza-Peptides - Researchers wishing to enter this area of backbone surrogacy will be well advised from now on to start their search with the



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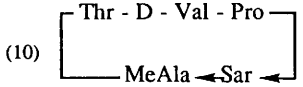

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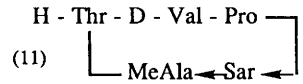
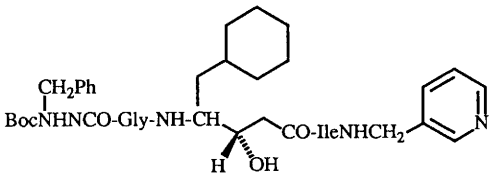


(12)



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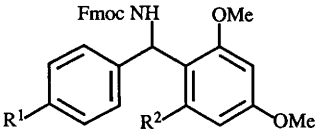
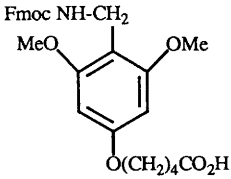
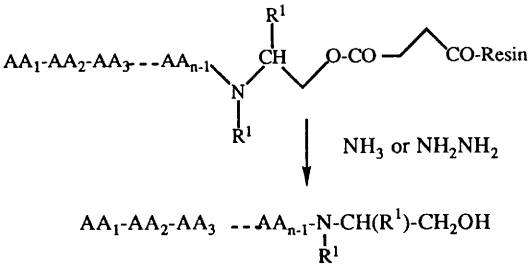


(14a) R^1=HO_2CCH_2O,R^2=H
(14b) R^1=OCH_3,R^2=CH_2CH_2CO-



(15)

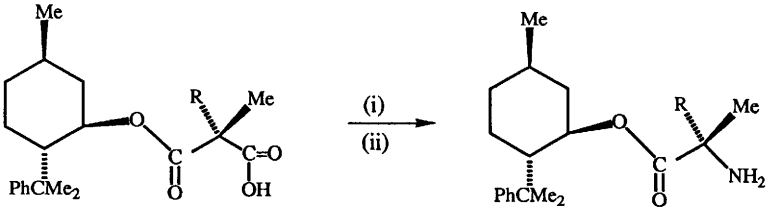


Scheme 7

authoritative review by Gante,[30] and as an example of the author's work, a renin inhibitor (13) containing an aza analogue at the N-terminal has been synthesised[31] and shown to inhibit renin with high specificity (IC_{50} = 1.5 x 10^{-6}M), slightly more active than pepstatin but less active than its counterpart with Boc-Phe at the N-terminal position.

2.15 C-Terminal Modifications - C-Terminal amides and alcohol groups often appear at the C-terminus of peptides in nature. The direct synthesis of the amide terminus from solid phase resins has been explored for some time, and in a comparison[32] of their stability towards TFA, the solid phase linking agents (14a) and (15) performed equally well. A new linker group (14b) has been attached[33] to a commercially available aminomethyl polystyrene resin, which on treatment with TFA/CH_2Cl_2 in the presence of scavengers gave C-terminal amides, e.g., of LHRH and the C-terminal hexapeptide of secretin. A bifunctional link has been incorporated[34] between appropriately protected amino alcohols and a resin to allow release of the C-terminal alcohol under the conditions summarised in Scheme 7.

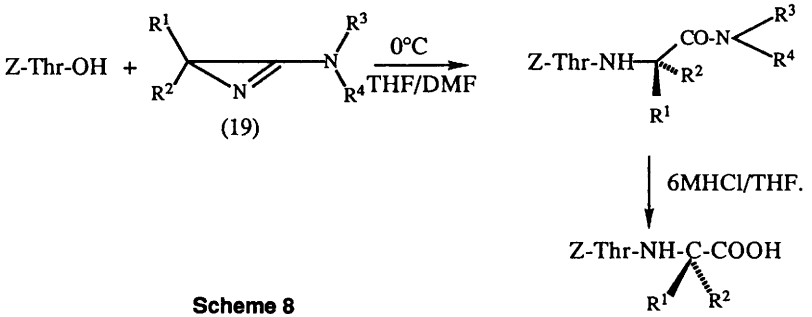
2.16 α,α-Di-Alkylated Glycine Analogues - Proof[35] that these sterically constrained residues inhibit enzymic activity has been unambiguously obtained from a study of the hydrolysis of Ac-Tyr-OEt by chymotrypsin, competitively inhibited in turn with (2R,3S)- and (2S,3R)-∇^{E}Phe-Phe(Or Leu)OMe where ∇^{E}Phe represents (E)-2,3-methanophenylalanine. The ester groups of the dipeptide and the ∇^{E}Phe-Phe bond were stable to enzymic attack. The most potent (the 2R,3S form) had an inhibition constant (K_i) of 0.16mM at 25°C confirming a configuration dependence at the ∇Phe residue, and also that this residue can account for the irreversible inactivation of chymotrypsin due to the electrophilic attack of the cyclopropane ring on the enzyme. Previously reported syntheses of cyclopropane amino acid analogues by the reaction of a precursor alkene with diazomethane to form the intermediate pyrazolines have now been reported with full experimental details for, 2,3-methanopyroglutamic acid[36] (±)2,3-methanoproline[37], (±)2,3-methanovaline and 2,3-methanoleucine[38]. However, this methodology proved inefficient for the preparation of 2,3-methanotyrosine, but the problems have been overcome[39] for both (E) and (Z) forms by cyclopropanation of the benzalmalonate 4-$MEMOC_6H_4CH=C(CO_2Et)_2$ using $(CH_3)_3SOI$/NaH/DMSO, followed by Curtius rearrangements for the introduction of an amino group. An enantioselective construction[40] of a quaternary carbon centre starting from the malonate (16) with $LiN(CHMe_2)_2$ and alkyl halides, gives a diastereoisomeric excess of (17), which can be considered a precursor of α-alkyl amino acids since Curtius rearrangement of an azide followed by hydrogenolysis yielded (18), which can be hydrolysed to the appropriate acid. Full details have been reported[41] on the use of substituted 3-amino-2H-aziridines (19) as synthons to overcome the problems of inserting



(16) R=H
(17) R=alkyl.

(18)

(i) $(PhO)_2PON_3$,Et_3N : $PhCH_2OH$
(ii) H_2/Pd/C.



Scheme 8

(Coupled further with(19))

sterically crowded acids into peptides. The early synthetic steps in the pathway towards a tetrapeptide are outlined in Scheme 8. The tetrapeptides produced have also been subjected to c.d. and n.m.r. analysis and the results show that α,α-disubstitution supports β-turns in very short oligopeptides.

The α,α-disubstituted residues have again been a focus of interest in their role as conformational restrictors, with a wide variety of physical techniques being used to study their conformation. C.d., i.r. and X-ray studies[42] have been brought to bear on leucinostatin A (20) and a tetrapeptide fragment Boc-Aib- Leu-Leu-Aib-OMe (Aib = α-aminoisobutyryl). I.r. studies on (20) suggested an intramolecularly H-bonded structure in $CDCl_3$, while c.d. indicated a helical conformation in lipophilic solvents. In the crystalline form the tetrapeptide's backbone was folded in a right-handed 3_{10}-helical conformation stabilised by two intramolecular H-bonds. An X-ray diffraction study[43] on Ac-$(Aib)_3$-OMe.$3H_2O$ and Ac-$(Aib)_2$-Iva-OMe.H_2O representing the 3-5 sequence of the antibiotics antiamoebins revealed that the former folded in a common helical type III β-bend, but the latter preferred a type II β-bend which is unusual for an -Aib-Aib- sequence. It is presumed that the type II β-bend is due to crystal packing as computational analysis showed such a structure to have about a 10 kcal/mol higher energy than the lowest energy conformation. The methyl amide of 2,3-methanopyroglutamic acid (2,3-MeGlp) and the TRH analogue [2,3-MeGlp^1]-TRH have been the subject of an X-ray and n.m.r. analysis[44]. In solution the pyrrolidone and imidazole side-chain rings in [(±)2,3-MeGlp^1]-TRH appeared to be in close orientation, but only the imidazole C_4H resonance was sensitive to the presence of the diastereoisomeric mixture. *Cis-trans* isomerisation around the His-Pro bond was not affected by the N-terminal modification, but the 2,3-MeGlp-His bond was considerably more stable to pyroglutamate aminopeptidase.

The utility of the Aib residue in the construction of relatively long helical segments as protein folding mimics has been confirmed[45] by an X-ray study of Boc-Aib-(Val-Ala-Leu-Aib)$_3$-OMe. In the crystal it adopts a helical structure with seven α-type H-bonds in the middle and 3_{10}-type H-bonds at either end. There does not appear to be distortion of the helix due to hydration in this example but in Boc-Ala-Leu-Aib-Ala-Leu-Aib-OMe.H_2O, the insertion[46] of the water molecule caused the apolar peptide to mimic an amphiphilic helix. Two molecules of Boc-Aib-Val-Aib-Aib-Val-Val-Val-Aib-Val-Aib-OMe have been shown to co-crystallise[47] with different helical conformations in a triclinic cell. One molecule is completely α-helical and the other has a mixed 3_{10}/α-helix conformation, with a helix reversal at both termini. A very similar situation has been reported[48] for Ac-$(Aib)_2$-(S)-Iva-$(Aib)_2$-OMe, where X-ray diffraction found two independent molecules (A and B) aligned in an anti-parallel arrangement, differing essentially in the handedness of their 3_{10}-helical structure. Selectively deuterated Aib-residues

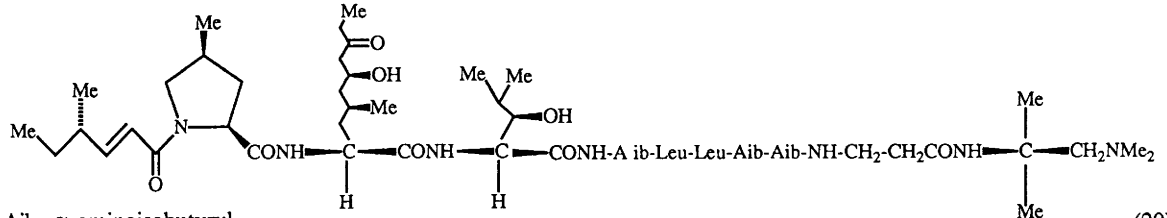
has enabled a detailed n.m.r. study to be performed[49] on Boc-Ala-Aib-Ala-OMe which is already known to adopt the β-turn type II structure. From n.m.r. data and molecular dynamic simulations two major conformational species seem to exist in solution.

Cyclopropyl amino acid residues have been rigorously studied using a number of physical methods. X-ray studies[50] on simple derivatives, concentrate on the conformational angles, and on extending the technique to homooligopeptides of 1-amino-1-cyclopropane carboxylate (Ac3c), the data[51] revealed that protected tri- and tetrapeptides fold into type I β-bends and distorted 3_{10}-helices, respectively, somewhat different to the corresponding size of peptide based on aminoisobutyrate, 1-amino-1-cyclopentane and 1-amino-1-cyclohexane carboxylates. The N-C^{α}-C′ bond angle was significantly expanded from tetrahedral value. Agreement between i.r., n.m.r. and theoretical calculations on Ac-$(Ac_3c)_n$-NHMe (n = 1-3) indicated[52] that type I′ β-bends and distorted 3_{10}-helices are stable conformations for the di- and tripeptides which is a similar situation to that found in X-ray studies[53] on a series of peptides rich in Ac3c residues. N.m.r.[54] and X-ray diffraction[55] studies on homopeptides containing 1-aminocyclopentane-1-carboxylic acid have also been reported.

3. Conformationally Restricted Cyclic and Bridged Analogues

Of relevance to this section and to sections on $C^{\alpha\alpha}$-dialkylated residues, tetrazolyl and thiated peptides, is the short review[56] on peptide surrogates which concentrates mainly on the author's own work.

3.1 Rings and Bridges formed *via* Amide Bonds - Molecular dynamics simulation have assisted in the design of potent and long-acting cyclic lactam analogues of α-melanotropin. Lactam analogue (22) was synthesised[57] from its linear precursor Ac-[Nle^4,Asp^5,D-Phe^7,Lys^{10}]α-MSH(4-10)NH_2 and was found to be exceptionally potent in the lizard skin (90 x activity of α-MSH) and mammalian melanoma tyrosinase (100 x α-MSH) assays and exhibited prolonged activity. The influence of ring size on the biological activity has been deduced[58] from cyclic analogues (22) with 23- and 24-membered rings and (21) with smaller sized lactam bridges. The highest activity was achieved by the 23-membered analogue which exhibited a 100-fold higher melanotropic activity over that of α-MSH showing some selectivity for lizard rather than frog melanocyte receptors. Increasing or decreasing ring size diminished biological activity, but the effect was more prolonged in the larger ring systems than the smaller ones. In contrast to their linear analogues, cyclic analogues of cholecystokinin (CCK_8) (24), in both their sulfated and non-sulfated forms displayed[59] high affinities for central-type binding sites (B-type), but all the peptides displayed low affinity for the pancreatic



Aib= α-aminoisobutyryl (20)

Ac-Nle-Asp-His-D-Phe-Arg-Trp-Lys-NH_2

(21)

Ac-Ser-Tyr-Ser-Nle-X-His-D-Phe-Arg-Trp-X^1-R

(22) X=Glu, X^1=Lys; X=Asp, X^1=Lys

(23) X=Glu, Asp X^1=2,3-diaminopropanoyl or 2,4 -diaminobutyryl

R=NH_2 or Gly-Pro-Val-NH_2

Boc-X-Tyr(R)-Nle-D-Lys-Trp-Nle-Asp-PheNH_2

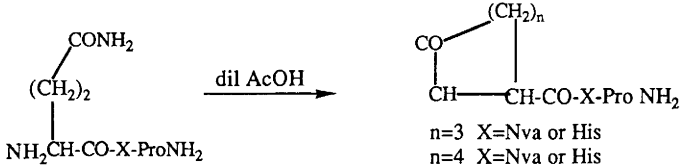
(24) (X=Glu,DGlu, γ-D-Glu and R=H,SO_3H)

Leu-X-Arg-Pro-Pro-Gly-Phe-Gly-Pro

(25) X=absent or Lys

H-X-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu

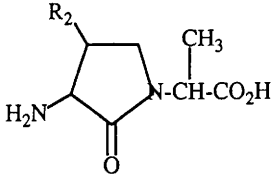
(26) X=Lys or Orn.



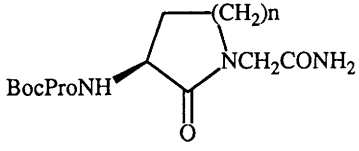
Scheme 9

receptors (A-type). Cyclic analogues of des-Arg^8[Leu^8]-bradykinin with either structural type (25) or (26) have been synthesised[60] using pentafluorophenyl esters and cyclised using high dilution conditions. Compound (25) released histamine from rat mast cells comparable to bradykinin but without myotropic and vascular effects. Scheme 9 represents attempts[61] at varying the ring size at the N-terminal position of thyrotropin releasing hormone (TRH) through lactamisation. δ-Lactam formation took place more easily than γ-lactam formation in aqueous acetic acid. The higher homologue [pHgu^1,Nva^2]-TRH showed a dose-dependent antagonistic effect against pentobarbital anaesthesia in mice but no binding to TRH receptors in rat brain. γ-Lactams with the model structure (27), representing cyclic mimics of Val-Ala, Ile-Ala and Leu-Ala dipeptides have been synthesised[62] enantiometrically pure from L-aspartic acid diester precursors. Cyclic lactam analogues of H-Pro-Leu-Gly-NH_2 whose synthesis was reported in last year's Report have now undergone X-ray crystallographic analysis[63]. Structures based on (28) in the crystalline state, show drastic changes in the backbone torsion angles in the region of the modified Leu-Gly sequence eventually promoting the onset of extended conformations.

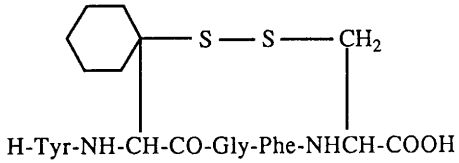
3.2 Bridges formed by Disulfide Bonds - Conformationally restricted analogues of opioid peptides still command interest in this sector. β-Mercapto,β,β-pentamethylene propanoic acid (Apmp) has been inserted[64] into the 2- and 5-positions in enkephalin as replacements for the penicillanic acids which have previously produced potent δ-receptor selective compounds. The cyclic component was produced by oxidative cyclisation followed by diastereoisomeric purification by preparative h.p.l.c. Analogues with Apmp in the 5-position (30) were approximately 5 orders of magnitude more potent than (29) in the mouse vas deferens assay, but all showed diminished δ-selectivity. Analogues (31) and (32) have been prepared[65] as photolabile analogues of enkephalin and somatostatin, respectively, using solid phase techniques. The analogue (31) displayed high affinity (IC_{50} = 95nM) and selectivity ($IC_{50}\mu/IC_{50}\delta$ = 1053) as an agonist at δ-receptors. Compound (32) displayed weak affinity (8% contraction at 300nM) at μ receptors but good selectivity ($IC_{50}\delta/IC_{50}\mu$ = 412). The most highly δ-selective opioid compound [(31) without its affinity label] has been compared conformationally[66] with its cysteine analogue H – Tyr – Cys – Gly – Phe – D – Cys – OH which has only moderate δ-selectivity, using the Amber programme. Conformers with Tyr and Phe aromatic rings in the vicinity of the disulfide bond were preferred, but for analogue (31) this conformation only came possible with a positive dihedral angle for the disulfide bond due to the presence of the β-methyls of Pen^2. Both chiralities of the disulfide bond in the cysteine analogues gave the preferred conformers. A second disulfide link [between Cys^{108} and Cys^{117} in 33] has been incorporated[67] *via* the linear



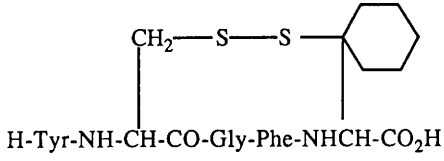
(27) R=Me, Me(Et)CH,Me_2CHCH_2-



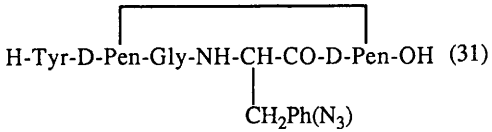
(28) n=1 or 2



(29) L or D



(30) D or L



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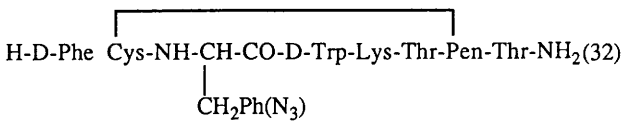

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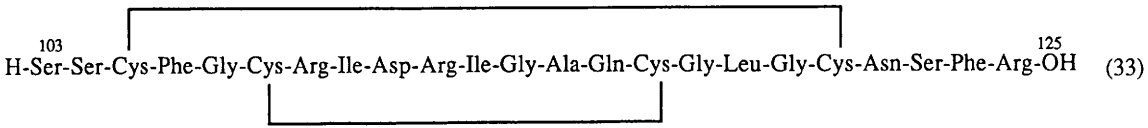


(33)

precursor, into atriopeptin (103-125) amide with no significant scrambling of the -S-S- bonds. Analogue (33) had EC_{50} values ranging from 0.05 to 3μM and bound selectively to a class of specific tissue binding sites not shown to be associated with any known second messenger system.

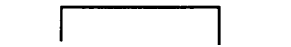
N.m.r. studies[68] on cyclic disulfide (34) showed that there was stabilisation of a β-turn conformation and it is interesting to note that the disulfide link was actually made while the linear precursor was still attached to the resin. Tl(III) trifluoroacetate oxidation of the precursor cysteine analogue on the resin gave 94% yield of the cyclic product. (I_2/H^+ only gave 52%). However, I_2/MeOH was the synthetic method[69] of choice for the synthesis of a series of cyclic cystine peptides ($H-\overline{Cys-(Gly)_n-Cys}-OH$) with n ranging from 0-4.

3.3 Miscellaneous Bridges and β-Turn Mimetics - Computer-assisted molecular modelling revealed[70] potential as a β-turn mimetic for the 11-membered ring bis-lactam (35) and has now been synthesised for further insertion into peptides. The latter step has already been realised[71] for a bicyclic analogue of (35) with its incorporation into the compound (36) using a Merrifield solid phase approach on a polystyrene resin and either an Fmoc- or Boc- protecting group strategy. 4-Alkylamino-3-cyano-6-azabicyclo[3.2.1]oct-3-enes (Ben derivatives) as γ-turn mimics were reported in preliminary form two years ago. The complete details of their synthesis have now appeared[72]. Conformational constrainers based on the piperazinones (37) and (38) have already been incorporated into enkephalin-type molecules. Enantiospecific syntheses of these species have now been reported[73] as exemplified by Scheme 10. The novel dipeptide (39) (Eaa) can be prepared[74] from (S,S)-$HO_2CCH(Me)NH(CH_2)_2NHCH(Me)CO_2H$ and has been incorporated into the cyclic peptides *cyclo*-(Sar-Eaa-Sar)$_3$ and *cyclo*-(Sar-Eaa-Sar-Eaa-Sar)$_2$.

Although not expected to adopt the type II β-turn, the ability to form smaller intramolecularly H-bonded turns exists if the unit (40), which contains a *cis* peptide linkage was incorporated as a di-alanyl mimetic. Both the *cis*- and *trans*-form of (40) have been prepared[75] and have been shown by ^1H n.m.r. to adopt a distorted chair conformation with the PhCONH equatorial. In the crystal the *cis*-form tends to prefer a boat conformation. Conformationally constrained 4-substituted proline derivatives have been synthesised[76] chirospecifically for the first time *via* a route starting with N-protected glutamic acid esters.

4. Dehydroamino Acid Analogues

Although interest in these analogues continues, there are only one or two reports involving the dehydro-units '*in situ*' within a biologically active molecule. In an n.m.r. study[77] on molecules first reported in 1986, the effect of Δ-Phe residues inserted in the 3rd and 5th positions of dermorphin and N-terminal fragments have



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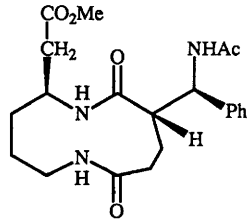

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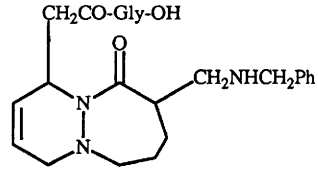

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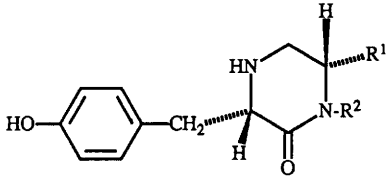
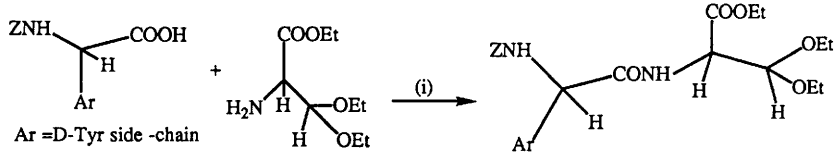


(37) $R^1 = CO_2Et$ $R^2 = H$

(38) $R^1 = H$, $R^2 = CH_2CO_2Et$



Scheme 10

(i) DCC/HOBt (ii) 70%TFA/H_2O (iii) $Pd(OH)_2/H_2$

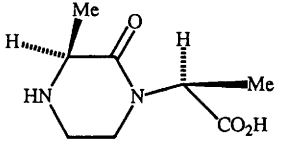
been investigated. In d_6-DMSO at 500 MHz all peptides adopted essentially random extended conformations, due to strong solvation. To circumvent the problem of lack of solubility in $CDCl_3$, 18-crown-6-ether complexes were used and the n.m.r. results showed ordered folded conformations, very similar behaviour to that observed in c.d. spectra. In general, there is more interest in model dehydropeptides as in an n.m.r. study[78] of Boc-Phe-Δ^z-Phe-Val-Phe-Δ^z-Phe-Val-OMe, again conformations were found to be solvent-dependent - in $CDCl_3$ there were folded helical conformations present - in d_6DMSO the extended conformations were favoured. In general, backbone conformational restraints introduced by an α-β double bond appear to be relatively weak. But an i.r. and n.m.r. report[79] on synthesised Boc-Pro-Δ^zX-Gly-NHEt, where X = Leu or Phe, shows that both peptides have a strong tendency to stabilise type II β-turns when the dehydro residue was present at the i + 2 position. The well accepted azlactone synthetic route has provided crystalline samples of Boc-L-Pro-ΔLeuNHCH$_3$[80] and Boc-Gly-ΔPheNHCH$_3$[81] and both adopt a type II β-bend structure. A similar conformational picture is created from n.m.r. studies[82] on the related Boc-X-Δ^zPheNHMe examples where X was varied from Ala, Gly, Pro to Val. In contrast to their saturated counterparts these dehydro peptides favour β-turn structures in $CDCl_3$ with Pro a better stabiliser of the β-turn than Val.

Unsaturated azlactones have been useful in the synthesis[83] of various didehydropeptides. N-Carboxydehydrotyrosine anhydride, made from the corresponding Z-ΔTyr derivatives has been employed[84] in a 'one-pot' synthesis of a dehydrooligopeptide, and in the making of [ΔTyr^1]- and [ΔTyr^1, ΔPhe^4]-enkephalins. A previously reported successful synthesis of dehydro-amino-acids using t-butyl oxamic esters has been extended[85] to dehydrodipeptides according to the key steps outlined in Scheme 11. Oxazolones such as (41) with R = 2-pyrimidyl, 4-methyl-2-pyrimidyl, 6-chloro-3-pyridazinyl were successful precursors[86] of the corresponding dehydroamino acids.

Only when the dehydro-amino acid residue is present in the N-terminal position in a series of dehydrotripeptides does heterogeneous-catalytic hydrogenation give a large diastereomeric excess[87] of the saturated analogue. A β-turn structure at the C-terminal end of the Δ^1 analogue has been implicated as a reason for the chiral induction.

5. Enzyme Inhibitors

5.1 Angiotensin Converting Enzyme (ACE) Inhibitors - From a series of compounds synthesised[88] as potential ACE inhibitors analogue (42) has been selected for clinical evaluation as an antihypertensive agent. It has an ID_{50} of 16μg/Kg. In the series (43) the compound with the best ACE inhibition potency[89] was (43, R^1 = Et, R^2 = (S)-Me, R^3 = CH_2CH_2-Ph) which had an ID_{50} of



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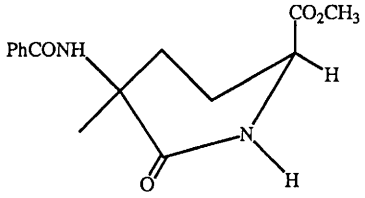
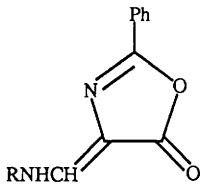


(40) cis-form



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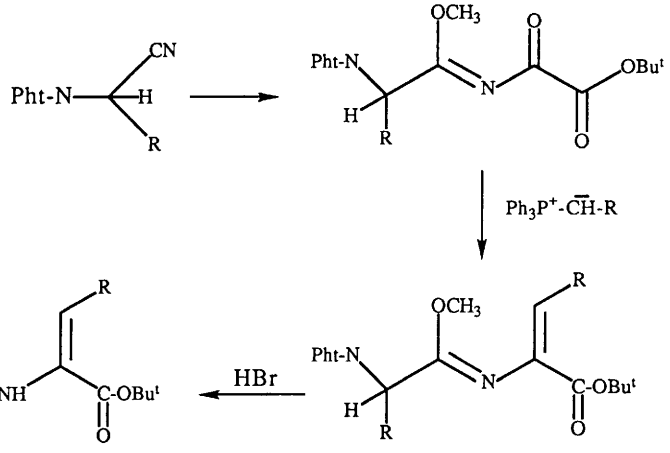


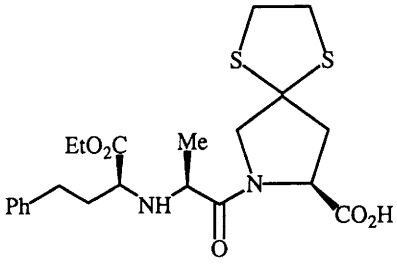
Scheme 11

0.24mg/Kg and produced a dose-dependent decrease in systolic blood pressure in spontaneously hypertensive rats. ACE inhibitors of the enalapril and enalaprilat family can now be synthesised[90] stereoselectively as a result of the use of (R)-NH_2CONH-$CH(CH_2CH_2Ph)CO_2H$ (44) as a chiral intermediate derived from the asymmetric hydrolysis of DL-5-phenethylhydantoin using microbial hydantoinase. Conversion of (44) to (R)-$ClCH(CH_2CH_2Ph)CO_2H$, gave the synthetic intermediate which was subjected to an S_N2 reaction with the appropriate C-protected L-amino acid derivative to give enalapril.

An ACE inhibitor (45) (A58365A) discovered in culture filtrate of *Streptomyces chromofuscus* has been synthesised[91] in eleven steps of which Scheme 12 represents key stages. An isomer of (45), structure (46) was also synthesised[92], but proved to be inactive as an ACE inhibitor. A biomimetic synthetic sequence[93], based on the condensation of 3 moles of suitably derivatised L-tyrosine and involving thallium III nitrate as an oxidising agent, has provided the same heterocyclic skeleton (47) as that of K-13 a novel inhibitor of angiotensin I converting enzyme.

5.2 Renin Inhibitors - Inhibition of the aspartic proteinase renin, as a means of controlling blood pressure still remains an active pharmaceutical goal, with obviously high stakes to play for. Mimicking the transition state at the scissile Leu-Val bond of the renin substrate remains the popular philosophy used in the design of inhibitors, and the success of the naturally occurring statine residue has provided a useful template from which other designer molecules have developed. Thus, a report[94] on a simple synthesis of statine (48) which relies solely on the chiral centre already present in the precursor is worth noting and is summarised in Scheme 13. A diastereoselective synthesis of cyclohexyl norstatine from Boc-L-phenylalaninol was a key part in the synthesis[95] of an orally potent human renin inhibitor (49) which has an $IC_{50} = 2.4 \times 10^{-9}M$ for isolated human renin and high and long-lasting activity *in vitro* and after oral administration.

Hydroxyethylene dipeptide isosteres have attracted much attention in this area with three research groups independently using a similar approach. Isostere (50) has been synthesised[96] from a lactone precursor (51), designated by X-ray crystallography to be (2S,4R,5S). Similarly excellent stereocontrol over all three chiral centres of (50) was achieved[97] *via* the intermediate bromo- or iodo-lactones (52). Bromo-lactonization, stereoselectively controlled by the chiral acyloxazolidinone (53) resulted[98] in a similar successful route. 3,4,6-Tri-O-Acetyl-D-glucal also seems[99] to be a useful synthon for controlling the stereochemistry at C_2 and C_5 *via* conversion to an amino lactone which then opens up to a derivative of (50). The sulfur-containing analogue (54) has been synthesised[100] stereochemically pure and has been incorporated into a number of renin inhibiting structures. As a development of a previous study in 1987, the azidomethyl inhibitor



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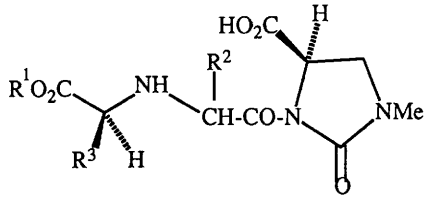

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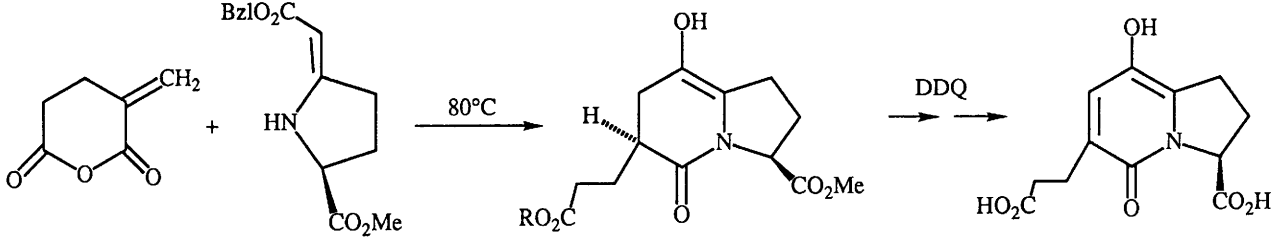
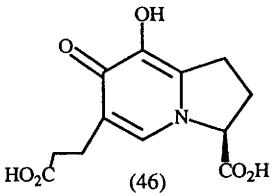


(45)

Scheme 12



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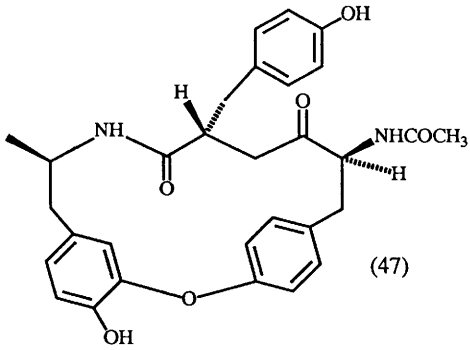

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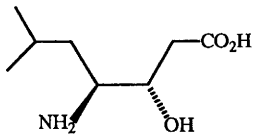

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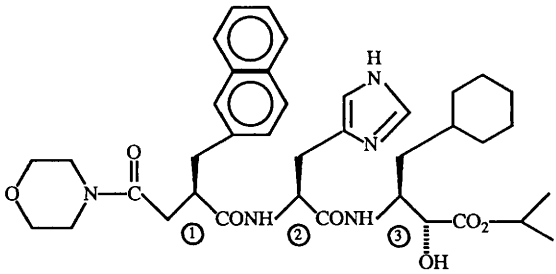
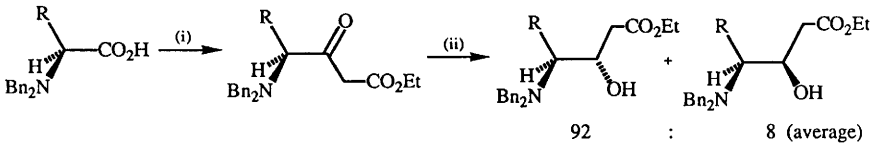
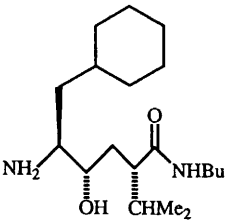


(49) in the -1R,2S, 3S, 4R-form.



(i) N,N' carbonyldiimidazole, then Pr^i $MgCl/CH_2(CO_2Et)COOH$
(ii) $NaBH_4$/MeOH - 20°C.

Scheme 13



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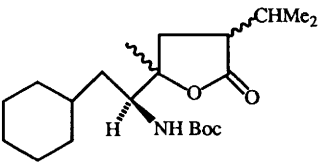

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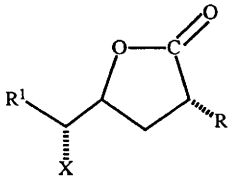
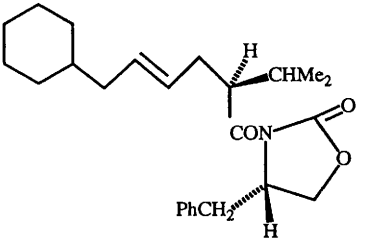


(52) X=Br or I



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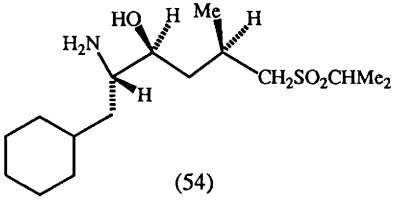

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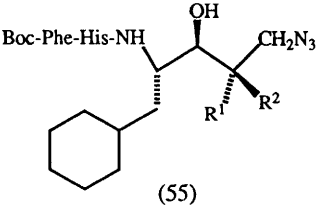
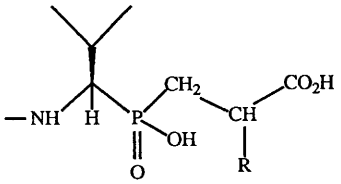


(55)

(55) has been synthesised[101], and confirms that the entire post-scissile site portion of an inhibitor can be replaced by the azidomethyl group. Substitution of OH and CH_2OH groups at the R^1/R^2 positions led to an inhibitor at the nanomolar range, but although improved transport across the intestine could be proved, liver extraction reduced the systemic levels. Phosphorus-containing derivatives such as (56) have been considered[102] as tetrahedral transition-state mimics of the scissile Leu^{10}-Val^{11} in angiotensinogen (5-14). When (56) with R = $CH_2CH(CHMe_2)CO$, was incorporated to give H-Pro-His-Pro-Phe-His-'56'-Ile-His-Lys-OH an IC_{50} value of 7.5 x 10^{-8}M was achieved, as the best of a series of inhibitors tested. The renin inhibitor peptide, H-Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys-OH has been subjected[103] to a full n.m.r. analysis, and the presence of a tight turn within the conformation of the molecule is strongly indicated.

5.3 Inhibitors of Other Enzymes - Statine derivatives with histidine side-chains (57) have also found[104] application in the synthesis of potent inhibitors of the fungal aspartic proteinase penicillopepsin. Inhibitor (57) (Bom = $PhCH_2OCH_2$) was 10 times more active than the comparable statine analogue in the same assay. Modifications at the C-terminal residue of inhibitors of neutral endopeptidase and aminopeptidase N have been carried out[105] in order to study the structure-activity relationships of cyclic amino-acid residues at the C-terminus as in (58). The most active inhibitors contained a *trans*-cyclopentyl β-amino acid (58, n = 3) and a *cis* or *trans* cyclohexyl β-amino-acid (58, n = 4), and elicited potent antinociceptive responses on both the jump latency and fore paw lick times. In order to improve the *in vivo* protection of enkephalins from enzymatic degradation, modifications[106] have been carried out on a new series of inhibitors based on kelatorphan [$HONHCOCH_2CH(CH_2Ph)CONHCH(CH_3)COOH$] a known inhibitor of enkephalin metabolism. Compounds containing variously substituted β-Ala structures at the C-terminus [$HONHCOCH_2CH(CH_2Ph)$-$CONHCH(R_1)CH(R_2)COOH$] all exhibited inhibition of neutral endopeptidase and dipeptidylaminopeptidase in the nanomolar range with compounds having $R^1 = CH_2Ph$, $R^2 = H$ and $R^1 = Ph$, $R^2 = H$ showing better prospects than the others, and are more potent than kelatorphan. The advantages of inhibiting enkephalinase A has been explored[107] using a series of dibenzylglutamic and some phenolic benzylamine derivatives. In $PhCH_2CH(CO_2R^1)CH_2CH(CH_2Ph)COR$, no change in potency was seen if R was changed from Gly to β-Ala to 4-aminobutyric acid (increasing chain length), but R = S-benzylcysteine improved activity 10-fold. Reversing the amide bond as e.g. in $PhCH_2CH(CO_2H)CH_2CH$-$(CH_2Ph)NHCO(CH_2)_nCO_2H$ gave a 15-fold decrease in potency.

Alanine racemase and D-Ala-D-Ala ligase catalyse key steps in the biosynthesis of bacterial peptidoglycan. The racemase has been studied as a potential means of designing novel antibacterials but the ligase has not been



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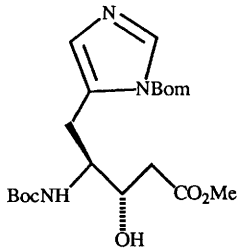

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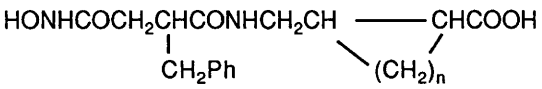

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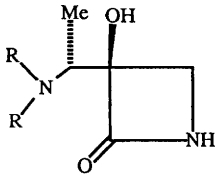
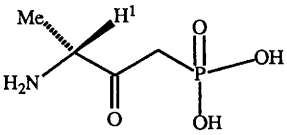


(59) R = $CH_2CH{=}CH_2$ or H



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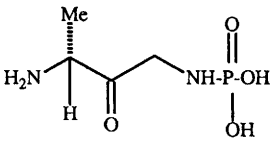

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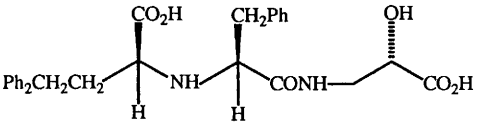


(62)

extensively studied. This situation has now been given attention[108] through the synthesis of analogues (59) of tabtoxinine. Neither the (3R) or (3S) form of (59) (R = H) were active against *S.aureus* and *E.coli* and showed no inhibitory activity. Following the same analogy regarding the intermediacy of D-alanyl phosphate in the D-Ala:D-Ala ligase mechanism several 3-amino-2-oxoalkyl phosphonic acids were synthesised[109]. Phosphonic acids (60) and (61) were effective ligase inhibitors but possessed no antibacterial activity.

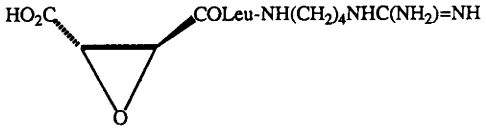
Inhibition of neutral endopeptidase, which is a zinc metallopeptidase homologous to thermolysin could potentiate the hypotensive activity of arterial natriuretic factors (ANF) which are inactivated by the enzyme. The rationale of this approach has been justified[110] from the inhibition properties of (62) which expresses antihypertensive activity in a rodent model.

The natural peptide protease inhibitor (EETI-II) from *Ecballium elaterium* seeds has been synthesised[111] using the BOP reagent in a solid phase protocol, and shown to be identical with the natural product. A 30-residue peptide (CMTI-III), a trypsin inhibitor, from *Cucurbita maxima* (synthesised in 1986) has been studied[112] with 500MHz n.m.r. 2D techniques. The main conformational features appear to be a β-turn involving residues 12-15 and a triple stranded β-sheet consisting of residues 8-10, 21-23 and 26-29. A crystal structure and molecular conformation has been reported[113] for the cysteine protease inhibitor E-64 (63), and has provided a possible inhibitory mechanism involving the Cys-25 side chain being alkylated by the epoxide ring in (63). A novel analogue (64) of proline has prospects for further study[114] as an inhibitor of prolyl-4-hydroxylase.

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

Papers published under this category remain at the productivity level of previous years. The scope covered again is wide-reaching and categorisation under appropriate sub-headings has been difficult. If the discerning reader can perceive some theme to the sub-classification, then receptor specificity might be gleaned to be the approximate guide. Synthetic methodology is reviewed authoritatively in another Chapter of this book and often is taken for granted in papers under this category. However, all researchers in this field would benefit from the synthetic challenges represented by the total synthesis of urogastrone[115] by the conventional methods and bombesin[116] by the solid phase procedure. The sequence of elephant growth hormone has been reported[117] and found to be highly homologous with the porcine form but to a much lower extent with that of the human sequence.

6.1 Peptides with 'Opioid Characteristics' - Designing new synthetic drugs of pharmacological interest in this area has been the subject of a review[118]. Polar tri-



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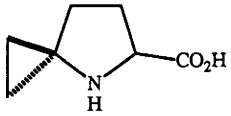

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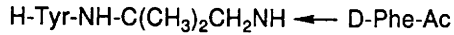


(65)

βEP Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys

(66) ----------Leu-Lys

(67) ---------(NHCH(CH_2OH)CH_2CH_2CO)$_4$-Pro ----------

-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH

-Leu-Lys-Leu-Leu-Gln-Lys-Leu-Leu-Gln-Lys-Leu-Phe-Lys-Gly-Lys-Glu-OH

----------OH

and tetrapeptide analogues based on [D-Arg^2]-enkephalin have been designed[119] to be excluded from the central nervous system, thus limiting their action to the peripheral opioid receptors. Tetrapeptides such as H-Tyr-D-Arg(H^+)-Gly-EtPhX(NH_2) (X varying from NO_2 to Cl to F) seemed to be more potent than the tripeptides of general structure H-Tyr-D-Arg(H^+)-Gly-N(Et)$(CH_2)_n$Ar, and within the series a number had the desired pharmacological profile to confirm peripheral selectivity. One of the most potent opioid agonists synthesised[120] up to now was obtained as part of a research effort on O-glycosyl enkephalins. O$^{1.5}$-(β-D-Galactopyranosyl) [D-Met^2,Hyp^5]-enkephalin amide proved to have the best activity *in vivo*. Fluorinated derivatives of tyrosine and phenylalanine have been incorporated[121] into the 1- and 4-positions of enkephalin using solid phase procedures. The main effects were seen with p-(F)Phe^4 where there was significant potentiation of the effects in guinea pig ileum (gpi) and mouse vas deferens (mvd) but replacement of F for OH in Tyr or the introduction of p-CF_3(Phe) reduced potency. To test the theory that the crucial recognition elements needed for active μ-ligands were the aromatic residues of Tyr and Phe, enkephalin analogues with only those two residues suitably spaced have been investigated[122]. Of the diamine spacers used between the residues, 1,2-ethanediamine representing the closest distance was better than the corresponding 1,3-propanediamine. The presence of a *gem*-dimethyl group next to the nitrogen attached to Tyr as in (65) increased activity substantially for the D-Phe derivative. Human preproenkephalin (100-111), H-Tyr-Gly-Gly-Phe-Met-Lys-Arg-Tyr-Gly-Gly-Phe-Met (contains two enkephalin sequences) has been synthesised[123] *via* the Merrifield technique, together with its Arg^6 and Lys^7 analogues. In the gpi and mvd assays the parent was less active than enkephalin, while the Arg^6 analogue was more active than enkephalin in gpi. All analogues were more potent in mvd assay (δ-receptor) than in gpi (μ-receptor representative). The two peptides (66) and (67) have been designed[124] as models for the linker domain (residues 6-12) and the amphiphilic α-helical domain (residues 13-29) in β-endorphin (β-EP). In binding studies both displayed a higher affinity for μ- and δ-receptors than for κ-receptors, and were 2-3 times more potent in the μ- and κ-receptor assays and about equipotent in δ-binding. They therefore provide further ideas to support the functional conformation of β-endorphin.

Glucopyranosyl groups have been attached[125] *via* the Glu^6-γ-carboxyl position using solution procedures, to produce glycosylated analogues of substance P(SP). The key step of coupling 2,3,4,6-*tetra*-O-acetyl-β-D-glucopyranosylamine to Fmoc-Glu-OBzl was catalysed by DCC/HOBt, with a little loss of the Fmoc during subsequent hydrogenolysis. [Glu^6]SP_{6-11} and [Glu(β-D-Glcp)^6]SP_{6-11} were determined in the usual assays and showed that introduction of the glucopyranosyl group did not affect the *in vitro* activity pattern of [Glu^6]SP_{6-11}. Selective agonists for SP and neurokinin (NKS) receptors have been studied[126] as a means of

deducing a better understanding of the functions of the three receptor types NK-1, NK-2 and NK-3 believed to be available. From a survey of the activities of [Sar^9,Met(O$_2$)^{11}]SP, [Nle^{10}]NKA(4-10) and [MePhe^7]-NKB it is suggested that receptor NK-1 mediates peripheral vasodilatations and exocrine secretions, NK-2 stimulates bronchial muscles and release of catecholamines and NK-3 promotes the release of acetylcholine in peripheral organs. Dimeric analogues (68) and (69) are representative of NKA(4-10) and NKB(4-10), respectively. These shortened, but dimeric sequences were fairly potent in gpi but not in the rat vas deferens assay. It is suggested[127] that dimerisation changed the receptor selection of the original sequence from NK-2 to NK-1.

L- and D-Neopentylglycine have been inserted[128] into the 5-position of dalargin, H-Tyr-D-Ala-Gly-Phe-X-Arg-OH. When X = L-neopentylglycine activity in the gpi assay was 20 times higher than dalargin while in the D-form only 4 times the activity was obtained. Deaminated, acetylated and dimethylated analogues of the analgesic dipeptides H-X-Trp(Nps)-OMe where X = Lys, Orn or Arg have been synthesised[129] and tested. Only the acetylated dipeptides exhibited a naloxone-reversible antinociceptive effect comparable to the original sequence. Only the H-Leu-Arg-Pro-Arg-OH analogue of tuftsin out of the three synthesised[130] had comparable analgesic activity to tuftsin suggesting the need for a Pro-Arg sequence for activity.

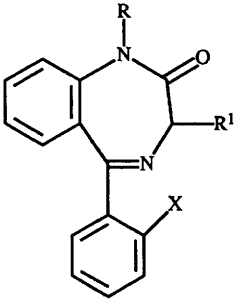
Dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH$_2$) analogues carrying a nett positive charge in their N-terminal message segment have been used[131] as test compounds to test the concept that μ- and δ-receptors are located in anionic and cationic membrane compartments. Gradual change of positive charge from 1+ to 3+ in a series of dermorphin(1-4) tetrapeptides produced enhancement of μ-receptor affinity at the expense of δ-receptor affinity. The most selective, [D-Arg^2,Lys^4]-dermorphin(1-4)-amide showed a selectivity ratio of $K_i\delta/K_i\mu$ = 11,400 more than that of the well known DAGO ($K_i\delta/K_i\mu$ = 1050). However, on a pessimistic note it is implied that because of its high +ve charge it will most likely not be able to cross the blood-brain barrier. No affinity for the κ-binding site has been obtained[132] for any D-substituted or N-methyl substituted dermorphin analogues involving positions 5, 6 or 7. However, provided a C-terminal amide group was present some analogues showed a reverse $IC_{50}\delta/IC_{50}\mu$ selectivity ratio. [Tyr^7]-Dermorphin, synthesised[133] on a Merrifield resin using Fmoc-protected amino acid trichlorophenyl esters/HOBt, was shown to be twice as potent as dermorphin in the gpi assay, 1.4 times in its analgesic activity in mice. [Thr^7]-Dermorphin was equipotent with dermorphin itself. In a series of replacements[134] at the D-Ala^2 position in dermorphin, in general lower activity in gpi was achieved, but four analogues, showed enhanced antidiarrheal potency, [D-Phe^2]-dermorphin being the best (2.7 x morphine) . Low-energy conformations have been calculated[135] for dermorphin, dermorphin (1-5) (H-Tyr-D-Ala-Phe-X-

CH_2CO-Asp-X-Phe-Val-Gly-Leu-Met-NH_2
|
CH_2CO-Asp-X-Phe-Val-Gly-Leu-Met-NH_2

(68) X=Ser (69) X=Phe

H-Tyr-X-Phe-Gly-Tyr-Y-X-NH_2

(70) X=Cys, Y=Pro (or a D-Cys^2 analogue)
(71) X=D-Cys, Y=no residue



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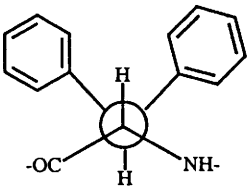

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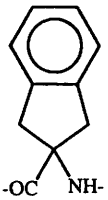

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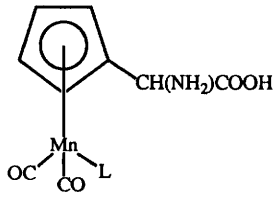


(75) L = CO or PPh_3

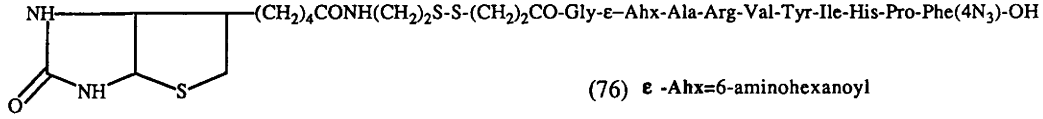
Tyr-OH', X = Gly) and analogues X = Leu, D-Gln, (2S)-2-amino-3-(adenin-9-yl) or -thymin-1-yl)propionic acid in relation to their μ-receptor binding results. Reduced potencies in the gpi assay were reported[136] for the cyclic dermorphin analogues (70) and (71). Deltorphin (H-Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2) which has an N-terminal resemblance to dermorphin, has been investigated[137] by ^1H n.m.r., and the conclusion drawn that an important difference between a δ- and μ-receptor is that the former can tolerate charges outside the region that recognises the β-turn in the N-terminal region of peptides. A heptapeptide, dermenkephalin has from the skin of the frog *Phyllomedusa sauvagei* been shown [138] to have the sequence H-Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2, which seems to be identical to the deltorphin sequence above.

6.2 Cholecystokinin Analogues - Glycine-29 has been replaced by a β-Ala residue in the C-terminal heptapeptide fragment of cholecystokinin[139], together with some residue-substitution by D-forms and C-terminal 2-phenylethylalcohol. None of the changes improved potency which strongly suggests that the C-terminal region is important to the binding to CCK-receptors. Minimal protection of side-chains, using Ser and Thr with side-chains unprotected, was successfully applied[140] in conjunction with the BOP-reagent to the solid phase synthesis of CCK-7, Ac-Tyr(SO_3H)-Met-Gly-Trp-Met-Thr(SO_3H)-PheNH_2. Sulfation of the hydroxyl groups on the resin was also successful and after this step, release from the PAM-resin was achieved by liquid NH_3. Removal of the Phe^{33}-NH_2 residue is known to introduce antagonist properties, so its influence has been further analysed[141] through the synthesis of several CCK(26-33) analogues having lipophilic amino acids in the sequence. Analogues Boc(Nle^{28},Nle^{31},X^{33})CCK-(27-33) with X = naphthylalanine or cyclohexylalanine displayed high affinities for central and peripheral CCK-receptors and were full agonists of CCK-8. This would imply that size and hydrophobicity might be the critical factor for optimal interaction of position-33 with its receptor. Tritium atoms have been introduced[142] into CCK-4, H-Trp-Met-Asp-[^3H]Phe-NH_2 by passing tritium gas over PdO in the presence of a tribromophenylalanine derivative. Replacement of the sulfated tyrosine residue with a non-hydrolysable equivalent group has been made possible[143] by the insertion of L- or D-Phe(*p*-$CH_2SO_3^-Na^+$) into CCK-8. Ac-L- or D-Phe(*p*$CH_2SO_3^-Na^+$)-Nle-Gly-Trp-Nle-Asp-PheNH_2 displayed high activity for peripheral and central CCK receptors ($K_i = 10^{-9}M$) and were full agonists in stimulation of pancreatic secretion. The activity of the sulfated insect neuropeptides leucosulfakinin (LSK) and LSK-II (sequences show homology with human gastrin II and cholecystokinin) is influenced[144] by the position of the Tyr(SO_3H) residue in the sequence. In [Tyr(SO_3H)^5,Phe^6,Nle^9]-LSK in which the sulfate moiety is one nearer the N-terminus the peptide retained 38% of the

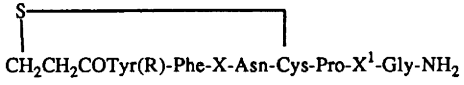
parent's threshold activity. Further movements either way led to reduced or complete loss of activity.

A 4th class of benzodiazepines have been discovered[145] which bind selectively to brain CCK-B and gastrin receptors. The general structures (72) disclosed in the study support the hypothesis that these core structures are amenable to the molecular design of ligands for a variety of peptide receptors. A 400MHz ^1H n.m.r. study[146] on cyclic analogues of CCK-8, such as Boc – X – Tyr(SO_3H) – Nle – D – Lys – Trp – Nle – Asp – Phe – NH_2 where X was varied from L- to D-Glu or to γ-D-Glu has enabled a correlation to be made between the increasing affinity for receptors shown by the γ-D-Glu analogue and (a) an enhancement in internal flexibility of the cyclic moiety, (b) an external orientation of the phenolic side-chain of tyrosine and (c) a restructuring of the C-terminal residues.

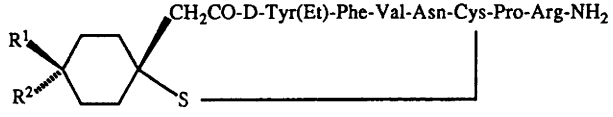
6.3 Angiotensin and Analogues - There have been a number of studies recorded this year on residue substitution in the angiotensin sequence. Increasing the conformational constraints at position 8 in H-Sar-Arg-Val-Tyr-Val-His-Pro-X-OH, through the use of, e.g., biphenylalanine (73) and 2-aminoindan-2-carboxylic acid (74) has given rise[147] to analogues with high affinity for rat uterus (K_i = 0.74-6.08nM) and brain (K_i = 0.46-1.82nM) receptors. Analogues containing (73) in its L-form showed 284% of the activity of angiotensin II, but incorporation of (74) and a diphenyl-alanine analogue produced antagonist properties. Replacement of Phe^8 with (75) also gave analogues[148] which were pure antagonists of angiotensin II, but confirmed a higher affinity than other bulky analogues at position-8. New active esters (2,3,5,6-tetrafluorophenyl) have been used[149] in the solution phase synthesis of analogues containing Ser or D-Ser at position-1. [Ser^1]- and [D-Ser^1]-Angiotensin II showed pressor activity 129 and 314% as potent as the parent. On the other hand [Ser^1,Leu^8]- and [D-Ser^1,Leu^8]-angiotensin II were effective antagonists. These results and others[150] which involve [$DOPA^1$]-, [Cys^1]-, [$MeAib^1$]-, [Aib^1Aib^2]- and [$GABA^1GABA^2$]-angiotensin II confirm that the N-terminal positions has an important influence on the biological activities of angiotensin. Zooming in on position-4 with *para*-substituted phenylalanine ranging from OH, NH_3^+, $B(OH)_2$, SO_3^-, NO_2 and halogen substituents showed[151] no correlation between the nature of the substituent in [Sar^1, *p*(X)-Phe^4]-angiotensin II and antagonistic activity. One trend deduced was that H-bonding promoters in the *para*-position promoted agonist properties, while hydrophobic substituents favoured antagonistic properties. At position-5 of angiotensin it has been reported[152] that for antagonist potency a lipophilic β-branched side-chain is not a necessity since 'straight chain' residues can be substituted for X in [Sar^1,X^5,Ile^8]-angiontensin II with retention or enhancement of antagonistic activities. A biotinylated probe (76) has been synthesised[153] to



(76) ε -Ahx=6-aminohexanoyl



(77) (R=Me, Et; X=Glu, Val; X^1=Arg, D-Arg)



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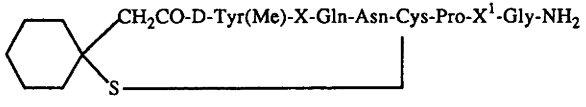
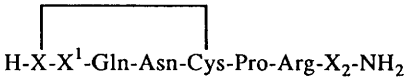


(79)

explore the angiotensin II receptor sites. The BOP reagent proved to be a successful reagent in the assembling of the units, and a ^{1}H n.m.r. survey suggested that the extended conformation of the molecule would allow simultaneous recognition of both streptavidin and hormone receptor.

6.4 Vasopressin Analogues - Solid phase synthesis has provided[154] a number of different combinations of substitutions into the 4/7, 4/8 and N-terminal positions in vasopressin(VP). From these analogues, 1-desamino[Pro^4,Lys^8]-VP, 1-desamino[HOPro^4,Lys^8]-VP and 1-desamino[Lys^4,HOPro^7]-ArgVP, the ε-amino lysine groups were converted to photoreactive azido compounds, which could be assayed for antidiuretic and pressor activities. 1-Desamino[Lys^4-(N$^\varepsilon$-4-azido-benzoyl),HOPro^7]-ArgVP was reported to have the highest antidiuretic activity of any photoreactive compound reported up to that time. Two analogues of Arg-vasopressin, [Cpp^1,D-Phe^2,Sar^7,Arg^8]- and [Cpp^1,D-Phe^2,MeAla^7,Arg^8]-VP where Cpp^1 represents β-mercapto-β,β-cyclopentamethylenepropanoyl, were shown[155] to exhibit antioxytocic, antivasopressor and antiglycogenolytic activities and while most of their binding properties are the same as VP, their affinities for the renal V_2 receptor are 60-90 times lower. [Nβ-Guanidoacetyl-α,β-diaminopropionyl^8]-VP has been synthesised[156] but has lower antidiuretic activity than [β-mercaptopropionyl^1,D-Arg^8]-VP. Highly specific and potent diuretic agonists have however materialised[157] from the 1-desamino analogues based on structure (77). Deamination significantly enhanced the diuretic properties and all analogues (except (77) with R=Me, X=Glu, X^1=Arg) were antagonists of the vasopressor responses to Arg-VP and of the uterine response to oxytocin. Highlighted as a unique compound, and a potent antiduiretic agent was the analogue (77) (R=Me, X=Val, X^1=D-Arg). Modifications[158] to the hydroxy group of tyrosine-2 in analogues of (77) with a cysteinyl group at the terminal position, also showed that both R=Me and Et on the tyrosine gave antiduiretic agonists, but antagonists of vasopressor responses to Arg-VP. The full antidiuretic agonist (SKαF 1019261) (78) (R^1=R^2=H) recently tested in man, has been slightly modified[159] by substitution of a methyl group into the cyclohexyl ring. Tests in animals revealed that *cis*-methyl analogue (78) (R^2=Me, R^1=H) had a reduced agonist activity while the *trans*-form (78) (R^2=H, R^1=Me) exhibits more activity. Sixteen vasopressin or vasotocin analogues, e.g., (79) with X=Phe or Ile, and X^1=Arg or Orn have been synthesised[160] using solid phase techniques, and all compounds exhibited potent oxytocic antagonism *in vitro* and *in vivo*.

In a series of analogues related to (78), C-terminal diaminoalkanes and aminoalkyl guanidines have been studied[161] as replacements for the peptide unit outside the cyclic hexapeptide ring. It seems that the 'exo-cyclic' segment can be replaced simply by a basic function at an optimal distance from the hexapeptide ring. Vasotocin analogues (80) with X=Cys or β-mercaptopropanoyl X^1=Tyr or



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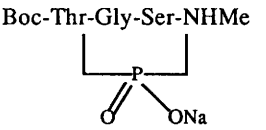

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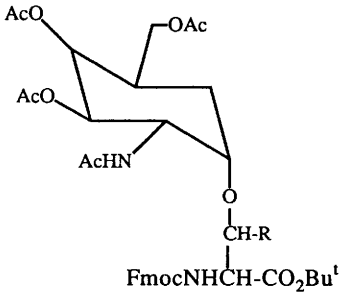
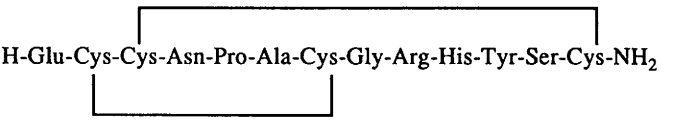


(82) R=H or Me



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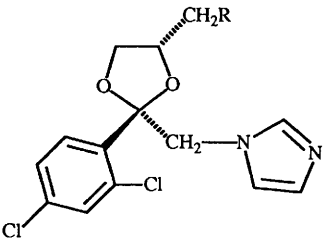


(84)

Phe, and X^2=*p*-NH_2-Phe or Tyr have been synthesised[162] and modified at the C-terminal residue to include iodo-tyrosine, *p*-N_3-Phe or *p*-biotinylPhe. These compounds could be used as probes in the isolation of vasotocin and V-2 vasopressin receptors since these were amongst the most potent compounds made to date. [Glu$(NHNH_2)^4$,Lys^8]-Vasopressin (and Glu$(NHNH_2)^4$ oxytocin) were the products of solution phase synthesis[163] using *N*-hydroxysuccinimide but showed reduced biological activities compared to the respective parent hormones with their glutamine side-chains.

6.5 O-Phosphorylated and Glycosylated Derivatives - Some increase in productivity under this category this year justifies a separate sub-section. Recent recognition of tyrosine phosphorylation as an important step in cellular processes has justified the development of synthetic methodology for making O-phospho-Tyr peptides. In the solution phase[164] Boc-Tyr(PO_3Bzl_2)-OH was used if the last step was a hydrogenolytic cleavage, while Boc-Tyr(PO_3Me_2)-OH also proved useful for the solution or solid phase. The latter protected derivative was amenable to incorporation[165] into H-Asn-Glu-Tyr(PO_3H_2)-Thr-Ala-OH, the phosphate Me groups being removed by Me_3SiBr, Me_3SiBr/PhSMe in TFA or CF_3SO_3H/-CF_3CO_2H/Me_2S/m-cresol. Boc-Tyr(PO_3Me_2)-OH was reported[166] also to be the derivative of choice in the Merrifield solid phase synthesis of H-Leu-Arg-Arg-Ala-(P)Tyr-Leu-Gly-OH, while in the Fmoc-polyamide protocol Fmoc-Tyr(PO_3Me_2)-OH fitted[167] the bill for the synthesis of H-Arg-Leu-Ile-Glu-Asp-Asn-Glu-(P)Tyr-Thr-Ala-Arg-Glu-Gly-OH which occurs in viral proteins.

The peptides H-Leu-Arg-Arg-Ala-X-Leu-Gly-OH where X = Ser(PO_3H_2) or Thr(PO_3H_2) have been prepared[168] using solid phase methodology with the appropriate residues protected as Boc-diphenylphosphono esters. The side-chain protection was not removed by HF or by CF_3SO_3H during cleavage from the resin but could be removed by catalytic hydrogenation. Phosphorylation of serine residues left unprotected on a peptide resin[169] has been achieved with $(BzlO)_2P(O)Cl$. Final deprotection of the protected peptide with TFA, cleaved the peptide off the resin and removed the benzyl groups.

Macrocyclisation of Boc-Thr-Gly-Ser-NHMe to give the cyclic phosphopeptide (81) was carried out[170] using the phosphitylating agent $[(Me_2CH)_2N]_2POCH_2C_6H_4pCl$, followed by oxidation to the phosphate triester and hydrogenolysis. N.m.r. data from (81) provided a full characterisation. The same phosphitylating agent and methodology has also been used[171] for the synthesis of H-Lys-Arg-Thr(P)-Leu-Arg-OH a phosphorylated analogue of the substrate to protein kinase C.

The Fmoc derivative (82) has found successful application[172] in the insertion of glycosylated hydroxy amino acids into a number of peptides with sequences related to the N-terminals of interleukin-2.

6.6 Miscellaneous Examples - Isosteres for the Cys-Cys disulfide link in atrial natriuretic factor analogues have been provided[173] through the enantiospecific synthesis of L-α-aminosuberic acid and its dehydro-analogue $NH_2CH(CO_2H)$-$CH_2CH{=}CHCH_2CH_2COOH$. A dicarba analogue of β-atrial natriuretic peptide (β-ANP) with an -S-S- link replaced[174] by α-α-aminosuberic acid has been synthesised. [Asu$^{7,23'}$]β-ANP(7-28) is the modified antiparallel dimer of human α-ANP, but with one link replaced by the carba bridge. However, the biological results confirm that it is only <1% as active as β-ANP, which suggests that the dimer to monomer conversion is critical for the activity of β-ANP. Other results seem to confirm that β-ANP is inherently inactive and can behave as a complete antagonist of α-ANP. Several atriopeptin (103-125) amide analogues have been studied[175] in which the cystine group has been varied from the natural position in the protein (between residue 105 and 121). The data obtained demonstrated that binding of atrial peptides to vasorelaxation receptors requires defined receptor/ligand interactions but that the nonvasorelaxant binding sites appear to tolerate changes in structure quite well. The sequence of cecropin D has been confirmed[176] by solid phase synthesis. Structure-activity studies on analogues, e.g., [Lys^1]-, [Gln^3,Leu^4]-cecropin D and cecropin D(9-37) indicated that a strongly basic N-terminus was a prerequisite to antibacterial activity. A hybrid analogue, cecropin A(1-11)D(12-37) was 5 to 55 times as active as cecropin D against six bacterial species.

α-Melanotropin(4-10) has been modified[177] at positions 5 and 10. Ac-[Nle^4,Asp^5,D-Phe^7,Dab^{10}]αMSH(4-10)NH_2 where Dab = 2,4-diaminobutyryl was the most potent analogue obtained to date in the lizard (*Anolis carolinensis*) melanophore assays. Analogues Ac-[Nle^4,X^7]-α-MSH(4-11)NH_2 with X = Phe, D-Phe, phenylglycine(L and D), L-1,2,3,4-tetrahydroisoquinoline carboxylic acid (Tic) have been surveyed[178] both conformationally using n.m.r. techniques and biologically in the usual assays. L-Phenylglycine in position 7 resulted in an enhanced ring stacking interaction between its phenyl ring and the indole ring in Trp^9. The tetrahydroisoquinoline carboxyl substitution led to the α-proton of His^6 interacting with the carbonyl of Glu^5. The power of n.m.r. FAB-MS, uv spectroscopy and tryptic mapping has revealed[179] how complex the synthesis of pure peptides can still be, due to incomplete deprotection and premature chain termination. In the example taken [Nle^{8,18},Tyr^{34}]-parathyroid hormone, synthesised by the Merrifield procedure, gave almost an equal amount of by product due to the formylation of the tryptophan residue. Use of the Tam procedure with an increased ratio of thiocresol/cresol avoided the formylation altogether. The structural requirements at the C-terminal end of gastrin tetrapeptide to give antagonist properties has come under scrutiny[180] with the synthesis of analogues containing β-homo residues. The most potent antagonists

incorporating a β-homo-Leu (-NH-CH(Bu^i)-CH_2CO-) residue were, Boc-Trp-Leu(β-homo)-Asp-$NHCH_2Ph$ (IC_{50} = 1μM, ED_{50} = 0.2mg/Kg), Boc-Trp-Leu(β-homo)-Asp-Phe-NH_2 (IC_{50} = 1.5μ~M, ED_{50} = 0.1mg/Kg) and Boc-Trp-Leu(β-homo)-Asp-D-Phe-NH_2 (IC_{50} = 2μM, ED_{50} = 0.1mg/Kg). A benzyl substituted oxoimidazolidine ring has been used[181] as a substitute for the pyroglutamyl ring in TRH (L-*p*Glu-L-His-α-Pro-NH_2), and showed potency 1.5-8 times greater than TRH, with its thyrotropin releasing activity about 1/16th that of TRH. Replacement of His^2 by 2-[β-4-methylpyrazol-1-yl] alanyl gave an analogue[182] with significant growth promoting activity in mice. In the proline rich polypeptide (PRP) H-Tyr-Val-Pro-Leu-Phe-Pro-OH, substitution of the D-analogues for Tyr and Phe in turn gave an analogue[183] containing D-Phe which had immunoregulatory activity. Conotoxin G1(83) is a neurotoxin peptide used by the marine snail *Conus geographus* to paralyse its prey. In order to find antagonists to its action a series of analogues of [des-Glu^1]conotoxin GI have been synthesised[184] and assessed for their ability to inhibit contractions in the mouse-diaphragm-with-phrenic-nerve assay. Results showed that total loss of paralytic activity occurred when Pro was replaced by Gly, Tyr by D-Tyr, or Gly by D-Phe, and change in length or replacement of one of the disulfide links also eliminated or greatly lowered paralytic activity.

Further advances in the purification of the LH-RH receptor have benefitted[185] from the availability of photoaffinity labels incorporated into the LH-RH sequence. In this work two active LH-RH analogues [D-Lys^6]-LH-RH and [D-Lys^6,desGly^{10}]-LH-RH ethyl amide provided the ε-amino-moiety as a convenient coupling group which were further biotinylated and derivatised *via* N-hydroxysuccinimide esters. The three conjugates reported were, p-Glu-His-Trp-Ser-Tyr-D-Lys-[$N^ε$-biot-Phe(N_3)]-Leu-Arg-Pro-X (X = Gly-NH_2 or NHEt) and p-Glu-His-Trp-Ser-Tyr-D-Lys-[$N^ε$-biot-Gly$(CH_2)_2NH_2$)-Leu-Arg-ProNHEt, the former having photolability, the latter was useful as a cross-linker and showed 50% specific binding to receptors. A series of potent antagonists of LH-RH have been based on the ketoconazole partial structure[186] (84) with R ranging from OH,OPh, phthalimido, 3-indolylamino, Z-pGlu-NH, Z-pGlu-His-NH, Z-Pro-His-NH to Bu^t-Trp-Pro-His-NH. Sequences chosen from within two regions of the three disulfide loops of epidermal growth factor (EGF) have been synthesised[187] to check on their receptor binding activity but not one of the three, Ac-Cys(Acm)-Val-Ile-Gly-Tyr-Ser-Gly-Asp-Cys(Acm)-NH_2, Ac-Cys(Acm)-Met-His-Ile-Glu-Ser-Leu-Asp-Ser-Tyr-Thr-Cys(Acm)-NH_2 nor Ac – Cys – Val – Ile – Gly – Tyr – Ser – Asp – Arg – Cys – NH_2 showed receptor binding. The advantages of multiple low pressure continuous flow solid phase techniques have been used fruitfully in the concurrent synthesis[188] of a HIV antigenic decapeptide H-Val-Tyr-Tyr-Arg-Asp-Ser-Arg-Asn-Pro-Leu-NH_2 and its nine omission analogues. Solid phase techniques have also enabled[189] an analogue

of Gramicidin A to be synthesised with naphthylalanine replacing the Trp residues at positions 9, 11, 13 and 15. Ir spectroscopy indicated that the naphthylalanine residues modify the monomer-dimer equilibrium and it is concluded that conductance of the gramicidin A channel is governed by the dipole moment of the aromatic rings and not by their bulkiness. Ionophore activity has also been monitored[190] for a series of alamethicin analogues, in which the many Aib residues in the molecule have been replaced by Ala or Leu. Ir and cd studies revealed that the Ala substitution facilitated a *trans*-conformation to a β-structure whereas the Leu substitution retained α-helical character and was thus a potential ionophore. While the results for the latter analogue showed that Aib was not a requisite to observe alamethicin-like behaviour the lifetime of the single channels was shorter.

Echistatin, a 49-residue polypeptide from the venom of the saw-scaled viper, *Chris carinatus* has been synthesised[191] in 4% overall yield by a solid phase protocol. The molecule has 4 cystine bridges and these were linked in the final oxidation stage by air oxidation at pH 8. Since the peptide inhibits fibrinogen-dependent platelet aggregation ($IC_{50} = 3.3 \times 10^{-8}M$), and also prevents aggregation by thrombin, epinephrine, and collagen, the significance of the 'adhesive' sequence Arg^{24}-Gly-Asp in echistatin was also of interest. Replacement of Arg^{24} with Orn or Ala reduced the inhibitory activity confirming the importance of arginine in the binding process. Cyclosporin A, the immunosuppressive drug used for the rejection of transplanted human organs contains the unique amino acid, (4R)-4-[(E)-butenyl]-4-N-dimethyl-L-Thr(MeBmt) which is known to be critically involved in its biological activity. The incorporation of the (4S)-$MeBmt^1$ residue in cyclosporin A has been reported[192] and was shown to have only 2-4% of the immunosuppressive activity of the parent molecule. N.m.r. data revealed that the changed chirality altered the side-chain conformation of MeBmt. The (4S)-epimer was also amongst a series of $MeBmt^1$ cyclosporin A analogues investigated[193] with molecular mechanics calculations in association with X-ray and n.m.r. conformational data. The analogues studied are listed in the Table together with the broad correlation made.

Table

	Boltzmann Population (of geometrically similar conformers)	Predicted Immuno-suppressive Activity (IS)	Observed (IS)
[4(S)$MeBmt^1$]CsA	8	12	2-4
des C-4 Me, i.e. [$MeBth^1$]CsA	24	37	10-13
Extra C atom, i.e. [$MeBm_2t$]CsA	22	34	20-30
Cyclosporin CsA	64	100	100

between computational and experimental results confirming that the cyclosporin A analogues would be less active than the parent. The antiarrhythmic peptide H-Gly-Pro-Hyp-Gly-Ala-Gly-OH has been the subject[194] of a cd spectroscopic study which involves correlation with a number of analogues with Hyp replacements at position 3.

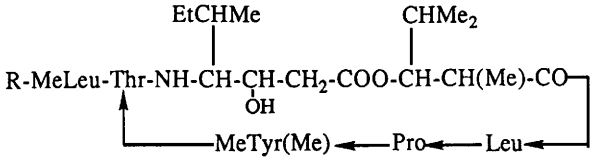
7. Conformational Information derived from Physical Methods

Reports accumulated under this sub-section again confirm the trend that detailed conformational data on small and medium-sized peptides are now readily accessed through advanced instrumental methods in both the solid and solution. Many of the experimentally determined data are also being correlated with computationally-derived energy calculations with a greater sense of confidence than was possible a few years ago. There is no record this year of a 'quantum-leap' into new technology, but the publications reflect the benefits of a 'maturing' process which annually adds a great deal of information to structure-activity relationships and receptor-binding structural requirements. Not all the peptides listed are biologically interesting but the data included in the publications could be of benefit to conformational studies.

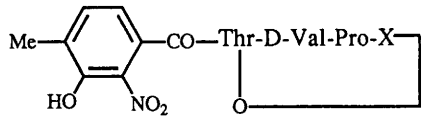
7.1 Nuclear Magnetic Resonance and Related Techniques - Studies[195] on the cyclodecapeptide antamanide can be highlighted as 'state of the art', in which the multidisciplinary approach has worked well to solve the conformation of the molecule. Homonuclear, heteronuclear 1D, 2D, proton, ^{13}C, and ^{15}N n.m.r. were used in combination with n.O.e. studies. The n.m.r. data were then used as constraints in calculations with the GROMOS molecular dynamics programme package to elucidate the most stable backbone conformations. It was discovered that there was a fast conformational equilibrium between up to 4 conformations which differ in a flip about the ϕ and ψ angles of the 2 amide bonds Ala^4-Phe^5 and Phe^9-Phe^{10}. The report also included the synthesis of γ-thiaproline residues substituted for Pro^3, Pro^7 and Pro^8 in the molecule. D-Valine-containing splenopentin *cyclo*-(Arg-Lys-Glu-D-Val-Tyr), and analogues containing an alanine residue in each position in turn and *cyclo*-(Ala_4-D-Ala) have been synthesised and analysed[196] by 2D-n.m.r. techniques. N.O.e. derived conformations were refined by molecular dynamic calculations *in vacuo* and with water. β^{II} Turns and γ-structured backbones with a D-residue in position (i+1) of the β-turn were the themes of the main conformations, while on the biological side substitution of D-Val and Glu for Ala proved detrimental. Multiple quantum proton-detected heteronuclear correlation n.m.r. techniques have been brought to bear[197] on *cyclo*-[Phe-Pro-Thr-Lys(Z)-Trp-Phe]. The presence of a *cis*-Phe-Pro bond and a β-turn

about Thr-Lys-Trp-Phe was confirmed with molecular dynamics calculations supporting a dynamic equilibrium of the backbone. Didemnin B, the most potent of a family of cyclodepsipeptides, that shows antitumour antiviral and immunosuppressive activity has been studied by two research groups. In $CDCl_3$ solution, after the usual n.m.r. data assignments[198] analysis of the ROE provided 41 constraints from which a family of closely related structures were calculated from distance geometry algorithms. Thr^6, Leu^7 and Pro^8 span a β-turn, with H-bonding between the Leu^3NH and Thr^6CO. The latter accounts for the linear portion of the molecule folding back over the depsipeptide ring. Many of the crystalline features in didemnin B were conserved in solution. The GROMOS programme and MOMO a novel personal computer-based interactive molecular graphics have also been used[199] using experimental distances as restraints. For didemnin B (85) three different conformations in the ratio (8:1:1) were discovered, and an extra H-bond was discovered between the lactoyl OH and the dimethyltyrosine O-atom. Main difference between didemnin A and B could be traced to a significant change in ψ angle of the D-MeLeu. In didemnin A the N-methyl group points inwards towards the ring. In another depsipeptide context, the pentapeptide lactone (86) of actinomycin D, distance constraints from 2D n.O.e. spectra in combination with minimum energy calculations revealed[200] two conformational forms. One conformer was all-*trans* with no H-bonds, while the other had D-Val-Pro and Pro-Sar as *cis*-bonds, the same as found previously in the crystal structure of (86) and in actinomycin D itself. The bicyclic decapeptide *cyclo*-(Glu-Leu-Pro-Gly-Ser-Ile-Pro-Ala)-*cyclo*(1γ→5β)Phe-Gly has been assessed[201] by 1D- and 2D-n.m.r. in the presence and absence of Ca^{2+} ions. Quite a compact structure stabilised by intramolecular H-bonds and turns was evident. The cyclic octapeptide *cyclo*-(L-Ala-D-Ala-L-Ada-D-Pro)$_2$ with Ada representing 2-amino-9-decenoic acid, and related to HC-toxin, did not reveal all its secrets from an n.m.r. study[202]. The peptide seemed to adopt different conformations depending on the solvent, but its ion-complexing and ion-transfer mechanism through membranes could not be adequately explained from the data. The conformations of *cyclo*(L-Leu-L-Pro)$_4$ and *cyclo*-(D-Leu-L-Pro)$_4$ as they relate to their ion-complexing/transport mechanisms have undergone n.m.r. scrutiny[203]. The latter diastereomer was found to take a C_2-symmetric conformation in $CDCl_3$, but an intramolecular conformational transformation was so rapid on the n.m.r. time scale that it appeared to take a C_4-symmetric form. The D-analogue also complexed with Ba^{2+} faster than its L-analogue as it did not require the isomerisation of bonds. Spin-lattice relaxation measurements have confirmed[204] a less mobile conformation for *cyclo*-(D-Phe-D-Pro-Ala-Pro) than larger cyclic peptides.

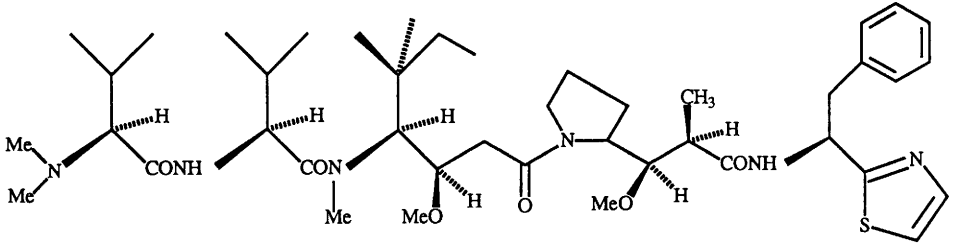
The question of whether defining the bioactive conformation of linear peptides remains such an elusive goal has been raised[205], and some of the answers from the area of opioid peptides reviewed. Receptor-selective substance P(SP)



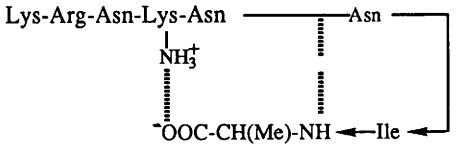
(85) R=H Didemnin A
R=$CH_3CH(OH)CO$-Pro Didemnin B



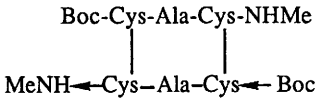
(86) X=MeVal or MeAla



(87)



(88)



(89)

analogues have been selected[206] for n.m.r. studies in d_6DMSO. Ac[Arg^6,Pro^9]SP(6-11) (selective for NK-1(SP-P) receptors) and [pGlu^6,MePhe^8]SP(6-11) (selectively activates NK-3(SP-N) receptors), showed differences in their conformation, the former had a *trans*-Phe^8-Pro^9 amide bond and a preferred type I β-turn, while the latter existed as a *cis-trans* mixture in which the *cis*-form adopted a type VI β-turn and the *trans* adopted a γ-turn. All the resonances in bombesin (pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2) and two fragments have been assigned[207] at 500MHz in d_6DMSO. In highly viscous mixtures of d_6DMSO/water, the existence of sequential n.O.e. effects, suggested some secondary structuring in the 6-13 C-terminal region. The C-terminal synthetic nonapeptide is in fact as biologically active as bombesin itself. Of special interest to other parts of this Chapter as well, are the comparisons reported[208] from simple model compounds, using n.m.r., i.r. and X-ray studies on the effect of pseudo-analogues on the conformation. It is concluded that (i) depsides, retro-amide, and 'reduced' amides rigidify the backbone inducing β-turns, (ii) C^{α}=C^{β} has little effect on geometry of the amide group, but the defined orientation of side-chain can alleviate steric hindrance hampering β-folding, and (iii) modifications such as elongation (β-Ala) or substitution of a hydrazino group result in more 'open' conformations.

The synthesis of (-) dolastatin 10 has been aided significantly by 400MHz data[209] which assisted in designating (87) as the absolute configuration. N.m.r. techniques have been used[210] extensively to rationalise the conformation of the C-terminal octapeptide of oxyntomodulin. Schematic diagram (88) summarises the conclusions that the ionisation state of the C-terminal COOH determines the conformation. Neurokinin B(NKB), H-Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-MetNH_2 and potent analogues formed by N-terminal changes and substitution at 2, 5 and 7 have been compared[211] by ^{1}H n.m.r. in d_6DMSO and MeOH. Postulated conformational features for potency at the NK-3 receptors, were (i) an extended structure over the 4 C-terminal residues, (ii) a C_7-orientation for Phe^6 and (iii) either an α-helical structure over residues 2 to 6 or a β-turn for 3 to 6. Changes in conformation due to pH changes in DMSO solution have been monitored[212] by a 2D-n.m.r. study of physalaemin. The peptide at pH 3.5 showed a random coil structure, but at pH 7.0 a folded structure around the charged Asp and Lys residues was revealed with a βI turn having Pro and Asn at the i+1 and i+2 positions. Changeover seemed to occur at pH 3.8. Mouse epidermal growth factor (mEGF) together with fragment sequences, e.g., mEGF(1-48) and mEGF(1-45), have been characterised[213]. Controlled proteolysis was followed by n.m.r. and the influence of the carboxy terminal sequences were also monitored. In the model glycopeptide of antifreeze glycoprotein, Ac-Thr-(α-GalNAc)-Ala-Ala-OMe the usual n.m.r. techniques strongly suggested[214] a H-bond between the GalNAc

amide proton and the ThrCO moiety which reduces the mobility of the sugar residue. A 2D, ^{1}H n.m.r. study[215] on the 1-34 fragment of human parathyroid hormone at 500MHz has provided a complete assignment of resonances but n.O.e. experiments failed to detect any secondary structural elements. The Pro^2 analogue (H-Arg-Pro-Asp-Val-Tyr-OH)of thymopentin has been analysed[216] by n.m.r. and in a study[217] of [D-Ala^2]-enkephalin in its zwitterionic form in solution at 500MHz, the data have been rationalised in terms of a predominance of two γ-bends, one around D-Ala the other around Phe. Synthesis of [Pro^3]-antiarrhythmic peptide (H-Gly-Pro-Pro-Gly-Ala-Gly-OH) has stimulated a c.d. and n.m.r. study[218] of its conformation. Both techniques confirmed that in solution the analogue exists predominantly in a polyproline II-like structure with no intramolecular H-bonding although in MeOH and CF_3CH_2OH there was a hint of a γ-turn found in c.d. spectra. Morphiceptin (H-Tyr-Pro-Phe-Pro-NH_2) and an analogue, H-Tyr-Pro-MePhe-D-Pro-NH_2 exist in solution[219] both as the all-*trans* form (55% and 65%, respectively) and a *cis*-form around the Tyr-Pro amide bond. Although other isomers are possible they could not be assigned using n.m.r. because of overlapping resonances. A receptor specific tachykinin analogue, senktide, succinyl-Asp-Phe-MePhe-Gly-Leu-Met-NH_2 showed up[220] as a distorted antiparallel H-bonded β-pleated sheet in n.m.r. correlations and in c.d. studies.

Precise information about exchanges in water of amide NH's in the insulin B23-B29 heptapeptide H-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-OH has indicated[221] a highly solvated structure around Phe^{24}, Phe^{25} and Tyr^{26}, a H-bond involving the Lys^{29}NH proton and a N-terminal NH_2 to C-terminal carboxylate salt bridge. Leu-Enkephalin, ^{17}O-enriched in Gly^2 and Gly^3, showed[222] ^{17}O-n.m.r. shifts in H_2O which were almost identical and independent of pH, thus suggesting that the hydration state of both oxygens is identical and pH independent. Evidence also showed that in CH_3CN/DMSO solutions Leu-enkephalin exists in the neutral form at pH 5-6, and that no specific 2→5 H-bond exists to an appreciable extent. The sensitivity of ^{17}O-chemical shifts to β-turn structure has been substantiated[223] from studies on [^{17}O]Ac-L-Pro-D-AlaNHMe and [^{17}O]Ac-L-ProOMe, both 40% enriched at the Ac-oxygen. Two distinct ^{17}O-resonances were also seen due to *cis-trans* Ac-Pro isomerism, so for small to medium sized peptides which can be selectively enriched the technique has potential. A complete ^{13}C-study and assignment has been reported[224] for tryptophan and H-Lys-Trp-Lys-OH.

7.2 X-Ray Crystallography - An authoratitative short review[225] on X-ray studies on peptides in the 10-16 residue range carried out by the reviewer in recent years is of interest to the subject matter of this Chapter. Although the symmetric perhydro analogue *cyclo*-(Val-Pro-Pro-Cha-Cha-Val-Pro-Pro-Cha-Cha) of

antamanide is biologically inactive, its crystal conformation was found[226] to be very similar to the active parent molecule. It is only when the conformation of their metal ion complexes were analysed that dramatic differences were seen, thus suggesting that the uncomplexed molecule is not the physiologically active form. X-ray data have been assessed[227] for a newly synthesised analogue of amaninamide, S-deoxy-[γ-(R)HOIle]-amaninamide, which has a γ-hydroxyisoleucine included for comparison with γ-amanitin. The X-ray data showed the OH group had the R-configuration, but since the activity of the molecule was not enhanced from that of the non-toxic amanullin it is very likely that the (S)-configuration is a prerequisite for maximal toxicity. Crystal structures of small fragments of angiotensinogen have been further investigated[228] as a means of augmenting the hypotheses for the renin substrate bioactive conformation. Conformational features revealed by an X-ray study[229] of Leu-enkephalin trihydrate, include a tightly folded conformation with two fused βIII(Gly^2-Gly^3) and βI(Gly^3-Phe^4) turns, with the Tyr^1, Phe^4 aromatic rings in an arrangement analogous to the tyramine/cyclohexenyl rings in morphine. This trihydrate conformation may therefore be a recognisable form at μ-receptor sites. The structure of the chemotactic peptide OHC-L-Met-L-Leu-L-PheOMe has been refined[230] to an R of 0.068. The backbone is folded at Leu without intramolecular bonds, and overall the structure differs from others proposed on the basis of other spectral data. The X-ray structure does however account for the amphiphilic behaviour of the peptide. An X-ray report[231] on the partial structure OHC-L-Met-L-Phe grown from aq MeOH also differs from a D-D structure previously reported. The L-L-form has parallel β-sheets rather than antiparallel ones. *Cis-cis-trans* amide conformations were found[232] in the crystal structure of *cyclo*-(β-Ala-L-Phe-L-Pro), while the structure revealed[233] for Bu^tCO-Alaψ[NHCO]Gly-$NHPr^i$ was an open conformation in contrast to the βII-folded form of the parent dipeptide. Similarities between the X-ray crystal structure[234] of (89) and data from solution confirm an antiparallel β-sheet conformation. Papers published on the following simple peptides also contain details to augment data from larger molecules. Thus X-ray data on Boc-Pro-Cys-NHMe[235], H-L-Phe-Gly-Gly-OH[236], H-L-Lys-L-Glu-OH[237], H-Gly-Gly-Sar-OH[238], L-pGlu-L-Ala-OH[239] and H-L-Pro-L-Ile-OH.H_2O[240] have been reported.

7.3 Circular Dichroism/Theoretical and Computational Methods - The development of multidisciplinary approaches to peptide conformation has meant that techniques covered in this sub-section have become subsumed into other parts of the Chapter. In a study on sequential polypeptides $(Arg\text{-}X\text{-}Gly)_n$ with X representative of Ala, Val, Leu, Ile, Nva or Nle circular dichroism however was the only technique of choice[241] to record the conformational behaviour in the

presence of increasing amounts of sodium dodecyl sulphate and dodecylphosphoryl choline. An equilibrium between α-helix, β-turn and random coil conformations was present in all examples with the side-chains of Ile and Nle favouring α-helical form rather more than their Val and Nva analogues. C.d., augmented by dynamic fluorescence, and n.m.r. have been utilised[242] to monitor changes in the conformation of the tetradecapeptide bombesin in buffer and with lysolecithin micelles. In buffer bombesin showed no ordered secondary structure, but a marked change in the c.d. spectra was observed in changing from buffer to lipid suspension, believed to be due to ordering of the molecules in the lipid phase.

The ECEPP (Empirical conformational Energy Programme for Peptides) has been applied[243] to seek out low-energy conformations of tetrapeptides which are components of larger peptides known to bind to CD-4 the receptor of monocytes. Tetrapeptides analysed included Ser-Ser-Asn-Tyr (from ribonuclease A), Thr-Thr-Asn-Tyr (from peptide T), Thr-Ile-Asn-Tyr (from polio virus coat protein), Ser-Ser-Ala-Tyr (from gp 120 coat protein of HIV) and synthetic peptide Asn-Thr-Lys-Thr. Using a 7kcal/mol cut-off approximately 20,000 conformations were computed for each tetrapeptide. It was found that the four most active tetrapeptides were 5-10 times as likely of being superimposable on the native structure of the 22-25 segment of ribonuclease A as the less active polio peptide. The ECEPP calculations on a model elastin tetrapeptide Ac-Val-Pro-Gly-Gly-NHM, show[244] the backbone bend to have high probability in the *trans*-form at the Pro-Gly and Gly-Gly portions which is in agreement with the tendency for a type II β-bend at Pro-Gly found experimentally. The relative merits of distance geometry (DG) and restrained molecular dynamics (MD) for deriving molecular conformation from atom-atom distance information from 2D-n.m.r. and X-ray structures have been assessed[245] for cyclosporin A. Theoretical and experimental data have been compared[246] also for the eosinophil chemotactic peptide analogue Val-Gly-Ala-Glu. Computed conformations were described as an ensemble of structures with the N- and C-terminii in close proximity. The charged state showed a marked effect on the calculations, with the ones assuming a dianionic form showing good agreement with n.m.r. However, the type I β-turn suggested from n.m.r. data was calculated to have a relatively high energy due to repulsions between the amide nitrogens Ala^3/Glu^4 and predicted to be an unlikely solution conformation. Experimental *cis-trans* isomer ratios in Pro-Pro-Pro in different ionisation states could not be predicted[247] using ECEPP calculations, probably due to the potential functions overestimating the electrostatic effects. N.m.r. relaxation experiments on cyclosporin A have provided the ^{13}C-relaxation times and n.O.e. parameters for a model-independent and model-dependent analysis[248] to describe the motions at 39 carbon atoms in the molecule. These confirmed the *trans*-geometry and the involvement of the CO associated with the NMe group in H-bonds. Conformational

energy analysis on chemotactic tripeptide HCO-Met-Leu-Phe, its analogues HCO-Ala-Leu-Phe, HCO-Met-Phe, and H-Met-Leu-Phe revealed[249] folded or puckered conformations in all four. The highly conserved sequence in thionins and viscotoxins, Ac-Arg-Asn-Cys-Tyr-Asn-NMA has also been analysed[250] and the results show the most likely conformation to be an amphipathic α-helix, with Tyr and Cys on the non-polar side and Arg and the two Asn on the polar side. An n.m.r. determination for this pentapeptide sequence as it exists in α1-purothionin agrees with this theoretical prediction.

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4
Cyclic, Modified, and Conjugated Peptides

BY P. M. HARDY

1 Introduction

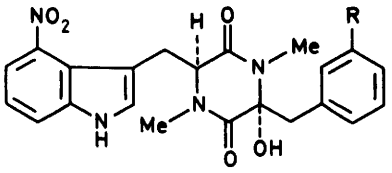
The subdivision of this chapter remains as last year except that cyclic hepta- and octapeptides are given a separate section. After taking over this chapter in volume 13 in 1980 (then entitled 'Peptides with structural features not typical of proteins,' although the coverage was the same), I first started vowing that this was positively my last report in about 1986. Having finally made the break in earnest, I note that my predecessor as author of this chapter, Barrie Bycroft, lasted eight years in the job, and I have now managed to pip him with ten. I only hope that SPR7 continues long enough for my successor to keep up the trend with twelve reports. Volume 13 had 137 references, whereas this report has 322 references. Clearly cyclic, modified and conjugate peptides are not lagging behind other areas of peptides in research effort expended.

2. Cyclic Peptides

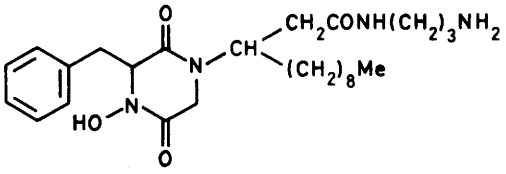
2.1. Naturally Occurring Dioxopiperazines (Cyclic Dipeptides)

– Two phytotoxins, thaxtomins A (1a) and B (1b), which induce scab-like lesions on immature potato tubers have been isolated from field grown and aseptically cultured potato tubers that had been infected by *Streptomyces scabies*. The synthetic analogue *cyclo*(Trp-Phe) is inactive, but its derivative *cyclo*(4-nitro Trp-Phe) shows thaxtomin-like activity. A new diketopiperazine hydroxamate derivative etzionin (2) has been obtained from an unidentified Red Sea tunicate. This has an unusual β-amino-dodecanamide side-chain, and has both cytotoxic and antifungal activity.[2]

Three recent papers concern cyclic dipeptides containing αβ-unsaturated amino acids. Megasporizine (3) has been extracted from *Penicillium megasporum*, one of the rarer penicillium species which nevertheless has a worldwide distribution.[3] X-ray studies have been made of three isomers of



(1) a; R = OH
b; R = H



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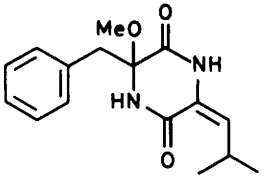
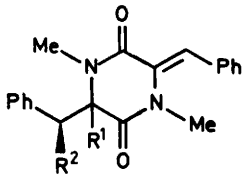
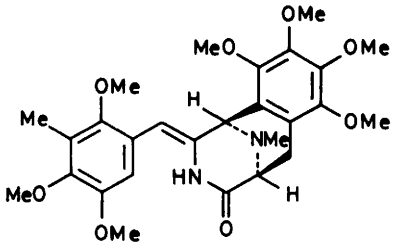


(3)



	R^1	R^2
a;	*threo* – OMe	H
b;	*erythro* – H	OMe
c;	*threo* – H	OMe
d;	*erythro* – OH	Br
e;	*threo* – OH	Br

(4)

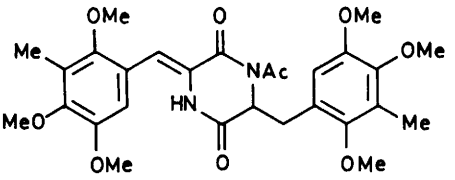


(5)

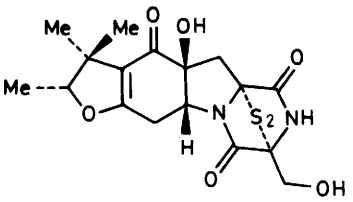
the lichen metabolite methylanhydropicroccelin (4a,b, and c) and two related bromohydrins (4d and e). In general the observed conformers are those with hydrogen extended towards the carbonyl group as far as the C^{α}–C^{β} bond in the benzyl side chain is concerned, but the erythro-bromohydrin is an exception for which there seems no clear steric reason. The tricyclic lactam (5) has been synthesised from the dioxopiperazine (6). The former was made as a step in the synthesis of the antitumour antibiotic saframycin.[5]

Several other publications also concern fused ring cyclic dipeptides. In the simplest of these, cyclo(trans-4-Hypro-Phe) has been isolated from a Jaspidae sponge from Fiji.[6] The fused polycycle phomalirazine (7) is a novel toxin from the phytopathogenic fungus Phoma lingam. It is active at 10^{-5} M in a cotyledon assay, whereas its co-metabolite sirodesmin PL (8), of which it is a likely precursor, is only active at 2×10^{-4} M.[7] Another novel epidithiodioxopiperazine, derived from the fungus Emericella heterothallica, has been named emethallicin A (9a). It is a potent inhibitor of compound 48/80-induced histamine release from mast cells and of 5-lipoxygenase.[8] The same source has also yielded emethallicins B (9b) and C (10), epitetrathiodioxopiperazines with similar biological activity.[9]

Fructigenines A (11a) and B (11b) have been isolated from Penicillium fructigenum. The former has growth inhibitory activity against Avena coleoptile and L-5178Y cells. These compounds are related to roquefortine, and do yield free tryptophan on acid hydrolysis.[10] The synthesis of 12α-and 12β-fumitremorgin C (12) have been reported. A comparison of their spectral data with those reported for natural fumitremorgin C showed greater similarity in the case of the 12α-isomer. However, complete identification was not possible as natural material was not available and no specific rotation has been documented.[11] The reaction of L-tryptophan methyl ester with butynone gives a cis-1,3-disubstituted-tetrahydro-β-carboline (13) which has been elaborated in six steps to demethoxy-fumitremorgin C.[12] Other synthetic studies on the fumitremorgins have also been described.[13]



(6)



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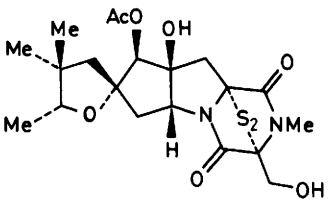

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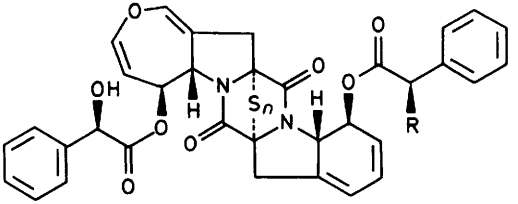
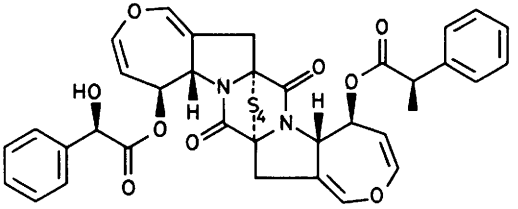


(9) **a**; n = 2, R = H
b; n = 4, R = H



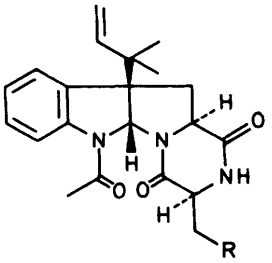
(10)

Further work on the biogenesis of the brevianamides has been carried out. A proposed intermediate (13) was prepared, but it does not autoxidise to either brevianamide A (14) or brevianamide B (15). Oxidation of (13) with m-CPBA followed by exposure of the incipient hydroxyindolenine to sodium methoxide does give a high yield of (15). Thus, unlike deoxybrevianamide A (16), which autoxidises to brevianamide B, the natural oxidation of (13) would seem to implicate a specific enzyme-mediated process.[14] The reactivity of bicyclomycin with eight amines has been examined. Methylamine, ethylamine, imidazole, benzimidazole, and N^{α}-benzoylhistidine amide all lead to ring-opened products. The secondary amines morpholine, ethyl piperazinecarboxylate, and N-methylpiperazine form adducts (17).[15]

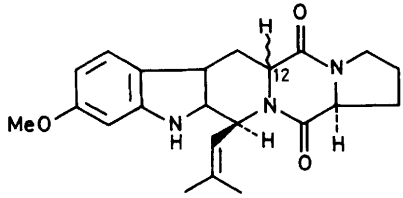
2.2 Other Dioxopiperazines – 1-Hydroxypiperazine-2,5-diones can be prepared in good yields by allowing N-benzyloxydipeptide esters (18) to cyclise and then deprotecting by catalytic hydrogenation.[16] Stereoselective syntheses of ornithine, 2,4-diaminopentanoic acid, and 2,4-diamino-6-methylheptanoic acid have been developed by the condensation of cyclo(Ac-Gly-Ac-L- or D-Ala) with the appropriate aldehyde in the presence of potassium t-butoxide with subsequent catalytic hydrogenation and acid hydrolysis.[17]. Only one application of the bis-lactim ether method has been reported this year; this involves the asymmetric synthesis of (2R,3S)-threo-3-arylserine.[18]

On reaction of Boc-Pro-OH with methyl 2-amino-2-deoxy-D-gluconate by the mixed anhydride method or via other activated derivatives, instead of the expected glycopeptide, (3R,6R)-bis[(1R,2S,3R)-tetrahydroxybutyl)]-2,5-dioxopiperazine was isolated in excellent yield. It is suggested that 2D networks of H-bonded molecules are present in solution, and these assist in fixing the orientations of the adjacent molecules, which promotes formation of the cyclic dipeptide.[19]

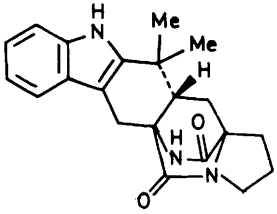
X-ray analysis of cyclo$(Ac_3c)_2$, where Ac_3C = 1-aminocyclopropane carboxylic acid, shows the six-membered ring to be almost planar. A similar examination of H-$(Ac_3c)_2$-OMe shows the N-terminal Ac_3c residue to adopt a novel type of C_5



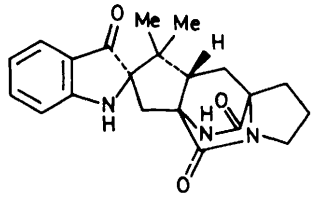
(11) **a**; R = Ph
b; R = $CHMe_2$



(12)



(13)



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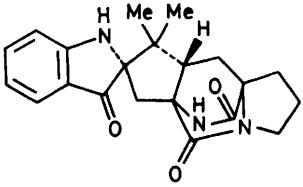

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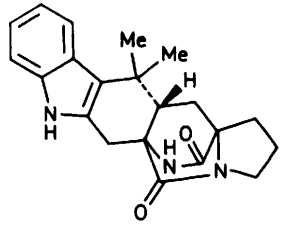

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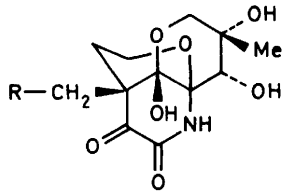
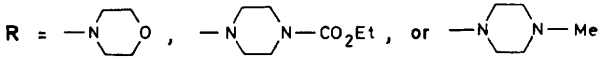


R = 

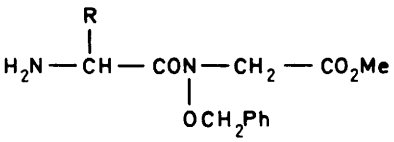
(17)

conformation.[20] Cyclo[Phe(Tos)-D-Phe] and cyclo[Phe(Tos)-D-Pro] have been prepared. The crystal structure of the former shows that the N-tosyl group alters the geometry of the cyclodipeptide ring by lengthening both the N-C bonds departing from the tosylated nitrogen and reducing the corresponding ring angle.[21] An X-ray analysis of cyclo(N-hydroxyGly-Ala) has also appeared.[22]

The complexation of cyclo(Pro^{17}O-Gly^{15}N) and cyclo(Gly^{17}O-Pro) with Co(II) ions has been studied by ^{17}O, ^{15}N and ^{14}N-n.m.r. spectroscopy in aqueous solution. The ^{17}O studies unequivocally show that the cobaltous ion binds to the peptide oxygen of both compounds. The ^{14}N and ^{15}N results do not indicate binding at either the Gly^{15}N or the Pro^{15}N sites.[23] Cyclo(Glu_2), cyclo{Glu-Glu(OMe)} and cyclo{Glu(OBzl)-Glu(OMe)} have been prepared,[24] and the lifetimes of radiolytically generated cyclic dehydrodipeptides (19) in aqueous solution have been studied by pulse radiolysis.[25]

2.3 Cyclic Tripeptides – Cyclo(β-Ala-Phe-Pro) has been made in 32% yield by a p-nitrophenyl ester cyclisation. X-ray work shows the backbone to resemble cyclo(Hyb-Phe-Pro) (Hyb = S-3-hydroxybutyric acid) in that it has a cis-cis-trans backbone conformation, whereas cyclo(MeAnt-Phe-Pro) (MeAnt = N-methylanthranilic acid) has an all cis backbone.[26] On treatment of Z-MeTau-Phe-D-Pro-OH with acetic anhydride and sodium acetate, (20) is formed in 90% yield. Catalytic hydrogenation of (20) gives cyclo(MeTau-Phe-D-Pro) via an amino-acyl insertion reaction after loss of the carbobenzoxy group. The crystal structure of this cyclic tripeptide analogue shows trans Phe-D-Pro and cis D-Pro-MeTau peptide bonds, with the SO_2NH adopting a cisoidal conformation.[27]

The acid ionisation constants of the series H-Cys-Gly_n-Cys-OH (n = 0 to 4) have been measured. There is first an upward and then a downward trend of the pK as the ring size increases. This is tentatively attributed to intramolecular salt bridge formation when $n \geqslant 1$.[28]



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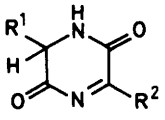

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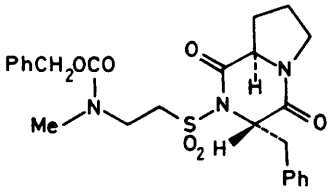

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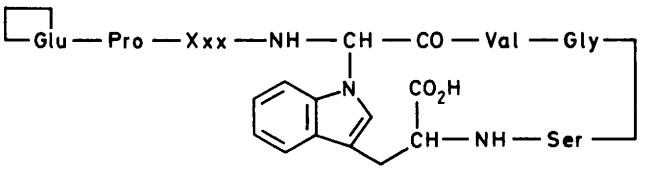

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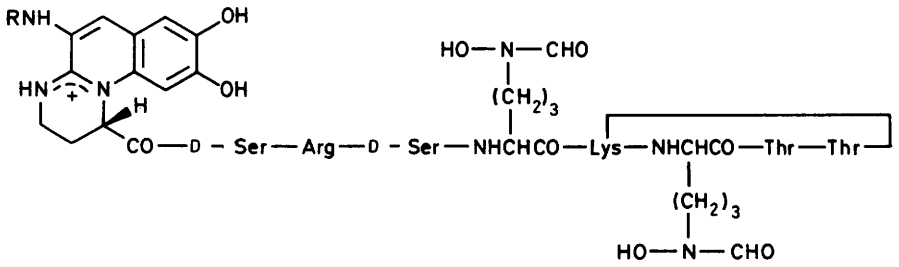


a; R = $-COCH_2CH_2COCO_2H$
b; R = $-COCH_2CH_2CO_2H$
c; R = $-COCH_2CH_2CONH_2$

(22)

2.4 Cyclic Tetrapeptides – Not surprisingly, most of the homodetic examples reported are as usual rich in proline. Cyclo{Glu(OMe)-Pro}$_2$ and cyclo(Glu-Pro)$_2$ complex with Ca(II) and Ba(II) ions without the participation of side chains. In the case of cyclo(Glu-Pro)$_2$, in the absence of metal ions intramolecular interaction of carboxyl groups was observed.[24] The crystal structure of cyclo(Pro-Gly)$_2$·3H$_2$O shows the Pro-Gly peptide bonds to be trans and the Gly-Pro bonds to be cis. The two independent copies of the cyclotetrapeptide found in the asymmetric unit have similar structures, both of which are consistent with the results of n.m.r. studies in solution.[29]

Measurements of spin-lattice relaxation in the rotating frame indicate that cyclo(D-Phe-D-Pro-Ala-Pro) is conformationally less mobile on the microsecond time scale than larger cyclic peptides previously studied.[30] Cyclo(D-Pro-Pro-D-Pro-Pro) has been prepared in 85% yield by a diphenylphosphoryl azide cyclisation. The product is a mixture of the two possible cis, trans, cis, trans stereoisomers, which are enantiomers. The mixture crystallises in enantiomerically pure separate crystals. This is the first example of a racemic peptide formed from one enantiomer not by racemisation, but by cis/trans isomerisation of peptide bonds.[31]

A simple and clear correlation between the ring conformation of cyclic tetrapeptides and their configurational sequences has been suggested. When there is a cis, trans, cis, trans backbone, and the neighbouring amino acids have the same configuration (i.e. LL or DD), then the two carbonyl oxygens acylating the amino acids are oriented on opposite (i.e. LD or DL) sides of the ring plane. When the configurations of the neighbouring amino acids are opposite, the carbonyl groups acylating these components are oriented on the same side of the ring.[32]

Analogues of H-Tyr-cyclo(N^{ε}-D-Lys-Gly-Phe-Leu) have been made containing one (Gly-Phe), two (Gly-Phe- and Phe-Leu), and all three amide bonds replaced by thioamide links. As the number of sulphur atoms increased, the compounds showed lower activity towards guinea-pig ileum, but increasing δ-selectivity. However, on binding assays, the mono- and di-thio analogues were

more μ-selective.[33] A series of cyclic analogues of CCK_{22-33} of the type Boc-Xxx-Tyr(R)-Nle-D-Lys-Trp-Nle-Asp-Phe-NH_2, where Xxx = Gly, D-Glu, or γ-D-Glu and R = H or SO_3H, have been prepared. All displayed low affinities for pancreatic receptors, but high affinities for central-type binding sites. The compound where Xxx - δ-D-Glu and R = SO_3H was the most potent (K_I = 0.56 nM) and selective for the central-type CCK receptors of the guinea pig.[34] A ^{1}H-n.m.r. conformational study of the three isomers where R = SO_3H ascribes an increase in affinity on going from Glu to δ-D-Glu to several factors.[35]

Three out of eight compounds of a series of [14]-membered ring cyclic peptides show cis-peptide bond conformers; H-Tyr-cyclo(D-A_2bu-Gly-D-Nal(1)-Leu) contains some D-A_2bu-Gly cis, H-Tyr-cyclo(D-A_2bu-Phe-gPhe-mLeu) contains some cis Phe-gPhe and mLeu-D-A_2bu, and H-Tyr-cyclo(D-Gly-Phe-gPhe-D-retro-Leu) some cis D-Gly-Phe and Phe-gPhe peptide bonds.[36]

Of another series of cyclic LH-RH antagonists, the most potent compound proved to be Ac-D-Phe(pCl)$_2$-D-Trp-Ser-Glu-D-Arg-Leu-Lys-Pro-D-Ala-NH_2, with an ED_{50} of 91.9 μg/kg in inhibition of ovulation. The corresponding linear peptide is only about one-third as potent.[37] Two novel cyclotetrapeptides have been isolated from the root bark of Lycium chinense, the source of an antifebrile oriental crude drug. These compounds, lyclumins A(21; Xxx = Tyr) and B(21; Xxx = Trp), which are renin and ACE inhibitors, have a novel link between the indole N^1 of Trp and the C^α of Gly.[38] The structures have been determined of pyoverdins C, D, and E (22a, b, and c) from Pseudomonas aeruginosa; they are peptidic siderophores obtained when the bacterium is grown in an iron-deficient medium. They are in fact identical with pyoverdins PaB, PaA, and Pa earlier described.[39]

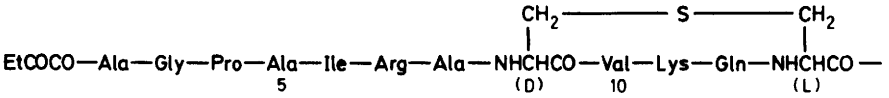
Two cyclic disulphides, H-Tyr-D-Pen-Gly-pN_3Phe-D-Pen-OH and H-D-Phe-Cys-pN_3Phe-D-Tyr-Lys-Thr-Pen-Thr·NH_2 have been evaluated. The former is δ-selective and the latter μ-selective. These conformationally constrained photoaffinity peptides may be useful tools to investigate the pharmacology of opioid receptors.[40] A cystine-bridged cyclopeptide, Ac-Cys-Pro-D-Val-Cys-NH_2 has been prepared by a solid phase synthesis, and

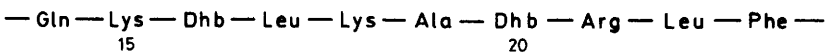
shown to adopt a type II β-turn. The S-S link was formed while the peptide was still attached to the polymer support. Treatment of the linear peptide containing two S-acetamidomethyl groups with thallium trifluoroacetate gave 94% of cyclic monomer, whereas oxidation with iodine gave only 52%.[41] Conformational analyses of H-Tyr-D-Pen-Gly-Phe-D-Pen-OH and H-Tyr-D-Cys-Gly-Phe-D-Cys-OH have been performed using AMBER and RNGCFM molecular mechanics programmes.[42]

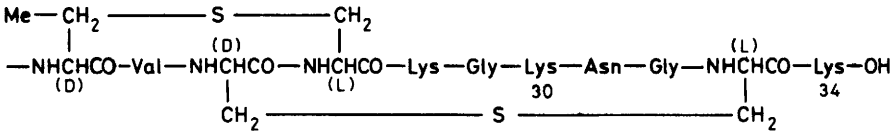
Of a series of cyclic enkephalin analogues containing α-amino-β-mercapto-β,β-pentamethylene-propionic acid (Apmp), the most potent were H-Tyr-D-Cys-Gly-Phe-L- or D-Apmp-OH. While displaying less δ-receptor selectivity than the corresponding penicillamine-containing peptides, they are an order of magnitude more potent.[43]

A new lantibiotic, Pep 5 (23) from Staphylococcus epidermidis, shows no homology to any of the eight compounds of this type so far known. The 2-oxobutyryl residue at the N-terminus originating from threonine of the prelantibiotic is counted as position 1. It contains sulphide rings involving five and eight amino acids as well as four amino acids.[44] Using 2D techniques, two groups have assigned the complete ^{1}H-n.m.r. spectrum of nisin. This both confirms the structure proposed on the basis of chemical degradation studies,[45] and indicates the molecule to adopt a well-defined 3D structure.[46]

Two degradation products of nisin formed on storage or by acid treatment have now been identified as (des-ΔAla^{33}, ΔAla^{33}-Lys^{34}; Ile^4-NH_2, pyruvoyl-Leu^6, Val^{32}-NH_2)-nisin and (desΔAla^{33}-Lys^{34}; Val^{32}-NH_2)-nisin. The former is biologically inactive, but the latter has comparable antimicrobial activity to nisin itself.[47] The conformations of two model compounds of ring A and a derivative of ring B of nisin have been examined by interactive n.m.r and computer simulation studies. The cyclotetrapeptide (24) has limited and specific conformational preferences, with indications of the presence of two β-turns. In the case of the cyclopentapeptides (25), the L-Ala isomer has three intramolecular H-bonds but the more flexible D-Ala isomer has none.[48]







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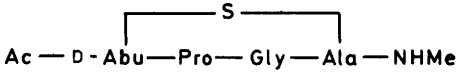

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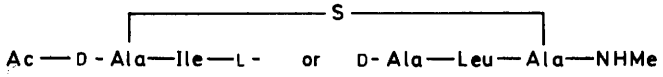

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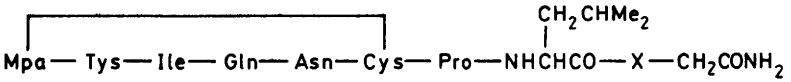
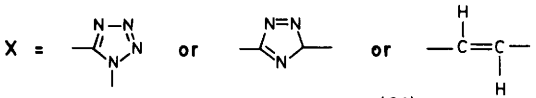


Mpa = 3-mercaptopropionic acid



(26)

2.5 Cyclic Pentapeptides – There are only three papers on this topic to report. Six cyclic analogues of splenopentin, the 32-36 sequence of the [49]-peptide thymopoietin III (H-Arg-Lys-Glu-Val-Tyr-OH), have been synthesised. These compounds comprise cyclo(Arg-Lys-Glu-D-Val-Tyr) and derivatives in which each amino acid in turn has been replaced by alanine (of the same configuration). As a model cyclo(Ala_4-D-Ala) was also prepared. The conformations in solution show $\beta II'\gamma$-structural backbones with the D-amino acid in the i + 1 position of the β-turn. The biological activity is strongly reduced by substituting Ala for D-Val or Glu.[49]

An E.I-m.s. study of four cyclic pentapeptides containing Gly, Pro, and Ala indicates that initial cleavage occurs at the N-C_α bond following the most non-planar peptide bond. Once the ring is opened, further fragmentation appears to follow the decomposition mechanisms observed for linear peptides.[50]

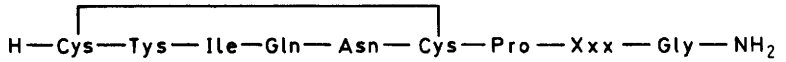
2.6 Cyclic Hexapeptides – A novel cyclohexapeptide has been extracted from Clerodendrum myricoides collected in Zaire, previously a source of macrocyclic spermidine alkaloids. Named cleromyrine 1, cyclo(Ala-Gly-Pro-Ile-Val-Phe) is one of the few naturally occuring homodetic cyclopeptides built exclusively of L-amino acids.[51]

Five cyclic hexapeptides containing repeated tripeptide units have been examined as ion complexing agents. Cyclo(D-Asp-D-Phe-Pro)$_2$ forms complexes with Ba(II) and Ca(II) ions, but cyclo(Asp-Phe-Pro)$_2$ and cyclo(Asp-Phe-Pro)$_2$ (where Asp = α-aminoadipic acid) do not.[52] Both cyclo(Leu-Phe-Pro)$_2$ and cyclo{Cys(Acm)-Phe-Pro}$_2$ adopt a C_2-symmetric conformation containing cis Phe-Pro bonds in chloroform and acetonitrile. Both also complex selectively with Ba(II) and Ca(II) ions in acetonitrile, the cysteine peptide having the higher binding constant.[53] Calculations of the relative energies of solvation of Ca(II) and Mg(II) ions in complexes with cyclo(Pro-Gly)$_3$, which binds calcium with an affinity comparable to those of naturally occurring proteins, show that the ion selectivity of the peptide resides in the difference of the solvation energies of the competing ions in water.[54]

Cyclo(Val-D-Val)$_3$·6TFA and cyclo(Phe-D-Phe)$_3$.8TFA have been prepared as analogues of enniatin and beauvericin. Their crystal structures show similar core diameters of about 7.0 Å.[55] Cyclo(D-Pro-Phe-Thr-Phe-Trp-Phe) has been made as a close analogue of the peptide '008' which inhibits the uptake of cholic acid by hepatocytes. A crude conformational model has been deduced for this compound on the basis of n.m.r. data, then refined by restrained molecular dynamics calculation. To mimic the solvent, the charges of the solvent-exposed NH protons were gradually reduced according to the temperature gradients. The structure thus obtained shows a close similarity to X-ray results. The main difference is the breaking of an intermolecular H bond of the Thr OH group on dissolution of the crystal and the formation of an intramolecular H bond. [56]

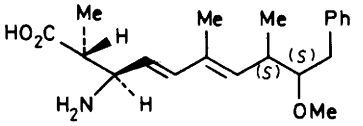
Laser desorption F.T.-m.s., a method that uses both positive and negative ion mass spectra obtained with collision-activated dissociation, has been developed for oligopeptide analysis. By examining the relationship between positive and negative ion spectra it is possible to distinguish linear from cyclic peptides. The spectra of cyclo(Lys-Thr-Phe-Pro-Trp-Tyr) and gramicidin S are cited as examples.[57]

A series of cyclic lactam analogues of α-melanotropin based on Ac[Nle^4, Xxx^5, D-Phe^7, Yyy^{10}, Gly^{11}]-α-MSH$_{4-13}$-NH$_2$ and Ac-[Nle^4, Xxx^5, D-Phe^7, Yyy^{10}]-α-MSH$_{4-10}$-NH$_2$ have been prepared, where Xxx = Glu or Asp and Yyy = Lys, Orn, Dab, or Dpr. The most potent compounds were obtained when Xxx = Asp and Yyy = Lys, being equipotent to α-MSH in the frog skin assay and 90-100 times more potent in the lizard skin bioassay.[58] Oxytocin analogues containing a pseudo Leu-Gly bond of defined cis or trans configuration have been made (26). The highest uterotonic activity (24% of that of the parent) was seen in the trans alkene. All three analogues showed a low but prolonged galactogenic activity.[59] The effect of non-coded amino acids in position 8 on the degradation of oxytocin analogues with α-chymotrypsin has been examined. These analogues, tabulated in (27), are cleaved only at the Tyr-Ile bond, whereas in oxytocin itself cleavage takes place at the Leu-Gly bond.[60]



Xxx = neo-pentylglycine
tert-leucine
cycloleucine
N-methyl-leucine
or —LeuΨ(CH_2S)

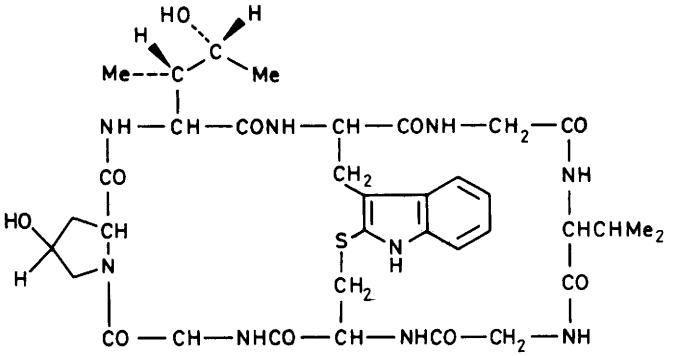
(27)



(28)

Table 1

	linear peptide concn. (mM)	ISOLATION YIELD % *metal ions* *present*	*absent*
cyclo [Gly_3Lys(Z)Lys(Z)GlyPhe$_2$]	0·6	72	58
cyclo [Sar_3Lys(Z)Lys(Z)GlyPhe$_2$]	1·2	41	14
cyclo [Sar_3Glu(OBzl)Glu(OBzl)GlyPhe$_2$]	1·2	46	15



(29)

2.7 Cyclic Hepta and Octapeptides – The absolute configuration at the 8- and 9-positions of Adda (28), a component of the cyclic heptapeptide cyanoviridin, have been determined; both are S. [61] The template effect of alkaline metal ions in promoting the cyclisation of linear peptides has been further examined. In the preparation of three cyclo-octapeptides, the cyclisation yields (using a carbodiimide/1-hydroxybenzotriazole method) were significantly increased in the presence of ten equivalents of an equimolar mixture of LiCl, NaCl, KCl, and CsCl (Table 1).[62]

Cyclo(D-Leu-Pro)$_4$ has been prepared and found to complex selectively with Ba(II) ions with a binding constant similar to that of cyclo(Leu-Pro)$_4$. However, the rate of complexation is much faster than that of the all-L compound as no isomerisation of peptide bonds is required for binding. The efficiency of cation transport through a $CHCl_3$ liquid membrane is similar for the two stereoisomers.[63] Three sarcosine-rich cyclo-octapeptides, cyclo[Lys(Z) or (Suc)-Sar-Leu-Sar-Leu-Sar-Leu-Sar] and cyclo[Glu(OMe)-Sar-Lys(Z)-Sar-Leu-Sar-Leu-Sar] (where Suc = succinic acid) have been found to selectively complex Cu(II) ions. Upon complexation, the trans peptide bonds of Sar residues isomerise to cis. Cyclo[Lys(Z)-Sar-Leu-Sar-Leu-Sar-Leu-Sar] transports Ca(II) ions through a lipid bilayer above the phase-transition temperature, but the other two macrocycles do not.[64] Cyclo(Ala-D-Ala-Ada-D-Pro)$_2$, where Ada = 2-amino-9-decanoic acid, has been found to form both peptide$_2$-cation and peptide-cation complexes, although the binding to monovalent cations is weak.[65]

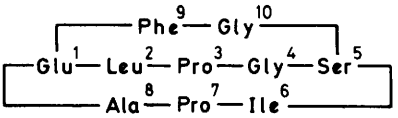
(S)-Deoxy[γ(R)-hydroxy-Ile^3]-amaninamide (29) has been synthesised. This molecule omits the dispensible features of the amatoxin molecule, but the configuration of the hydroxy amino-acid has been inverted from (S)- to (R). The inhibitory capacity of this new analogue is only about one percent of that of the amanitins. This seems to be due to the hydroxy amino-acid, as X-ray indicates a great similarity of the peptide backbone to that of the natural amatoxins.[66] The crystal structures of O-methyl-S-oxo-α-amanitin and the (S)-sulphoxide of O-methyl-α-amanitin have been determined. Both compounds have very rigid conformations which are almost identical. The

distorted 'T' shape conformation found is dominated by five rather strong intramolecular H-bonds.[67]

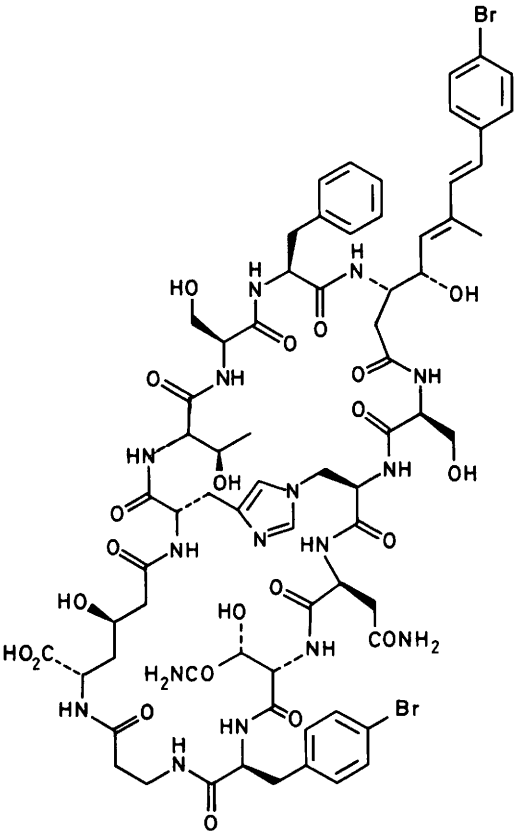
2.8 Higher Cyclic Peptides – Four papers concern cyclolinopeptide A, cyclo(Pro$_2$-Phe$_2$-Leu-Ile$_2$-Leu-Val), from linseed. The ^{1}H-n.m.r. spectrum in several solvents is reported to show very broad lines, indicating the presence of chemical exchange among several conformers. However, it proved possible to freeze a single conformational state in $CDCl_3$ at 214 °K. Most spectral parameters are consistent with the main features of the solid state structure published in 1987, which delineated 5 transannular hydrogen bonds with two β-turns, 1 γ-turn, one α-turn, and one C_{17} ring structure.[68] An independent study of cyclolinopeptide A in dmso-d_6 solution is consistent with a conformation including a type II β-turn centred at Pro-Pro and a γ-turn centred at Ile^6, with two intramolecular H-bonds.[69] Analogues of cyclolinopeptide A have been made in which Pro^1, Pro^2, Phe^3, and Phe^4 have in turn been replaced by Ala. A preliminary ^{1}H-n.m.r. study on the [Ala^2]-analogue in dmso-d_6 indicates a complex system of exchanging isomers.[70]

An immunologically active cyclic decapeptide, cyclo(Gly-Val-Trp-Thr-Val-Trp-Gly-Thr-Ile-Ala), has been isolated from the latex of Jatropha multifida, a shrub endemic in South America. The latex is used in folkloric medicine for the treatment of skin ailments. The amino acid configurations are as yet unknown. It is the first report of a cyclic decapeptide in higher plants.[71] The heterodetic bicyclic decapeptide (30) has been prepared. In CD_3CN it possesses quite a compact structure, the Pro^3 amide bond being cis and the Pro^7 one trans. There is a cavity in which several CO groups are embedded formed by the heterodetic 7-membered ring and the Ala^8-Ser^5 segment of the 8-membered ring. It forms a 1:1 calcium complex that has a less compact structure.[72]

A 500 MHz study of antamanide, cyclo(Val^1-Pro^2-Pro^3-Ala^4-Phe^5-Phe^6-Pro^7-Pro^8-Phe^9-Phe^{10}), shows a fast equilibrium between up to four conformations resulting from amide-bond flips about the Ala^4-Phe^5 and Phe^9-Phe^{10} links.[73] Three new antamanide derivatives in which Pro^3, Pro^7, or Pro^8 have been substituted


Phe 9 — Gly 10
Glu 1 — Leu 2 — Pro 3 — Gly 4 — Ser 5
Ala 8 — Pro 7 — Ile 6

(30)


Br
HO
H
N
O
OH
NH
HN
N
HO2C
H2NCO
CONH2
Br

(31)

by γ-thiaproline (Thp) have been prepared and subjected to X-ray examination. The Thp^{7} and Thp^{8} analogues are isomorphous with antamanide; the Thp^{3} compound has a similar backbone, but with differences of up to 12° near the substituted residue.[73] The crystal structure of the biologically inactive per-hydrogenated antamanide, i.e. [Cha^{5,6,9}]-antamanide (where Cha = cyclohexylalanine) has also been reported. The free peptide has a similar conformation to antamanide, but the Li(I) complexes have very different conformations. This suggests that the antitoxic activity of antamanide may be linked to the complexed form.[74]

New analogues of the [11]-cyclopeptide cyclosporin A (CA) have been prepared by using particular compounds in selective feeding of the fungus Tolypocladium inflatum; [AllylGly^{2}]-CA, [MeCha^{1}]-CA and [D-Ser^{8}]-CA all exhibit high immunosuppressive effects.[75] [(2S,3R,4S]-MeBmt^{1}]-CA has been more conventionally prepared, but has only low immunosuppressive activity although n.m.r. indicates a similar macrocyclic ring conformation to CA itself.[76] An enzyme preparation isolated from Tolypocladium inflatum (now designated Beauveria nivea) is able to synthesise cyclosporin in vitro. CA analogues not obtainable from the fungus can be prepared using this enzyme if other amino acids are added. [N-Me(+)-2-amino-3-hydroxy-4,4-di-methyloctanoic acid^{1}]-CA, [L-norvaline^{2,5},N-methyl-L-norvaline^{11}]-CA, [L-allo Ile^{5},N-Me-L-norvaline^{11}]-CA, [D-2-aminobutyric acid^{8}]-CA, and [β-chloro-D-Ala^{8}]-CA prepared in this way showed moderate immunosuppressive activity, but [allo Ile^{5,11}]-CA had only weak activity.[77]

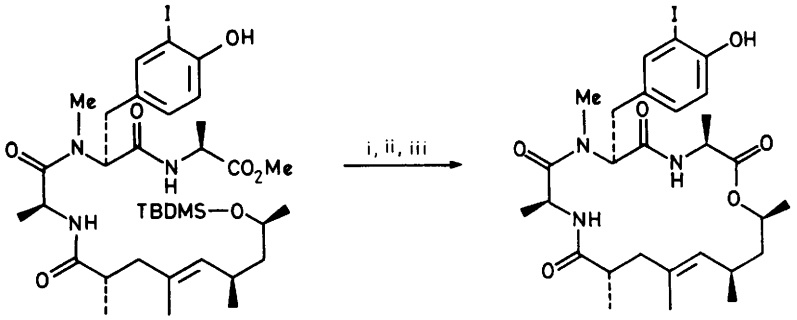
A novel cyclo-[12]-peptide has been isolated from a marine sponge of the genus Theonella. This antifungal compound, theonellamide F (31), contains the unprecedented histidinoalanine bridge and five unusual amino acid residues.[78] The cyclo-[15]-peptide cyclo(Val-Pro-Gly-Val-Gly)$_3$, which contains the repeating peptide sequence of tropoelastin, has been shown by a 2D-NOESY study to have a repeating type II Pro^{2}-Gly^{3} β-turn.

2.9 Cyclodepsipeptides – The total synthesis of geodiamolide A, isolated in 1987, has been achieved. Condensation of a tripeptide fragment with an 8-hydroxynonenic acid component gave (32), which, after removal of the methyl ester and TBDMS groups, was cyclised in low yield (Scheme 1). This ring closure proved difficult, 'the only reagent to effect this macrolactonisation being based on dicyclohexylcarbodiimide.'[80] A n.m.r. study of jasplakinolide (33), isolated in 1986, indicates a mixture of six conformers, but there are no intramolecular hydrogen bonds.[78] Complexation studies of jasplakinolide with Li, Na, and K ions have shown that Li alone binds to the cyclodepsipeptide, with only one of the two major solution conformations participating.[82]

Earlier work on the synthesis of AM-toxin II has experienced problems in obtaining preferential monomeric cyclisation and effective subsequent ΔAla formation. A new approach seems to give improved yields on these steps. Here cyclisation of H-Ser(Bzl)-Ala-Hmb-Phe-OSu (Su = 1-succinimidyl) in pyridine gave cyclic monomer in 59% yield. Removal of the benzyl group and dehydration of the Ser with DCC-CuCl gave [Phe^3]-AM-toxin II in 24% yield. AM toxin II itself(where App replaces Phe) was prepared using this route in similar yield.[83]

A cyclopentadepsipeptide antibiotic hypeptin (34) active against anaerobic bacteria has been isolated from a strain of Pseudomonas. It is rich in hydroxy amino acids, containing β-HyAsn, β-HyLeu, and β-HyTyr. The latter is destroyed on acid hydrolysis and the stereochemical assignment of the β-HyLeu is tentative.[84] The entomopathogenic fungus Matarhizium anisopliae has yielded another destruxin, destruxin E2 (35a). A chlorohydrin derivative (35b) was also characterised.[85] The solution conformation of destruxin A (35c) in $CDCl_3$ closely resembles its crystal conformation, and is similar to that of destruxin B (35d) and roseotoxin B determined earlier.[56]

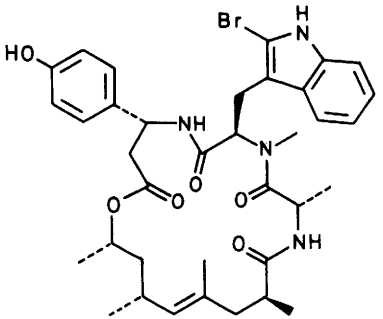
The stereochemistry of the fatty acid component of the cyclodepsipeptide complex CDPC 3510 from Fusarium sporotrichiodes (first reported in 1985) has now been unravelled; the complete structures are therefore as shown in (36).[87] Cyclo(Val-Lac)$_3$ and cyclo(D-Val-Lac)$_3$ show completely



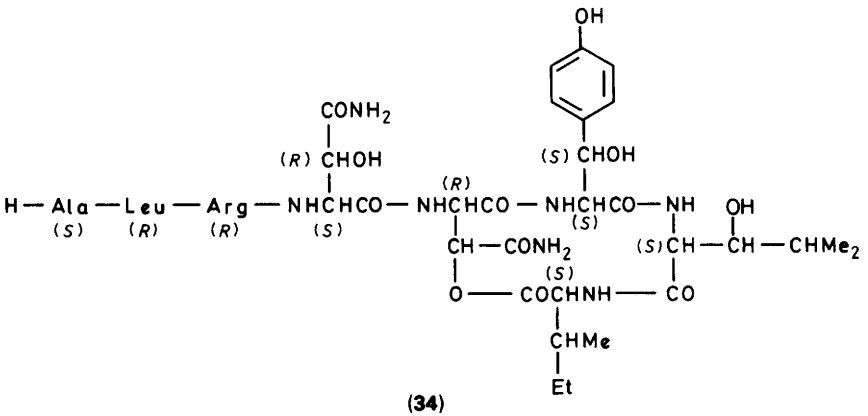
(32)

Reagents: i, 5% HF — MeCN, 25 °C; ii, LiOH, THF — MeOH — H_2O (3:1:1); iii, DCC, DMAP

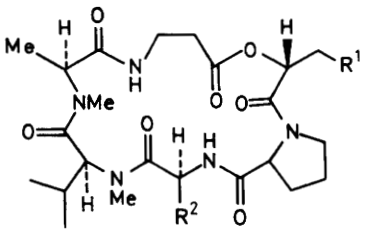
Scheme 1



(33)

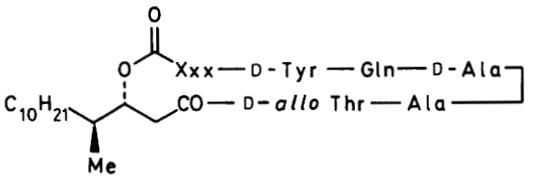


(34)



	R^1	R^2
a;	$-CH(O)CH_2$	$-CHMe_2$
b;	$-CH(OH)CH_2Cl$	$-CHMe_2$
c;	$-CH=CH_2$	$-CHMeEt$
d;	$-CHMe_2$	$-CHMeEt$

(35)



Xxx = Leu 60 %
Ile 30 %
Val 10 %

(36)

Reagents: i, Tos—OH, 80 °C, dioxan, 2h

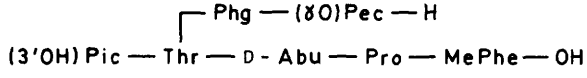
Scheme 2

different behaviour towards alkali cations. The latter forms complexes with Li, Na, and K ions, but the former lacks any complexing ability.[88]

An actinomycin-related peptide lactone has been prepared from the corresponding cyclic peptide by an N,O-acyl shift in 63% yield (Scheme 2). N,O-Acyl shifts have hitherto only been used for specific cleavage or seen as unwanted side reactions during manipulations under acidic conditions.[89] In $CDCl_3$ solution, the conformation of the pentapeptide lactone fragment of actinomycin D contains all trans peptide bonds, but in CD_3COCD_3 there are two cis and two trans amides. The latter conformation is the same as that found in actinomycin D itself.[90]

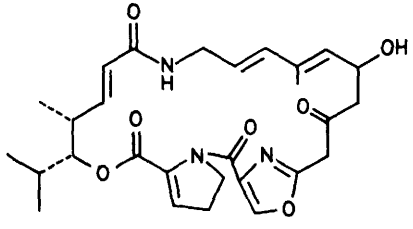
The cyclisation of a linear precursor (37) of virginiamycin S_1, using Woodwards reagent K has been examined. The best yield obtained was 25% using CH_2Cl_2 as solvent in the presence of air rather than nitrogen. However, 17% epimerisation occurred.[91] Both the (S)-4-pipecolic acid and 3-hydroxypicolinic acid portions of virginiamycin S_1, incorporate (RS)-[6-^{13}C,6-^{15}N]-lysine with retention of the labelled nitrogen in biosynthetic experiments. Thus the cyclisation of lysine in both cases occurs with the loss of the α-N and the retention of the ε-N. In addition, the 3-hydroxypicolinic acid unit incorporates deuterium from (2RS, 5R)-[5-^{2}H]-lysine but not from (2RS, 5S)-[5-^{2}H]-lysine. The 5-pro-R hydrogen of lysine is thus retained in the biogenesis of this unit. Experiments with possible dehydroproline precursors indicate that in the biosynthesis of virginiamycin M_1(38),(S)-proline is incorporated to form virginiamycin M_2 which then undergoes hydroxylation with retention of configuration and elimination of water to yield virginiamycin M_1.[93]

The synthesis of viscosin (39), an antiviral and antimicrobial compound from Pseudomonas viscosa sequenced in 1970, has been reported. The final cyclisation was effected between the D-Ser and Ile residues using BOP-Cl in 24% yield, the Ser and Glu side chains being protected as benzyl derivatives subsequently cleaved by Pd/C and ammonium formate.[94] The crystal structure of FR 900359 from Ardisia crenata [see SPR

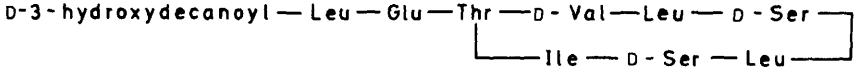


Pic = Picolinic acid
Pec = δ-Oxopipecolinic acid

(37)



(38)



(39)

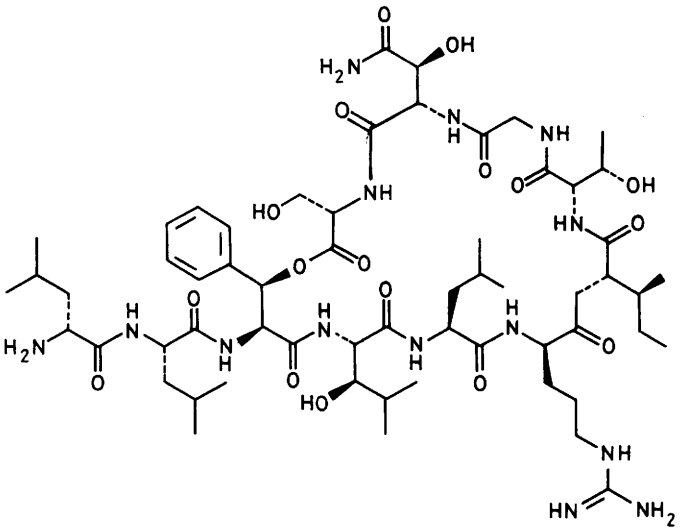
21, (47)] indicates five intramolecular hydrogen bonds, but only one of these is transannular. There are two cis peptide bonds, and the N-Me-dehydroAla residue falls in the stable region of a Ramachandran plot. It is vulnerable to nucleophilic attack and may be involved in the biological activity of the depsipeptide.[95]

A number of papers have appeared on the didemnins. These are summarised in Table 2

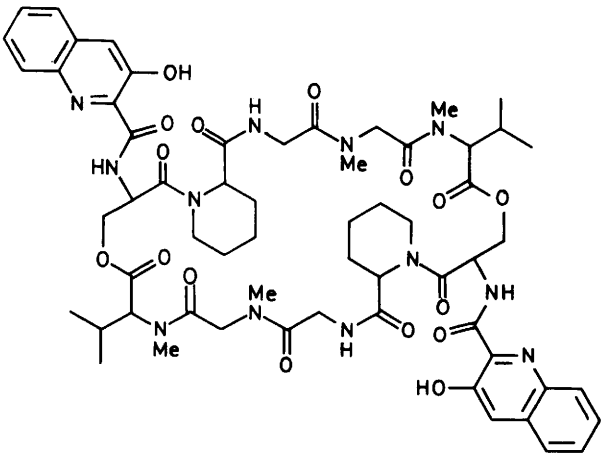
Table 2. Studies on the didemnins

	Refs
Synthesis of didemnins A and B	96
Synthesis of nordidemnin B	97
Synthesis of the didemnin macrocycle	98
Solution conformation of didemnin B	99
Solution conformation of didemnins A and B	100
Complete ^{1}H-and ^{13}C-n.m.r spectral assignments of didemnin B and nordidemnin B	101
Comparative study of didemnin B and iso-didemnin 1	102

A new cyclodepsipeptide containing nine amino-acids in the macrocyclic ring, lysobactin (40), has been obtained from Lysobacter sp ATCC 53042. Its antibiotic efficiency in vivo compares favourably with vancomycin but it is more toxic. It contains two D-residues, the Arg and the N-terminal Leu. After one cycle of the Edman degradation, the antibacterial activity is lost. Reacylation with D-Ala improves the potency tenfold over reacylation with L-Ala; the D-Ala compound is nearly as potent as the original antibiotic.[103] Another novel large ring cyclodepsipeptide, this time with ten ring components, has been isolated from a Nocardioides species collected in soil from Mexico. This antibiotic, named sandramycin (41), is closely related to the luzopeptins, and is of similar biological activity. It contains a repeated pentapeptide unit.[104]



(40)

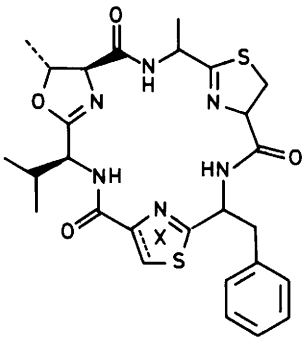


(41)

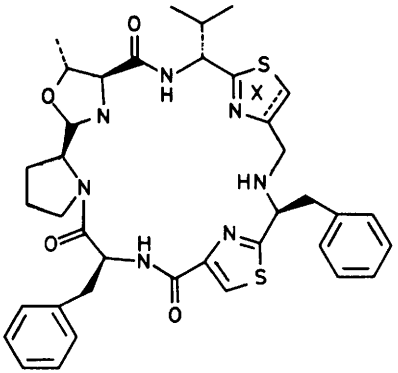
Valinomycin has been synthesised using isopropenyl chloroformate for ester bond formation. A mixed anhydride is initially generated, and this forms esters in the presence of hydroxy-component and DMAP. Isopropenyl esters are not formed as the acylation of DMAP releases the enolate of acetone.[105]

2.10 Cyclic Peptides Containing Thiazole and Oxazoline Rings – Two new cyclic hexapeptides, bistratomides A and B (42) have been characterized from the ascidian Lissoclinum bistratum. They display similar cytotoxicity to the patellamides. The peptides are formed within the obligate algal symbiont Prochloron, but clearly differ from peptides isolated from the same Prochloron of L. patella.[103] Two groups have reported the same new cyclic peptides from L. patella. These comprise lissoclinamides 4 and 5 (43) and patellamide D (44).[107,108] These peptides and others reported previously from this source are, as indicated above, in fact found within the algal symbiont Prochloron.[107] The crystal structure of patellamide D has been determined, and computer modelling indicates that the energy minimised conformation of this peptide closely resembles that found in the crystalline form. An antibiotic rich in dehydroalanine (it contains five such residues), thioxamycin (45), has been extracted from a Streptomyces species. It is active against anaerobic bacteria, and of the known antibiotics, it is most closely related to the sulphomycins.[109]

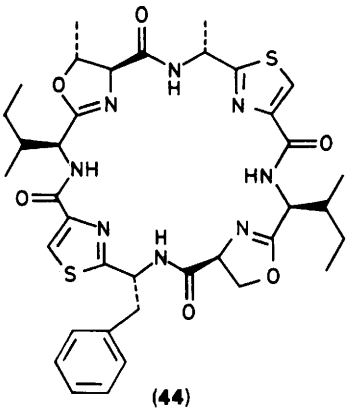
The conformation of ulithiacyclamide has been studied by n.m.r. and molecular mechanics energy minimisation. It assumes two kinds of conformations depending on the solvent. In $CDCl_3$ and C_6D_6 the four peptide bond NH's are all located on the interior of the structure, but in $(CD_3)_2SO$ two protrude to the outer part of the ring structure. Two saddle-shaped structures best satisfy all the n.m.r. criteria. The ^1H- and ^{13}C-n.m.r. spectra of both nosiheptide[111] and thiostrepton[112] have been assigned by use of 2D techniques on unlabelled samples and biosynthetically multiple-labelled samples from stable isotope feeding experiments.



A; X = thiazoline
B; X = thiazole
(42)



4; X = thiazoline
5; X = thiazole
(43)



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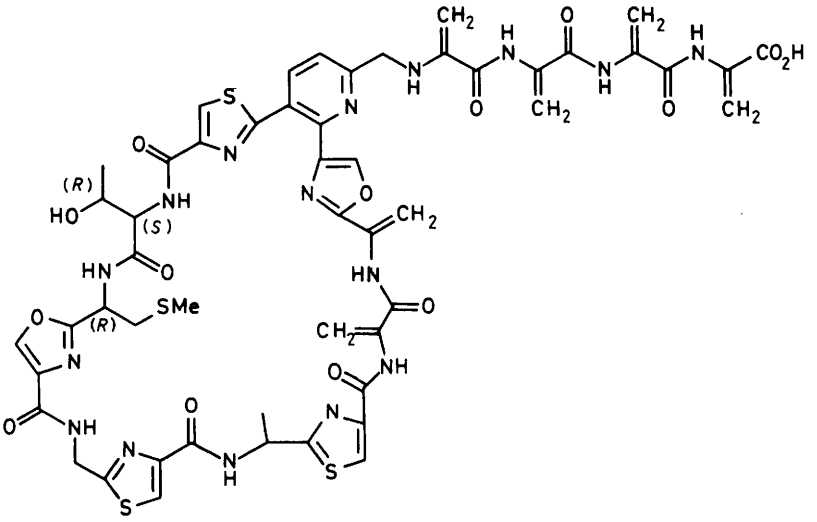
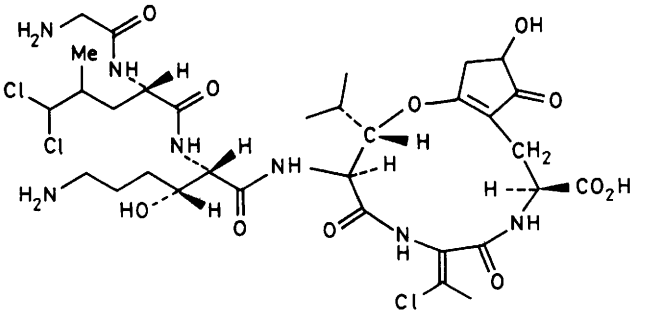


(45)

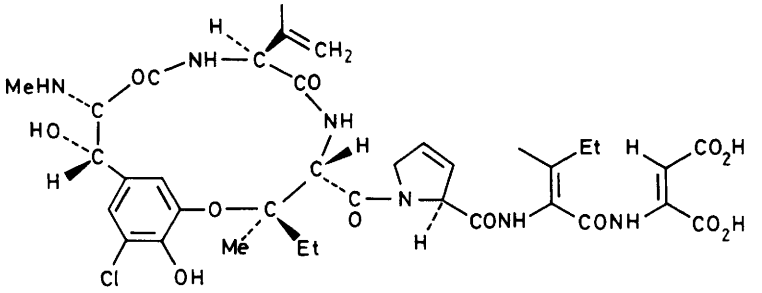


(46)

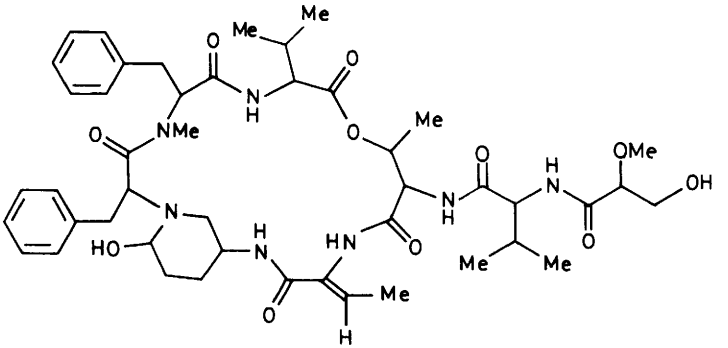
2.11 Cyclic Peptides Containing Other Non-protein Ring Components – A number of interesting new natural products in this category have been described. A causal agent of the Victoria blight disease of oats has been isolated from Helminthosporium victoriae. The active compound is HV-Toxin M (46), which contains δ,δ-dichloroleucine and β-chlorodehydroalanine.[113] The consumption of lupins infected with the fungus Phomopsis leptostramiformis has been identified as the cause of lupinosis, a mycotoxicosis of sheep, cattle, and horses. Lupinosis causes severe liver damage. The main toxin is phomopsin A (47), whose structure has been determined by X-ray analysis.[114]

Dolostatin 13 (48) has been obtained from the sea hare Dolabella auricularia. It appears only remotely related to the earlier isolated dolostatins 10 and 12. The Δ-Abu unit has been tentatively assigned the Z-configuration, but the other asymmetric centres remain undefined. The 3-amino-6-hydroxy-2-piperidone unit, presumably derived from a Phe-Glu dipeptide precursor, dehydrates on standing in chloroform solution.[115] No stereochemical assignments have yet been made either to a new lipopeptide antifungal agent (49) from a filamentous fungus ATCC 20868. This polyhydroxylated compound is a structural analogue of echinocandin D, and has potent anti-Candida activity.[116]

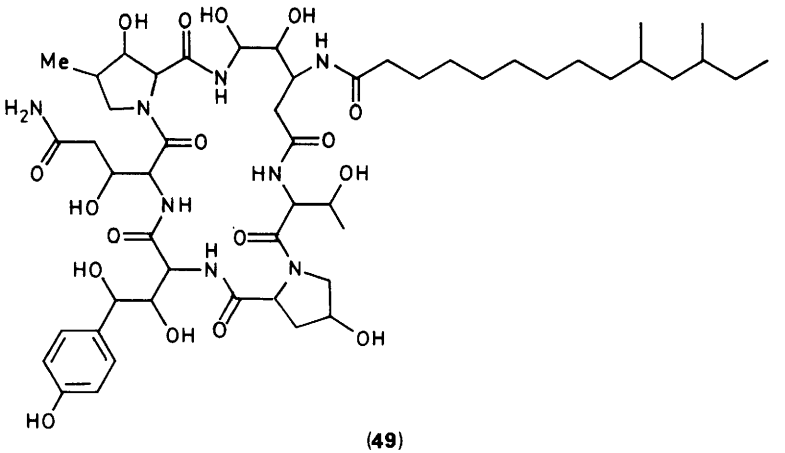
Two further toxic peptides from the blue-green alga Microcystis aeruginosa have been characterised. Cyclo (D-Ala-Leu-D-MAsp-Arg-Adda-D-iGln-Mdha) was found in a Norwegian specimen, and cyclo(D-Ala-Arg-D-isoAsp-Arg-Adda-D-iGln-ΔAla) from a Canadian sample (MAsp = β-methylaspartic acid, MDha = N-methyldehydroalanine, and Adda = 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid).[117] From another blue-green alga, the Hawaiian terrestrial Anabaena sp strain BQ-16-1, has been isolated the cardioactive puwainaphycin C (50). It possesses the unusual β-amino acid 3-amino-14-chloro-2-hydroxy-4-methyl-palmitic acid. From the same source the inactive puwainaphycin D has also been extracted; this differs only in having the C-terminal of the two Thr residues replaced by Val.[118]



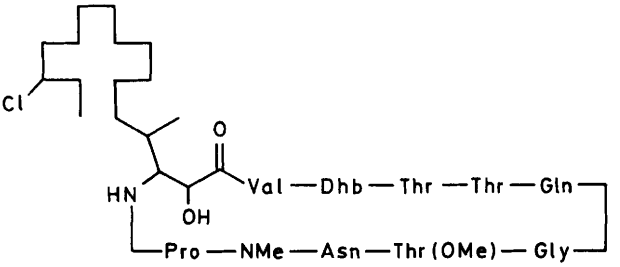
(47)



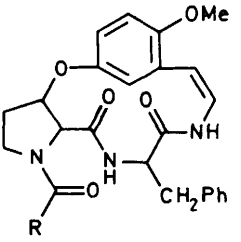
(48)

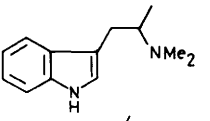


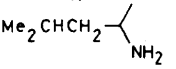
(49)


Cl
O
HN
OH
Val — Dhb — Thr — Thr — Gln
Pro — NMe — Asn — Thr (OMe) — Gly

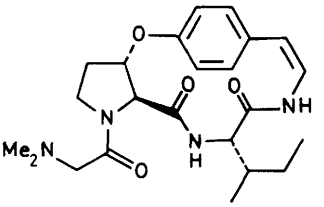
(50)


OMe
O
O
O
NH
N
N
H
O
CH2Ph
R

a; R = 
NMe2
N
H

b; R = 
Me2CHCH2
NH2

(51)


O
O
O
NH
N
N
H
Me2N
O

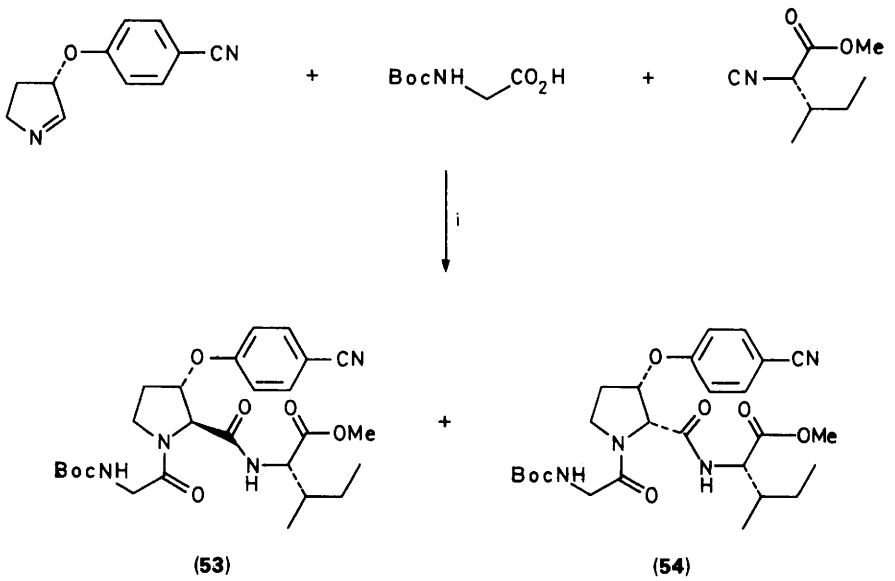
(52)

Two new cyclopeptide alkaloids have been isolated, ruganosine B (51a) from Zizyphinus rugosa [119] and nummularine S (51b) from Z. nummularia.[120] Nummularine F (52) has been synthesised using an Ugi reaction to generate the intermediate (53) (Scheme 3). The cis product (54) did not isomerise to (53) under either acidic (acetic acid in THF) or basic (DBU in MeOH) conditions, but did isomerise if applied to a preparative tlc plate and eluted with MeOH. If other more polar solvents are used in this Ugi reaction, more cis-isomer (54) is formed.[121] Another potential precursor to nummularine F, (55), has been prepared by cyclisation of a pentafluorophenyl ester (of HyPro) under catalytic hydrogenation conditions.[122]

The stereochemistry of the antibiotic biphenamycin (56), discovered in 1985, has now been assigned by using conformational information derived from n.m.r. studies.[123] Another ansa-tripeptide (57) has been prepared using a pentafluorophenyl ester cyclisation. Formation of either of the amide bonds by this method gave an 80% yield of the cyclic monomer.[124] Three new syntheses of the ACE inhibitor K13 (58) have been reported, [125, 126, 127] and two new syntheses of the immunopotentiating cyclopeptide OF 4949-III (59) have been developed.[125, 128]

The antitumour antibiotic WF-3161 (60), isolated in 1983, has now been synthesised. The most suitable site for ring closure by the pentafluorophenyl ester method was chosen on the basis of a model compound where all four possible sites were examined (61). As a result the WF-3161 ring was closed to the amino group of Phe, giving 74% of the desired product and 12% of a diastereoisomer. If formation of the Pfp ester is catalysed by DMAP, the diastereoisomer is found almost exclusively on cyclisation.[129] Avellanins A and B (62), new pressor-active metabolites from Hamigera avellanea, have also been synthesised. Coupling to methyl anthranilate in this work was best effected with Fmoc-Val- or Leu-Cl.[130]

The cyclotetrapeptide analogue (63) has been made, and its tetra-deprotonated form found to co-ordinate to cobalt to give a square-planar Co(III) complex containing a significantly non-planar amide group. Square-planar Co(III) complexes are rare,

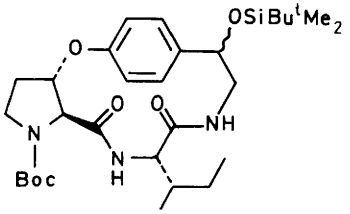


(53) **(54)**

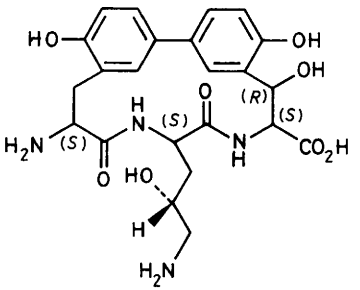
1 : 1·4

Reagent : i, toluene

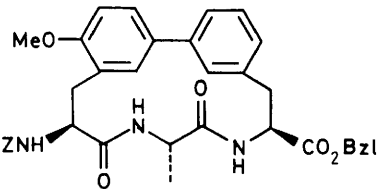
Scheme 3



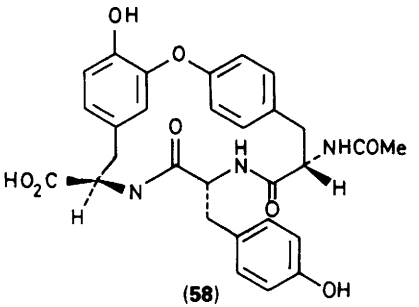
(55)



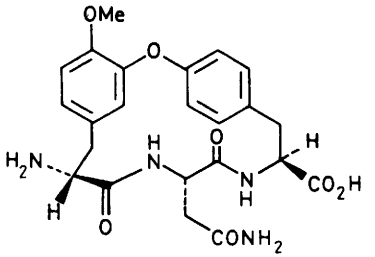
(56)


MeO
ZNH
O
H
N
O
N
H
CO2Bzl
O

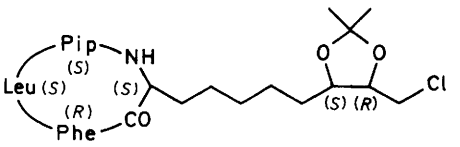
(57)


OH
O
HO2C
N
O
H
N
H
O
NHCOMe
H
OH

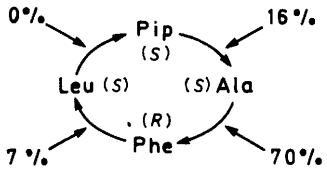
(58)


OMe
O
H2N
H
O
N
H
O
H
CO2H
CONH2

(59)

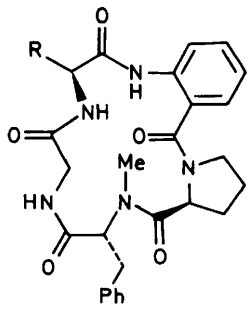

Pip
(S)
Leu (S)
(R)
Phe
NH
(S)
CO
O
O
(S) (R)
Cl

(60)



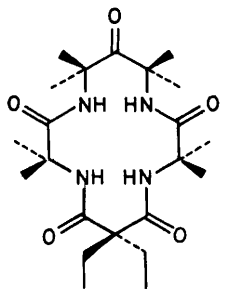
Percentages indicate yield on ring closure to form a given amide bond

(61)



A; R = — CHMeEt
B; R = — $CHMe_2$

(62)



(63)

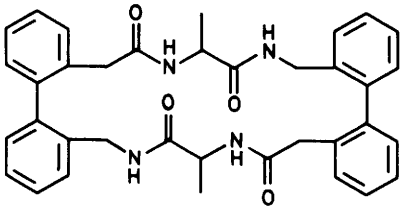
and this is the first macrocyclic example.[131] Another cyclotetrapeptide analogue (64) has been constructed and found to exist as a mixture of two conformers in $CHCl_3$ and three in DMSO.[132] A similar, but more rigid, compound (65) containing phenoxathiin has also been prepared, as has its corresponding cyclic monomer (66). In DMSO (65) adopts a stretched, probably flexible, conformation without permanent intramolecular H bonds; (66) exhibits two equilibrating conformations containing both cis and trans amide bonds.[133]

A cyclic tripeptide (67) containing a phosphodiester linkage has been synthesised (Scheme 4) as a model for the intermolecular Ser-Thr phosphodiester linkage in flavodoxin from Azotobacter. The α-CH of the Thr in (67) has a similar chemical shift to that of the corresponding proton in flavodoxin, but the β-CH_2 protons of the Ser differ in chemical shift.[134] The absolute configuration of the three hitherto undefined asymmetric centres of the bicyclo-octapeptide moroidin (see SPR 19, p212) has now been solved by a combination of 2D n.m.r. and molecular modelling techniques. The peptide backbone is in fact composed of all L-amino acids, and the Trp-His link is between the C_2 of Trp and the N_1 of the imidazole ring.[135] Six new antagonists of [Arg^8]-vasopressin have been prepared (68).[136]

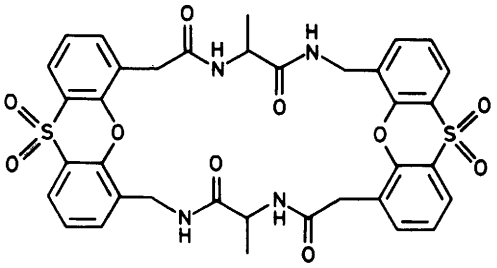
3 Modified Linear Peptides

3.1 Enzyme Inhibitors – A new carboxyl proteinase inhibitor tyrostatin, N-isovaleryl-Tyr-Leu-Tyrosinal has been isolated from a species of the actinomycete Kitasatosporia. It is also inhibits such cysteine proteinases as papain.[137] A new leupeptin analogue, propionyl or acetyl-Thr-Thr-Arginal, which shows strong inhibitory activity towards proteases, has been extracted from a Streptomyces griseus strain isolated from soil collected in China.

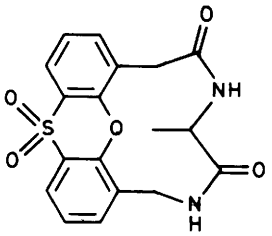
Three crystal structures of enzyme-peptide inhibitor complexes have been reported. In one, Boc-His-Pro-Phe-His-Sta or Achpa-Leu-Phe-NH_2 is found to bind to aspartic proteinase in an extended conformation in the active site cleft, the statine OH interacting strongly with the catalytic aspartates. The results show that statine should be considered a dipeptide



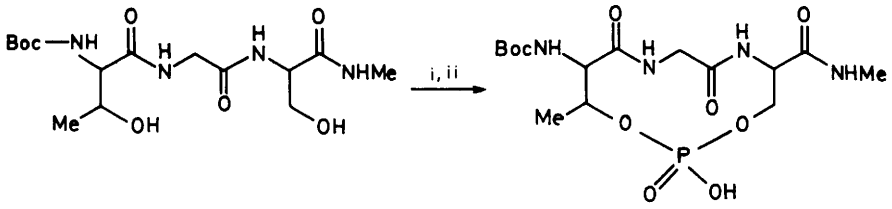
(64)



(65)



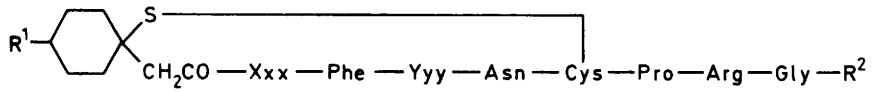
(66)



(67)

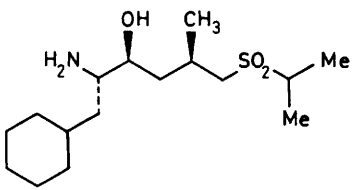
Reagents: i, $[(Me_2CH)_2N]_2POCH_2C_6H_4p\text{-}Cl$, 1*H*-tetrazole; ii, Pd/C, Bu^tOH, H_2O, NaAc, H_2

Scheme 4



	R^1	Xxx	Yyy	R^2
1;	Me_3C	Tyr	Abu	NH_2
2;	Ph	Tyr	Abu	NH_2
3;	H	Tyr(Me)	Glu(NBu_2)	NBu_2
4;	H	Tyr(Me)	Glu(NHEt)	NHEt
5;	H	Tyr(Me)	Glu(NEt_2)	NEt_2
6;	H	D-Tyr(Et)	Glu(NEt_2)	NH_2

(68)



(69)

analogue, despite the lack of a side chain.[139] In a second, the poor penetration of the Ile side chain of Z-Ala-Ile-boronic acid into the binding pocket makes possible an additional favourable interaction between the His-57 $\underline{N}^{\varepsilon}$ of porcine pancreatic elastase and the boron atom. This replacement facilitates a nucleophilic attack by His-57 on the inhibitor.[140] It is perhaps appropriate to note that a ^{15}N-n.m.r. study of a complex of α-lytic protease with the boronic acid analogue inhibitor MeOSuc-Ala_2-Pro-boroPhe-OH in the crystalline state using magic angle spinning indicatesthat the structure of the complex is the same as that in solution, involving again a histidine-boron adduct.[141] In the third, Ac-Ala-Pro-Val-$CF_2CONHCH_2CH_2Ph$ binds to porcine pancreatic elastase in an antiparallel β-pleated sheet conformation, the O^{γ} atom of the catalytic Ser^{195} forming a hemiacetal complex. The H-bonding catalytic tetrad of the enzyme remains structurally intact.[142]

An aspartic protease inhibitor containing a new histidine side chain analogue of statine, Iva-Val-Val-HiSta-OMe, has proved a very potent inhibitor of penicillopepsin (K_i4.5nM). It is ten times more active than the comparable statine compound, but weaker than analogues with Lys and Orn side chains in place of that of His.[143] A sulphur containing Cha-Ala isostere (69) has been prepared, and successfully incorporated into a number of renin-inhibiting compounds.[144] Other new renin and ACE inhibitors are summarised in Table 3. As before, only the most potent compound from each publication is given. Full details of the synthesis of spirapil and spirapilat and their glycyl and lysyl analogues have now been reported.[152]

3.2 Dehydropeptides – A number of oligopeptides containing dehydrotyrosine have been prepared, the largest being the enkephalin analogues Boc-ΔTyr(Mom)-Gly_2-Phe or ΔPhe-Leu-OMe, where Mom =methoxymethyl. These syntheses utilise the $\underline{N}$ - carboxyanhydride of ΔTyr(Mom).[153] The radical addition to di- and tripeptides containing a Ala residue by the alkylmercury halide/$NaBH_4$ method has been found to go in good chemical yield but low diastereoselectivity to give saturated peptides.

Table 3 *Novel enzyme inhibitors*

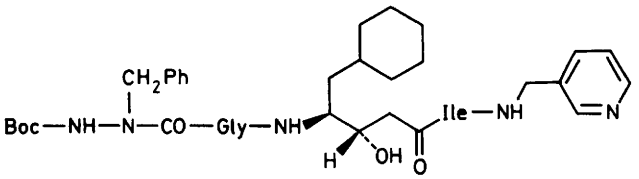
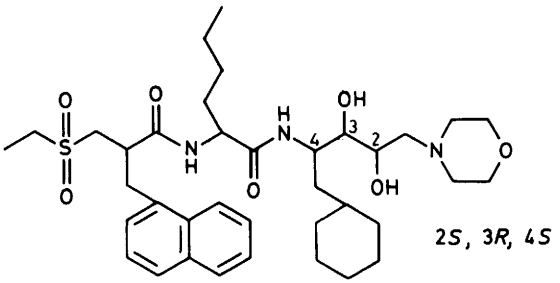
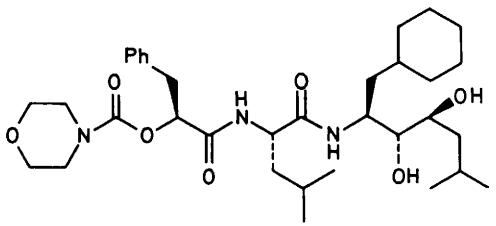
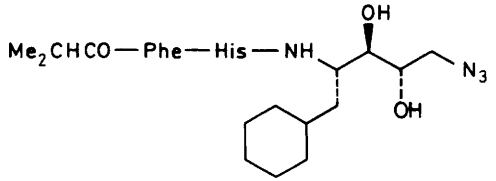
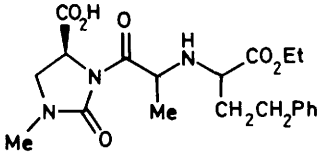
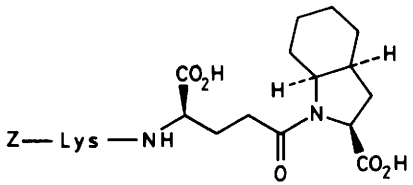
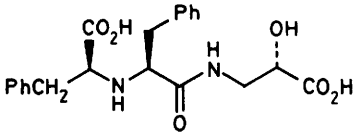
Compound	IC_{50}	*Ref.*
 renin inhibitor	1·5μM	145
 orally active renin inhibitor	3·3nm	146
 orally active renin inhibitor	5nm	147
 renin inhibitor	0·55nm	148

Table 3 - continued

Compound	IC_{50}	*Ref.*
 ACE inhibitor		149
 ACE inhibitor	3·5nM	150
 neutral endopeptidase inhibitor	11nM	151

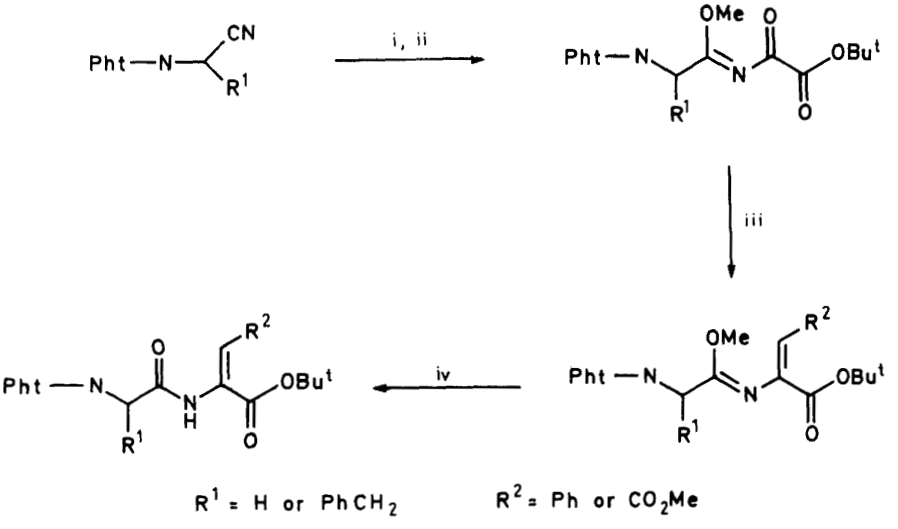
Addition of the isopropyl radical to Z-Ala-Pro-OMe gave the best diastereoisomoric excess (28%), the chemical yield being 74%.[154]

The condensation of phosphorus ylids with t-butyl oxamic esters derived from aminonitriles gives 2-aza-1,3-dienes. Subsequent reaction with hydrobromic acid gives protected dehydrodipeptides (Scheme 5). In all cases the Z-configuration is produced, and where R'=$PhCH_2$ the optical purity is only 32-35%.[155] The enzymatic coupling of Z-ΔGlu(OMe)OMe with some leucine derivatives has been explored (Scheme 6). The best yield was obtained with the phenylhydrazide.[156]

The crystal structures of Boc-Pro-ΔLeu-NHMe[157] and Boc-Gly-Phe-NHMe[158] have been reported. A 500 MHz study has been made of dermorphin and of its N-terminal pentapeptide in which the fifth and third aromatic residues have been substituted by ΔPhe. In d_6-dmso all these peptides adopt an essentially random extended conformation. The complexes of the pentapeptides with 18-crown-6 ester in $CDCl_3$ adopt ordered folded comformations, behaviour that closely parallels c.d. observations in MeOH.[159] A 270 MHz examination of Boc-Phe-Δ^zPhe-Val-Δ^zPhe-Δ^zPhe-Val-OMe shows a population of folded helical conformations in $CDCl_3$, but in d_6-dmso the peptide again favours an extended conformation.[160] The solution conformations of Boc-Xxx-Δ^zPhe-NHMe (where Xxx = Ala, Gly, Pro or Val) have also been reported.[161]

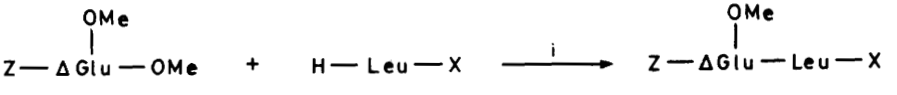
The heterogeneous catalytic hydrogenation of tripeptides containing an N-terminal dehydro-amino acid gives products with diastereoisomeric excesses of 65-81%. Bulkier C-terminal amino acids give the higher figures. If the dehydro residues are central or C-terminal, the d.e.'s are reduced to the 15-17% and 2.4-6.4% ranges respectively.[162] A study of the effects of solvent and temperature on the stereoselectivity of asymmetric hydrogenation of dehydrodipeptides using $Rh(I)(Ph_2PCH_2)_2CH(CH_2)_2NMe_2$ as the catalyst shows the highest stereoselectivity in aqueous methanol at 20-40 °C.[163]

3.3 Peptides Containing α,α-Dialkylamino Acids – The preferred conformations of peptides from $C^{\alpha\alpha}$-dialkylated residues, tetrazolyl peptides, γ-and δ-lactam containing peptides, and



Reagents: i, MeOH—HCl; ii, $ClCOCO_2Bu^t$; iii, $Ph_3P^+—\bar{C}HR^2$; iv, HBr

Scheme 5



Reagent: i, papain

X	Maximum yield	pH
NHPh	77%	8
NHNHPh	13%	8
$OCHPh_2$	10%	6

Scheme 6

peptides have been reviewed.[164] A number of tetrapeptides of the general structure Z-Thr-Xxx-Yyy-Val-OBzl containing two central α,α-dialkyl amino acids have been synthesised by application of the 'azirine/oxazolone' method. The conformation of the products show that α,α-disubstitution favours the formation of β-turns even in these short oligopeptides.[165]

Assignments previously made of the Cβ signals in the ^{13}C-n.m.r. spectra of Aib-containing peptides have been checked by the use of stereoselectively deuterated Aib residues prepared by the bis-lactim ether method. These residues have been built into Boc-Ala-Aib-Ala-OMe and Boc-[Glu(OBzl)]$_4$-Aib-[Glu(OBzl)]$_7$-OPEGM, where PEGM = polyethylene glycol monomethyl ether.[166] Both isomers of (E)-2,3-methanophenylalanine (^{E}Phe) have been incorporated into dipeptides, and both (2R,3S)-and (2S,3R)-^{E}Phe-Phe(or Leu)-OMe were found to inhibit the hydrolysis of Ac-Tyr-OEt by chymotrypsin.[167] [(±)Glp^1]-TRH has also been prepared, and found to be less easily hydrolysed by pyroglutamate aminopeptidase than is TRH itself. The cis-content of this analogue (10-15% in water at 20 °C) is comparable to that found in TRH.[168]

This years crop of X-ray studies of peptides containing α,α-dialkylamino acids are summarised in Table 4.

TABLE 4 Crystal structure of peptides containing α,α-dialkylamino acids.

Peptide	Ref.
Z-Ala-Aib-OH	169
Ac-(Aib)$_3$-OMe·3H$_2$O	170
Ac-(Aib)$_2$-(R)Iva-OMe·H$_2$0	170
H-(Ac$_3$c)$_2$-OMe, Fmoc-(Ac$_3$c)$_2$-OMe·MeOH, Ac-(Ac$_3$c)$_2$-OMe, pBrBz-(Ac$_3$c)$_3$-OMe·H$_2$O and Boc-(Ac$_3$c)$_4$-OMe·2H$_2$O (where Ac$_3$c = 1-aminocyclopropyl-1-carboxylic acid)	171
Boc-Aib-Leu$_2$Aib-OMe	172
Leucinostatin	172,173
Ac-Aib$_2$-(S)Iva-Aib$_2$-OMe	174

Boc-Ala-Leu-Aib-Ala-Leu-Aib-OMe·H_2O	175
Boc-Aib-Val-Aib_2-Val_3-Aib-Val-Aib-OMe	176
Boc-Aib-$(Val\text{-}Ala\text{-}Leu\text{-}Aib)_3$-OMe	177

3.4.Amide-Bond Analogues – Five groups have reported methods for preparing hydroxyethylene dipeptide isosteres and/or their γ-lactone precursors.[178-182] A new reagent (70) has been developed for converting peptides to endothiopeptides. It is claimed to give higher yields than Lawessons reagent (71), increased selectivity, and no racemisation. It is also more soluble in organic solvents and uses milder conditions.[183] A general synthesis of ψ[CH(alkyl)NH] pseudopeptides with defined stereochemistry at the new asymmetric centre has been described, and is exemplified by the synthesis of such compounds as (72) and (73).[184]

Some ketomethylene and hydroxyethylene analogues of di-and tripeptides have been examined as aminopeptidase inhibitors. The most potent compound of the series was H-Lysψ[$COCH_2$](R,S)-Phe-OH, which is of comparable activity to the natural product arphenamine A, H-Argψ[$COCH_2$]Phe-OH.[185] An X-ray study of a variety of pseudopeptide analogues of model dipeptides has been published covering 2 depsi, 10 N-methylated, 3 reduced, 3 retro, 1 αβ-dehydro, and 1 hydrazino compound.[186]

Other studies on amide bond analogues are listed in Table 5.

TABLE 5 Amide Bond Analogues

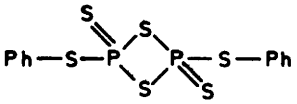
Peptides	Ref.
Three retro-inverso analogues of Boc[Nle^{28},Nle^{31}]-CCK7	187
Retro analogues of Bu^tCO-Ala-Gly-NHiPr	188
Boc-Tyr-Gly_2-Pheψ[CSNH]Leu-OBzl (X-ray)	189
(Leu^{26}ψ[CH_2O]Leu^{27})-N-Ac-$GRP_{20-27}NH_2$ (GRP = gastrin-releasing peptide)	190

Cinnamoyl-(1-^{13}C-Phe)Ψ[$COCH_2$]Gly-Pro_2-OH (collagenase inhibitor)	191
H-Asn[Ψtrans CH=CH]Val-OH and H-Ser[Ψtrans CH=CH]Asn-OH	192
Retro-inverso analogue of Ac-Phe-Phe-Val-OH	193

3.5 γ-Glutamyl Peptides – Two glutathione analogues have been isolated from rat liver. One, H-γ-Glu-Ser-Gly-OH, is a new natural compound; the other, H-γ-Glu-α-aminobutyryl-Gly-OH, is opthalmic acid.[194] The synthesis of γ-glutamyltaurine from L-glutamine and taurine has been carried out using γ-glutamyl-transpeptidase from Penicillium roquefortii in 36% yield. Earlier work has shown that γ-Glu-Tau has vitamin A like and taurine-like biological activities, antagonises the effects of cortisone, thyroxine, and prednisolone, as well as increasing renin activity in plasma and having a protective effect against X-rays.[195] γ-Glutamyltyrosine methyl ester has been similarly synthesised from glutamine and tyrosine methyl ester using γ-glutamyltranspeptidase from E. Coli. This dipeptide was prepared as a more soluble source of tyrosine for transfusion solutions.[196]

Pulse-chase experiments with $^{35}SO_4^{2-}$ fed for ten minutes to onion and garlic show the label to appear in γ-Glu peptides within 15 minutes, reaching a maximum after one hour. The label was not detected in flavour precursors (S-alk(en)yl-L-cysteine sulphoxides) until after six hours. It was concluded that glutathione and other γ-Glu peptides are intermediates in the biosynthetic pathway to these flavour precursors.[197] B/E linked scan sputtered ion mass spectrometry has been found a useful technique to distinguish the α- or γ-linking of N-terminal Glu residues or the α-or β-linking of N-terminal Asp residues in oligopeptides. Tests were carried out on ten pairs of Glu oligopeptides and five pairs of Asp dipeptides.[198]

The new immunoactive peptide RP56142 (74) has been prepared on a five hundred gram scale from L-2,6-diaminopimelic acid.[199]



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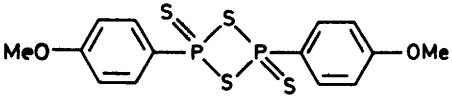

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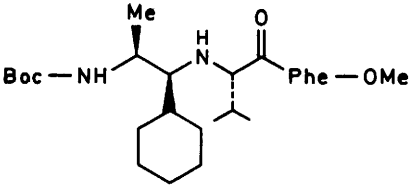

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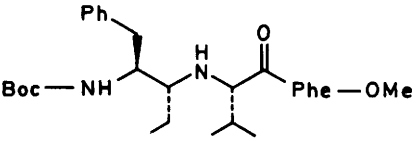

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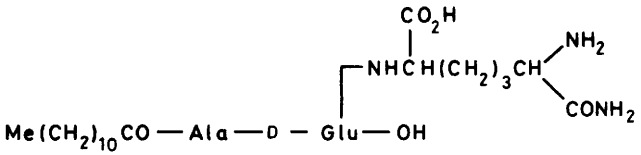


(74)

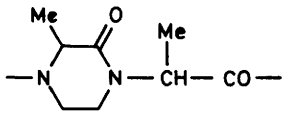
3.6 Conformationally Constrained Peptides – Two cyclic peptides have been synthesised that contain piperazine rings in the backbone. These compounds, cyclo(Sar-Eaa-Sar)$_3$ and cyclo (Sar-Eaa)$_4$, where Eaa is (75), were prepared by 1-succinimidyl ester cyclisations in 25% and 43% yields respectively. These compounds are designed as potential binding sites for phenyl groups.[200] The conformationally restricted analogues (76) and (77) have been prepared as γ-turn templates. They react with amino acid esters in dmso over 2-7 days to give e.g. (78). When R' = -CHMeEt in (78), acid hydrolysis gives isoleucine containing less than 0.20% H-allo-Ile-OH. Further elaboration of (76) has given (79).[201]

The chirospecific synthesis of 4-alkyl substituted γ-lactam-bridged dipeptides from L-aspartic acid has been developed. The last two stages of the synthesis are depicted in Scheme 7.[202] A β-turn mimetic (80) previously described in 1988 has now been incorporated into some oligopeptides using Merrifield solid phase peptide synthesis methods.[203]

Incubation of isopenicillin N synthase with [(5S)-5-amino-5-carboxypentanoyl]-L-homoCys-Cys-OH results in the formation of a novel bicyclic β-lactam (81) which contains an intramolecular disulphide bridge.[204] The crystal structure of four analogues of the C-terminal tripeptide of oxytocin, H-Pro-Leu-Gly-NH_2, containing backbone rings have been determined (82-84).[205,206]

3.7 Phosphonopeptides – A novel bovine liver alkaline phosphatase inhibitor, alphostatin, has been isolated from Bacillus megaterium. This has the sequence H-Ile$_2$-Ser(OPO_3H_2)-Gln-Glu-OH, and does not effect other phosphatases.[207] Five analogues of S-t-butyl glutathione have been prepared containing 4-amino-4-phosphonobutanoic acid in place of Glu and aminoethylphosphonic acid in place of Gly. Simultaneous deprotection of amino, carboxylic, and phosphonic groups was achieved with hexamethyldisilane and iodine.[208]

The free tripeptide bialaphos (85) has been synthesised for the first time by the use of enzymes which provide selective hydrolyses of phosphonates in the presence of ethoxycarbonyl and



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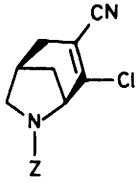

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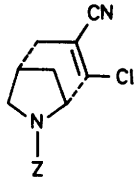

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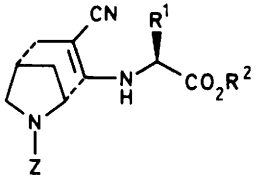

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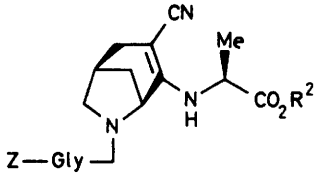

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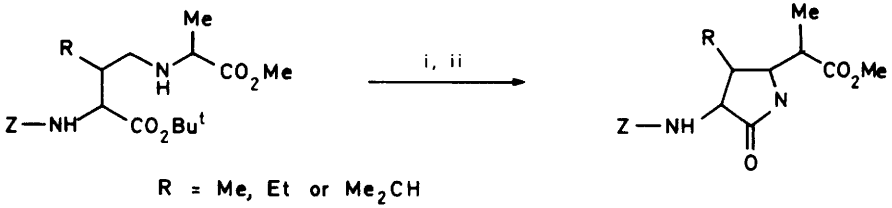
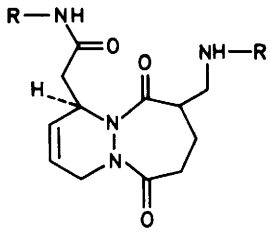


R = Me, Et or Me_2CH

Reagents: i, HCO_2H / 70°C ; ii, pyridine - DMF - 60°C

Scheme 7



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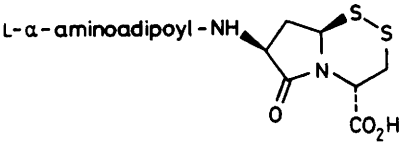

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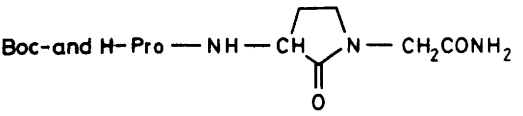

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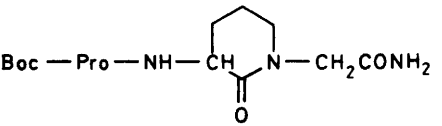

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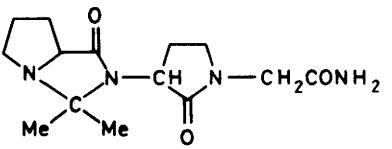

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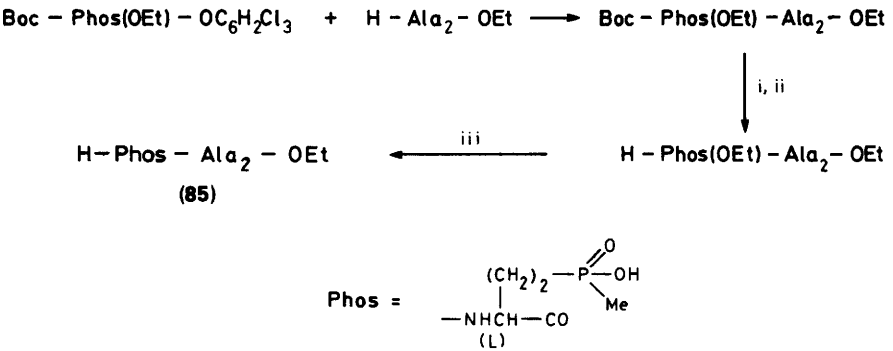
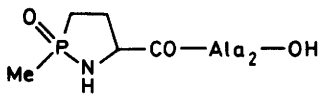


Reagents: i, alkaline mesintericopeptidase; ii, HCl—CH_3CO_2H; iii, phosphodiesterase 1

Scheme 8



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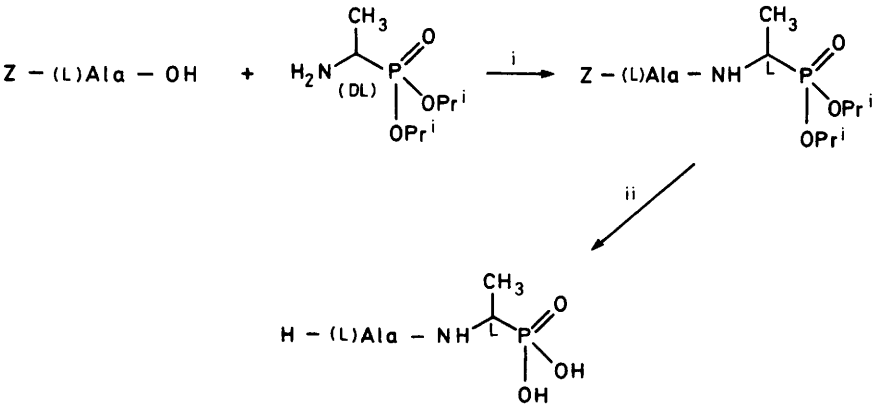


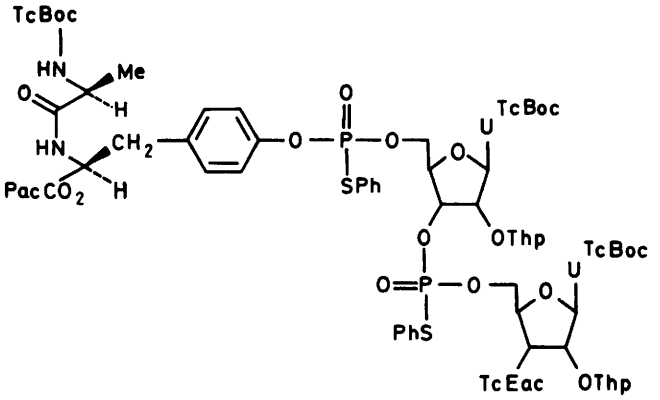
Reagents: i, papain; ii, HBr —CH_3CO_2H

Scheme 9

peptide bonds (Scheme 8). A cyclic analogue (86) was also prepared which showed fairly good anti-tumour activity, but low herbicidal activity.[209] Papain in powdered form has been found to efficiently catalyse the synthesis of alafosfalin in $CH_3CN:H_2O$ with high stereoselectivity (Scheme 9).[210]

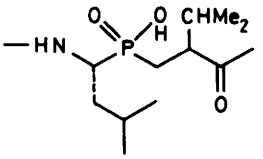
The heptapeptide H-Leu-Arg_2-Ala-Tyr(PO_3H_2)-Leu-Gly-OH has been prepared by a solid phase method using Boc-Tyr(Me_2PO_3)-OH to introduce the O-phosphotyrosine residue. The methyl phosphate was cleaved at the end of the synthesis by HBr in acetic acid.[211] A naturally occurring sequence from the autophosphorylated Rous sarcoma virus, H-Asn-Glu-Tyr(PO_3H_2)-Thr-Ala-OH, has been prepared in a solution phase synthesis, also using Boc-Tyr(Me_2PO_3)-OH. In this case phosphate deprotection was best achieved (in 44% yield) using 1M $BrMe_3Si$/thioanisole/Tfa.[212] The same group has also made H-Tyr(PO_3H_2)-Leu-Gly-OH using benzyl protection for the phosphate. In these latter studies it was observed that O-phosphotyrosine is unstable to liquid HF at 0^0C, and is also sensitive to conventional Boc cleavage conditions with Tfa.[213]

The novel protected amino acid N^{α}-Boc-N^{1}-(Me_2PO_3)-Trp-OH has been used in the solution phase synthesis of H-Glu-Trp(PO_3H_2)-Leu-OH. In this case demethylation was effected with CF_3SO_3H/Tfa/m-cresol/thioanisole.[214] The solid phase syntheses of H-Leu-Arg_2-Ala-Ser-or Thr(PO_3H_2)-Leu-Gly-OH have been reported. The Ser and Thr were incorporated as N-Boc-diphenylphosphonoesters, and the phosphonoesters were later cleaved by catalytic hydrogenation.[215] A dinucleotide dipeptide H-Ala-Tyr-(pUpU)-OH has been synthesised via the phosphorothioate intermediate (87). Virion RNA of poliovirus type I is known to be covalently linked to a genome-protein VPg through the 5'-OH group of RNA and the phenolic OH of tyrosine.[216] Of some transition-state analogues of human renin inhibitors containing phosphinic acid derivatives at the scissile bond, the most potent were H-Pro-His-Pro-Phe-His-Xxx-Ile-His-Lys (IC_{50} 7.5×10^{-8}M) and Z-Arg-Pro-Phe-His-Xxx-Ile-His-NH_2 (IC_{50} 1.0×10^{-7}M), where Xxx = (88).[217] The N-alkoxyphosphinyl dipeptide (89a) has been prepared. On stirring in n-butanol at 110 ^0C for 6h, it forms cyclo(Pro_2) in 42-48%



Pac = $PhCOCH_2$
TcBoc = $CCl_3—CMe_2OCO—$
TcEac = $CCl_3—CH_2—OCO—$

(87)



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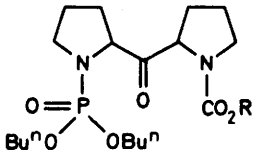


(89) a; R = H
b; R = Bu^n

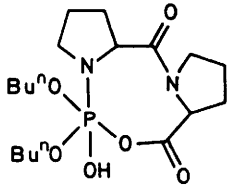
yield and the butyl ester (89b) in 28% yield. These products are suggested to arise via the pentacoordinate phosphorus intermediate (90).[218]

3.8 Peptides Containing Modified Protein Constituents – Azapeptides have been reviewed.[219] Peptides containing substituted Phe or Tyr residues are listed in Table 6 below.

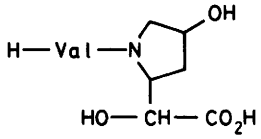
TABLE 6 Peptides Containing Substituted Aromatic Residues

Peptides	Ref
[Phe^2,$IodoTyr^9$]-vasotocin	220
[$desNH_2'$,pN_3Phe^9]-vasotocin	220
[$desNH_2'$,$biotinylPhe^9$]-vasotocin	220
[(2',3',4',5',6',-Br_5)Phe^8]-angiotensin II	221
H-m-FPhe-Ala-Ala-OH	222
[4-N_3(3,5-$_3$H)Phe^{10}]-$PKI_{6-22}NH_2$ (PKI=the heat stable inhibitor protein of cAMP-dependent protein kinase)	223
[o-$FTyr^1$]-[m-$FTyr^1$]-[p-$FPhe^1$],[o-F-Phe^4]-, [m-$fPhe^4$]-[p-CF_3Phe^4]-and[p-$FPhe^4$]Leu enkephalins	224

TRH analogues have been prepared in which pyroGlu has been replaced by 1-N-substituted-(S)-2-oxo-imidazolidine-4-carboxylic acid (Oic). The most potent CNS activities were shown by 1-benzyl-Oic-His-Pro-NH_2 (1.5-8.0 x those of TRH). Moreover, the thyrotropin-releasing activity of this analogue was only 1/16 th that of TRH.[225] A method has been developed for converting C-terminal proline residues in peptides into diethyl pyrrolidine-2-phosphonates. It involves oxidative decarboxylation followed by treatment of the resulting N-protected carbinolamide with triethyl phosphate/BF_3. The proline, however, is racemised during the process.[226]



(90)



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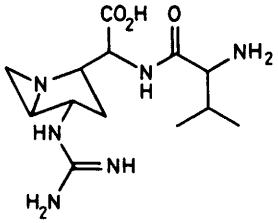
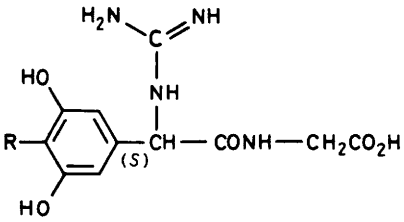


(92)



(93) A; R = Me_2CH
B; R = Et

The nitrosation of the hindered amide bonds of Z-Ile-Val-OMe and Z-Val-Leu-OMe has been examined. Using NO_2BF_4/Me_2S/pyridine or $NOBF_4$/pyridine in cold CH_3CN yields were quantitative.[227] Treatment of Pht-Gly-Gly-Val-OMe with N-bromosuccinimide/hν/CH_2CL_2 gives the α-bromo derivative of the C-terminal glycine residue. However, similar treatment of N-benzoyl-Gly-Gly-OMe brominates the N-terminal glycine. The bromides are not sufficiently stable for isolation and purification. They can be converted to the corresponding α-methoxyGly or α-deutero Gly derivatives with methanol or tri-n-BuSnD.[228]

Electrolysis in methanol of Z-or Boc-N-protected oligopeptides using anodes of Pt or glassy carbon causes substitution of the α-carboxyl group by methoxyl, i.e. forming N,O-acetals. Yields can be as high as 98%. These N,O-acetals react with trialkyl phosphite/$TiCl_4$ to give products where the alkoxy group has been replaced by a phosphodiester group. If such electrolyses are carried out in acetic acid instead of methanol, the α-carboxyl group of N-protected oligopeptides is replaced by a hydroxyl group.[229]

The crystal structure of Boc-Leu-Asu-Phe-NH_2 (where Asu = aminosuccinyl) shows a type II' β-bend conformation.[230] A c.d.study of some Asu-containing tri-and tetra-peptides shows that on going from polar to non-polar solvents there is a shift from unfolded to a folded conformation, the latter again incorporating a type II' β-bend.[231]

N-methylation at any of the last three amino acids at the C-terminus of dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) reverses μ/δ selectivity and gives compounds with less potent antinociceptive activity.[232]

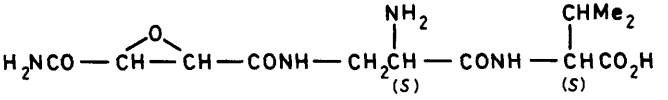
3.9 Peptides Containing Other Unusual Amino Acids – Several new dipeptide antibiotics falling into this category have been sequenced. Streptomyces antibioticus has yielded (91); the valine is of the L-configuration, but the other asymmetric centres have not yet been defined.[233] Ficellomycin was first obtained from Streptomyces ficellus in 1976, but has only now been assigned structure (92). The aziridine ring is thought to

be responsible for the antimicrobial activity.[234] The resorcinomycins A and B (93) have been isolated from Streptoverticillium roseoverticillatum, and show strong activity against a variety of acid-fast bacteria.[235]

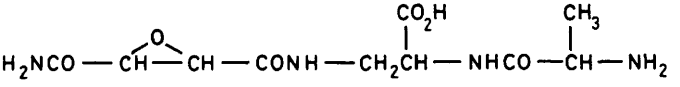
A new antifungal compound CB-25-I (94) has been obtained from Serratia plymuthica.[236] Its structure is partly related to Sch 37137 (95) produced by a Micromonospora.[237] Two new nikkomycins, ΨZ and ΨJ (96), have been extracted from Streptomyces tendae. They differ from nikkomycins Z and J, whose biological activity is higher, in having C-glycosidic instead of N-glycosidic linkages.[238] Other Streptomyces species have yielded antibiotic compounds with more extended peptide chains. The mureidomycins A,B,C, and D (97) from S. flavidovirens contain an unusual enamine sugar moiety, and they are specifically active against Pseudomonas aeruginosa.[239] Another new complex of antibiotics also selective against P. aeruginosa has been characterised from S. Coeruleorubidus. These nucleoside peptide hybrids, the pacidomycins (98), are closely structurally related to the mureidomycins.[240]

The strongly cytostatic depsipeptide dolastatin 15 from the Indian ocean sea hare Dolabella auicularia has been assigned structure (99). The pyrrolidone methyl vinyl ether group also appears in constituents of the Lyngba genus of blue-green algae. The sea hare may be obtaining and/or structurally modifying consituents of such cyanobacteria.[241] The absolute configuration of dolastatin 10 (100), the most potent antineoplastic substance known to date, has been proved by synthesis.[242]

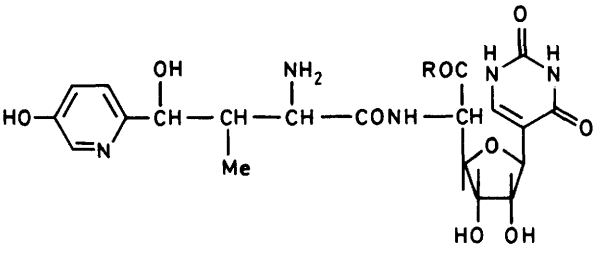
Three series of oligopeptides related to netropsin and distamycin have been synthesised. The first series comprises six alkylating agents (101). All six form complexes, but only some (101,b,c, and b) give rise to covalent adducts. Compounds (101,c and d) are active on melphalan-resistant L1210 leukaemia in mice.[243] The second series involves two oligopeptide sequences joined by a lipophilic linker, as depicted in (102). When X = 1,2,6, or 8, both the antitumour and anti-vaccinia virus activities were enhanced (up to 30 fold) relative to netropsin and distamycin.[244] In the third series, two compounds



(94)

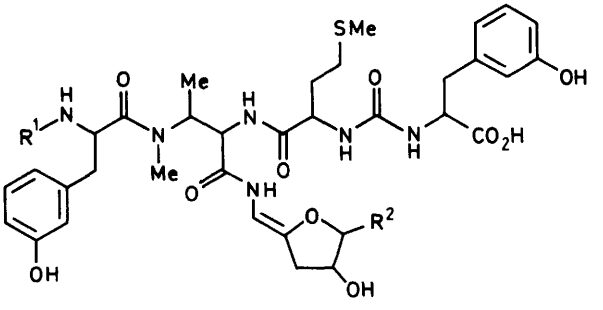


(95)



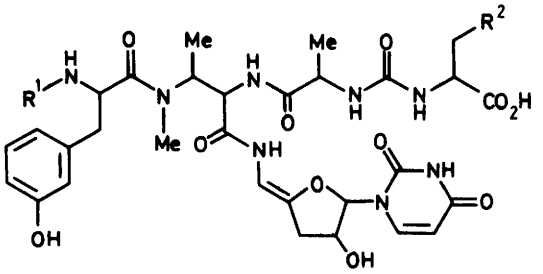
ψ Z; R = OH
ψ J; R = Glu

(96)

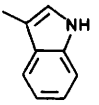


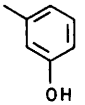
	R^1	R^2
A;	H —	uracil
B;	H —	dihydrouracil
C;	H —Gly—	uracil
D;	H —Gly—	dihydrouracil

(97)

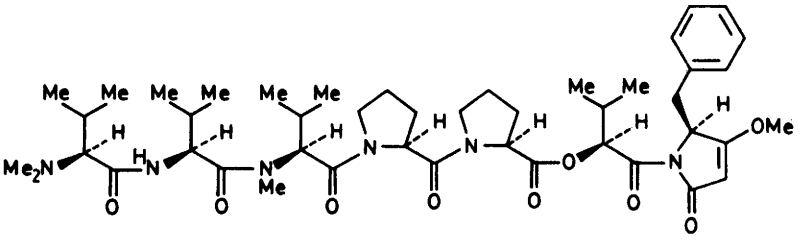


	R^1	R^2
1;	H - Ala -	A
2;	H - Ala -	Ph
3;	H - Ala -	B
4;	H	A
5;	H	Ph
6;	H - Gly -	A
7;	H - Gly -	Ph

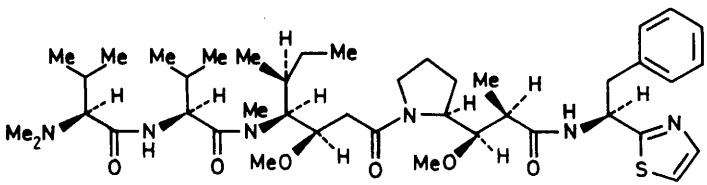
A = 

B = 

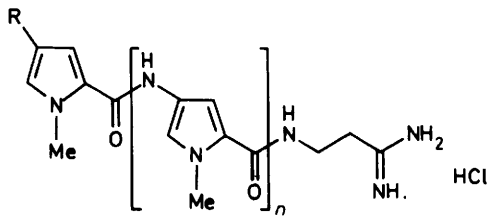
(98)

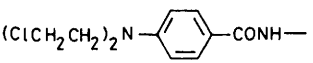
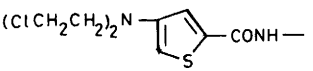
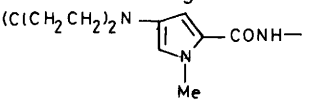


(99)

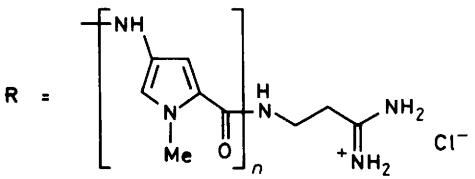


(100)



	R	*n*
A;	HCONH	2
B;	$(ClCH_2CH_2)_2N-$	2
C;	$(ClCH_2CH_2)_2N-$	3
D;		2
E;		2
F;		1

(101)



Series A; $R-CO(CH_2)_x-COR$; $n = 2$; $x = 1-10$
Series B; $R-CO(CH_2)_2-COR$; $n = 1$ or 3

(102)

(103) showed modest inhibitory activity on tumour cell proliferation.[245]

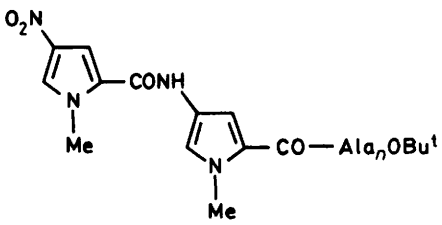
The immunomodulating peptide FR 900490 (104), isolated in 1988 from Discosia sp., has been synthesised. A key step was the formation of the imine (105) (Scheme 10) and its stereoselective reduction with $NaBH_3CN$.[246] A functionalized tripeptide (106) equivalent to the right half of the cyclic hexapeptide echinocandin has also been constructed in a highly stereoselective manner,[247] and the direct enzymic synthesis of 10-oxa-6α-methoxy-isopenicillin N (107) from (108) in 51% yield has been accomplished using isopenicillin N synthase.[248]

Dipeptides containing C-terminal α-amino glycine have been prepared by using the adducts derived from an amino acid amide, benzotriazole, and glyoxylic acid. An example is shown in Scheme 11.[249] A five stage conversion of C-terminal glycine residues in dipeptides into taurine has been developed, the lowest yield on any step being 81%.[250] The dipeptide D-β-amino-glutaryl-Ala-OH has been found to be a more potent inhibitor of γ-glutamylcyclotransferase than the D-D isomer; D-β-aminoglutaryl-L-alaninol is much less active.[251]

The reactions in aqueous solution of N-acylated tetrapeptides containing a 2,4-diaminobutyric acid (Dab) or a 2,3-diaminopropionic acid (Dap) residue have been explored.At pH 9.74 and 60°C, transpeptidation predominates (70-80%) over direct cleavage (Scheme 12), the reaction being strongly catalysed by phosphate and bicarbonate buffers. The resistance of lysine to transpeptidation under physiological conditions may explain the absence of Dab and Dap from proteins.[252]

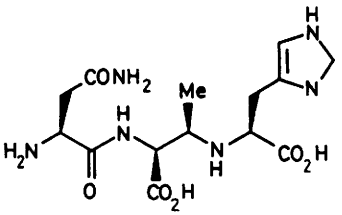
N-Dansyl-Tyr-Val-α-hydroxyGly-OH has been prepared by condensing Dns-Tyr(OBu^t)-Val-NH_2 with glyoxylic acid and treating the product with trifluoroacetic acid. The diastereoisomers were separated by RP-HPLC, but no stereochemical assignments made. Conversion back into Dns-Tyr-Val-NH_2 by α-amidating enzyme occurred with only one of the two isomers. Dns-Val-Tyr-N(OH)CH_2CO_2H is unaffected by the enzyme.[253]

Some other peptides containing unusual amino acids are listed in Table 7 below.

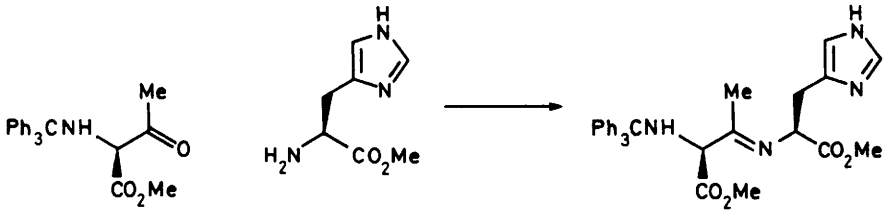


n = 1 or 2

(103)

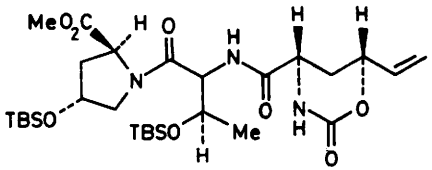


(104)



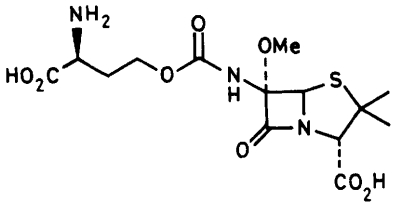
(105)

Scheme 10

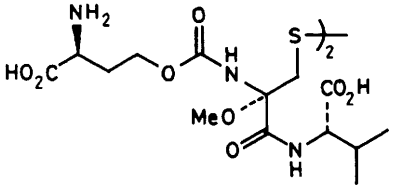


TBS = Bu^tMe_2Si—

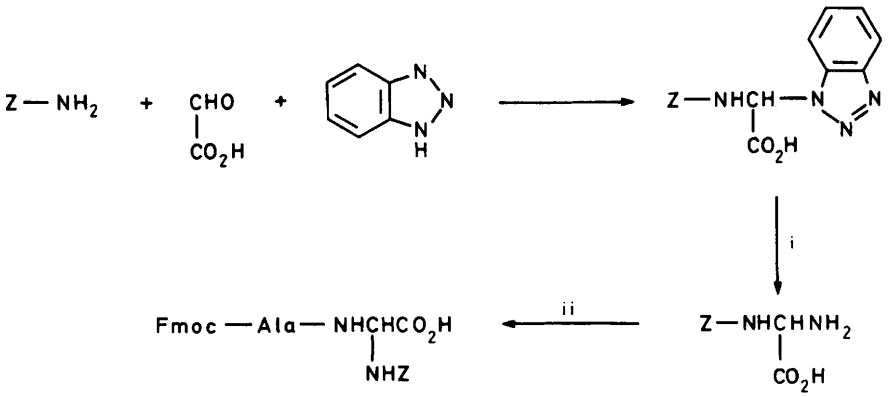
(106)



(107)

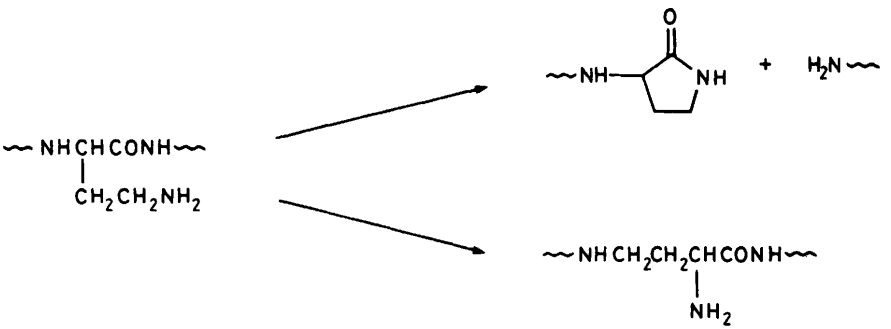


(108)

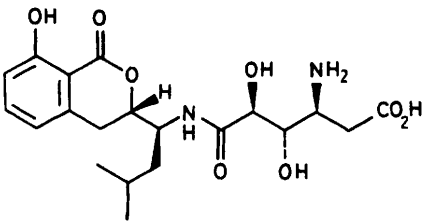


Reagents : i, NH_3; ii, Fmoc — Ala — OPfp

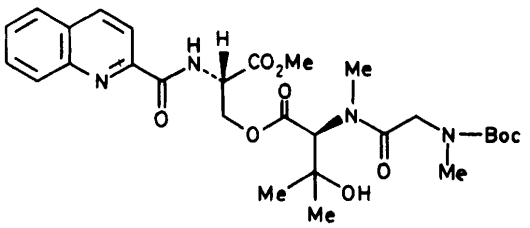
Scheme 11



Scheme 12



(109)



(110)

TABLE 7 Peptides Containing Other Non-Protein Amino Acids

H-Tyr-D-Arg-Phe-β-Ala-OH (analgesic)	254
Boc-[Nle^{28},Nle^{31},Naa or Cha^{33}]-CCK$_{27-33}$ (Naa = Naphthylalanine, Cha = cyclohexylalanine	255
[Naa^{9,11,13,15}]-gramicidin A	256
Ac-Tauryl-Phe-OMe (X-ray)	257
[β-HomoPhe^{3}]-[β-HomoPro^{7}]-[Cpp',Tyr(Me)^{2}, β-HomoPhe^{3}]-and[Cpp^{1},Tyr(Me)^{2}β-HomoPro^{7}]-arginine vasopressin (Cpp = β-mercapto-3,3-cyclopentamethylene propionic acid)	258
Boc-Tyr-Leu-β-HomoAsp-X (where X = NH_2, $NHCH_2Ph$, $NHCH_2CH_2Ph$, or D-Phe-NH_2) (gastrin agonists)	259
I-Naphthylpropionyl-[Naa^{6}]-LH-RH$_{4-9}$NHEt (antagonist)	260
Ac-Ama-Val-Ama-NHEt (Ama = aminomalomic acid)	261
[β-Ala^{29}]-CCK$_{27-33}$ analogues	262
Boc-(Nle-D-Nle)$_3$-Nle-D-MeNle-Nle-D-Nle-Nle-OMe	263
[Asu^{7,23}]-β-atrial natriuretic peptide$_{7-28}$ (Asu=L-α-aminosuberic acid)	264
[Sar,Nle^{5},Ile^{8}]-and[Sar^{1}(β-Me)Phe(S,S)^{5},Ile^{8}]-angiotensin II	265
Ac-Su-Gln-Asn-Xxx-Val-Val-NH_2 (where Xxx = 3-hydroxy-4-amino-5-phenylpentanoic acid, TyrΨ[CH_2N]Pro or PheΨ[CH_2N]Pro (analogues of the consensus retroviral protease cleavage sequence)	266

Finally, syntheses of the gastroprotective substance from Bacillus pumilis (109),[267] and a model tripeptide segment of luzopeptin (110)[268] have been reported.

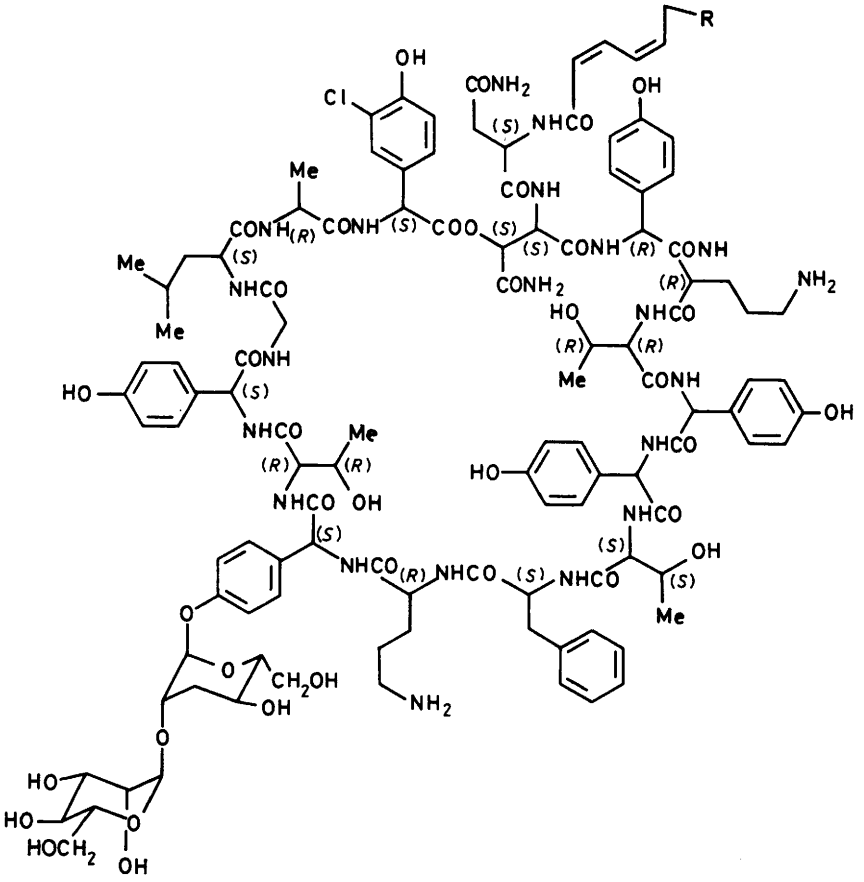
4 Conjugate Peptides

4.1 Glycopeptide Antibiotics – It has been proposed that vancomycin - ristocetin like glycopeptides should be named dalbheptides (from D-Ala-D-Ala-binding antibiotics with heptapeptide structures).[269] Three new glycopeptide antibiotics

have been described. Ramaplanin (111) from Actinoplanes sp. ATCC 33076 is a macrocyclic cyclodepsipeptide which contains dimannose and five residues of p-hydroxy-phenylglycine. It is active against ampicillin and methicillin resistant Gram positive bacteria.[270] Eremomycin, from an Actinomycete INA-238, differs from orienticin A only in the position of a chlorine atom. It is more active and less toxic than vancomycin and ristomycin. It does not cause local tissue damage and can be used for intramuscular injection; it is now on clinical trial.[271] The third new compound A42867, from a Nocardia species, differs from vancomycin only in the sugar portion (here it is rhamnosylglucose), the presence of the β-anomer of vancosamine, and in having only one chlorine atom in the peptide core. Its biological activity is similar to that of other ristocetin-vancomycin antibiotics.[272] Two less lipophilic analogues of teicoplanin have also been isolated from Actinoplanes teichomyceticus; they contain 6-methyloctanoic and n-nonanoic acids.[273]

It has been confirmed that A 82846B and chloro-orienticin A are identical. Their corresponding acid hydrolysis products des-epi-vancosamine A 82846B and chloro-orienticin B, and des-epi-vancosaminylglucose A 82486B and chloro-orienticin C, respectively, are also identical.[274] Vancomycin hexapeptide and aglucovancomycin hexapeptide have been prepared by using Edman degradation to remove the N-methyl-leucine. They lack any significant binding capability for Ac-D-Ala-D-Ala. This is attributed to protonation of the amino-terminus at the pH (5.6) of these studies.[275]

The dehalogenation of teicoplanin has been studied. The loss of the 22-Cl causes a decrease in binding strength to Ac_2Lys-D-Ala-D-Ala, and the removal of both chlorine further lowers the binding affinity. Preparation of the monochloro-compound was achieved using $NaBH_4$-$PdCl_2$ in MeOH.[276] The condensation of the carboxyl function of teicoplanin A_2, its pseodoaglycone, or its aglycone with various amines does not affect their ability to bind to Ac_2Lys-D-Ala-D-Ala. Most basic amides of teicoplanin A_2 are more active than the parent against Gram positive organisms, both in vitro and in vivo.[277]



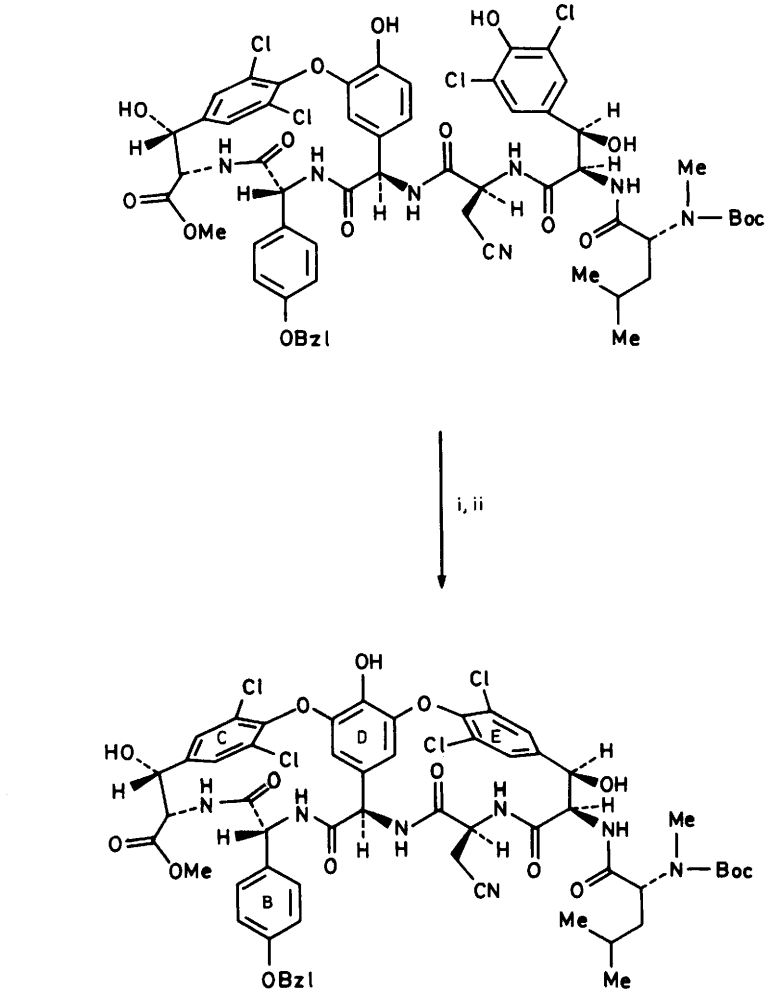
(111) A_1; R = Et
A_2; R = CHMe
A_3; R = CH_2CHMe_2

Proton-proton n.O.e. effects have been used to study the conformations of ristocetins A and Ψ (the latter lacks five of the six sugars of the former). In the ristocetin Ψ Ac_2Lys-D-Ala-D-Ala complex the aromatic ring of residue one folds over the H-bond network formed between the carboxylate anion of the tripeptide and the amide backbone of the antibiotic. Both ristocetins A and Ψ have been found, in the presence and absence of cell-wall analogues, to form dimers.[278]

A series of peptide derivatives of teicoplanin A2 and deglucoteicoplanin have been prepared by condensation of the carboxyl-63 function with amino-acid and dipeptide derivatives. The N^{ω}-nitro-Arg derivative was found to bind to Ac_2Lys-D-Ala-D-Ala 3.5 times as strongly as teicoplanin A2 itself.[279] Aminoacyl derivatives of the ristocetin aglycone have been made using N-Boc amino acid pentafluorophenyl esters, with subsequent removal of the Boc group. The antibacterial activity of these compounds was close to that of the aglycone parent, although the N-β-Ala derivative is of lower activity.[280]

A macrocyclic diether (112) which constitutes the basic skeleton of vancomycin has been synthesised; the last stage of this synthesis is depicted in Scheme 13. This work clearly shows the possibility of the total synthesis of vancomycin via biomimetic oxidative phenolic coupling.[281] Two monomacrocyclic compounds corresponding to vancomycin fragments have independently been prepared. One contains rings B,C, and D (of 112), and the other rings D and E.[282] Structurally related to the glycopeptide antibiotics, but lacking carbohydrate, is complestatin (113), from *Streptomyces lavendulae*. It is the most potent known inhibitor of the haemolysis of sensitised erythrocytes by the complement system. The glycopeptide antibiotics show no anti-complement activity, and complestatin only has very weak antibacterial activity.[283]

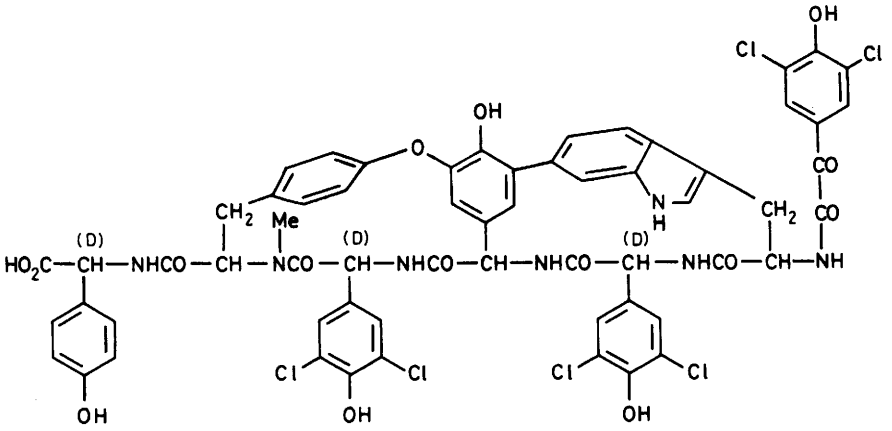
4.2 Other Glycopeptides – A number of oligopeptides with a single monosaccharide attached have been synthesised for a variety of reasons. [Glu(β-D-Glcp)^6]-substance P_{6-11} is one such compound, but the introduction of the sugar does not greatly affect the *in vitro* activity of the parent hexapeptide.[284] $Q^{1.5}$-



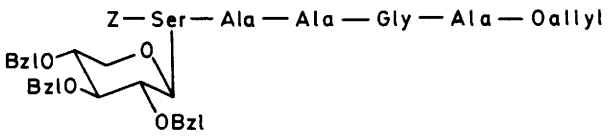
(112)

Reagents: i, $Tl(NO_3)_3$; ii, $CrCl_2$

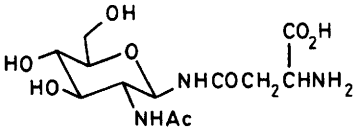
Scheme 13



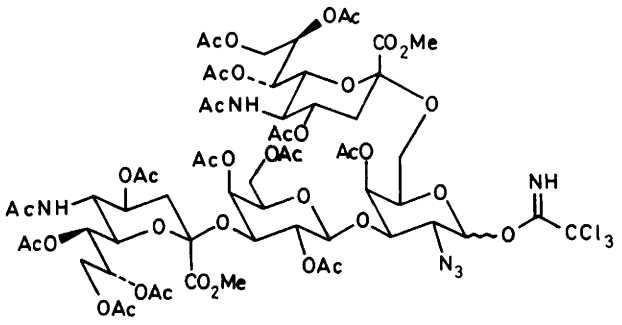
(113)



(114)



(115)



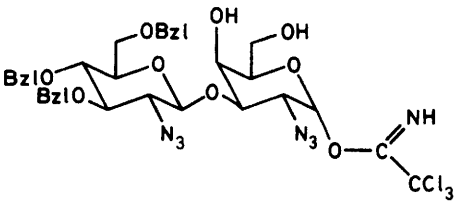
(116)

(β-D-Gal$_p$)[D-Met^2,Hyp^5]-enkephalin amide, however, has proved one of the most potent *in vivo* opioid agonists yet achieved.[285] Allyl esters have been used as carboxy protecting groups in the synthesis of (114); they are removed by Pd (0) - catalysed allyl transfer to morpholine.[286] An alternative synthesis of 6-O-(Tyr-Gly-Gly-Phe-Leu)-D-glucopyranose has been effected. In the key step Boc-Phe-Met-OPcp was coupled with D-glucose in the presence of imidazole to give 40% of the glucoside.[287]

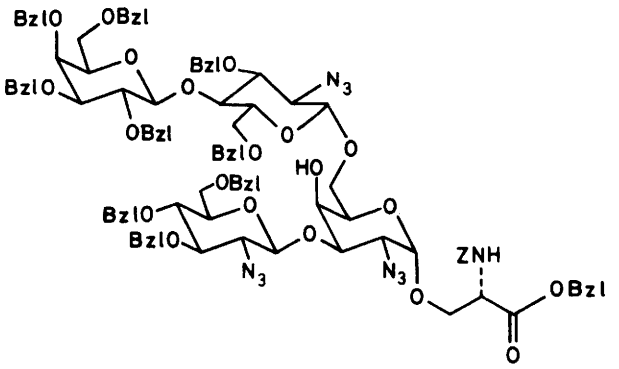
The ^{1}H-n.m.r. spectrum of Ac-Thr(α-GalNAc)-Ala-Ala-OMe has been examined as a model of antifreeze glycoprotein. The temperature dependence of amide proton chemical shifts strongly suggest the presence of a GalNAc-NH to Thr-CO intramolecular H-bond.[288] To test the specificity of glycosyl transferases the decapeptide H-Ala-Pro-Thr(α-D-GalNAc)-Ser$_3$-Thr-Lys$_2$-Thr-OH has been prepared, being part of the N-terminus of interleukin 2. Also prepared was H-Ala-Pro-Thr(β-D-Gal-(1-3)-α-D-GalNAc)-Ser$_2$-OH.[289]

A segment of ancofoetal fibronectin, H-Val-Thr(β-D-Gal$_p$(1-3)-α-D-GalNAc$_p$)-His-Pro-Gly-Tyr-OH, has been prepared by a solid phase method, using Fmoc-amino acids, acetyl protection for sugars, and a super-acid-sensitive resin. His and Tyr side chains were benzyl protected.[290] The sialylglycopeptide α-D-Neu$_p$-Ac-(2-6)-β-D-Gal$_p$(1-4)-β-D-Glc$_p$NAc-(1-4N)-L-Asn-OH has been prepared from (115) using an immobilised multienzyme system that regenerates UDP-α-D-galactose *in situ* to add the galactose residue (in 26% yield). Subsequent incubation with soluble α-(2-6)-sialyltransferase and CMP-Neu5Ac gave the sialyl-N-acetyllactosaminyl-asparagine (in 38% yield).[291]

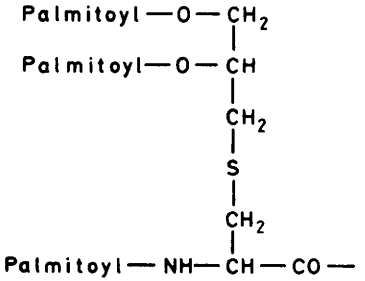
Two examples of the synthesis of amino acids attached to branched chain tetrasaccharides have been reported. Both are directed towards mucin-type glycoprotein models. In one case the tetrasaccharide derivative (116) was coupled with Z-Ser-OBzl in the presence of $BF_3.Et_2O$ at 15 ^{0}C to give 12% of the α-glyco amino acid and 32% of the corresponding β-anomer, separable by column chromatography. The azide group was subsequently converted to the 2-acetamido derivative.[292] In the other case (117) was coupled with Z-Ser-OBzl, again using $BF_3.Et_2O$ as



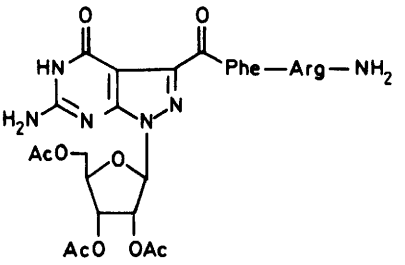
(117)



(118)



(120)



(119)

C_6F_{13} or $C_8F_{17}-CH_2CO-Gly-Xxx-Gly-NH_2$ or O—decyl

(121)

catalyst, in 88% yield. Further coupling with another protected disaccharide trichloracetimidate gave (118) in 80% yield.[293]

Glyceraldehyde has been found to react faster with the α-amino group of tripeptides than with dipeptides. Tripeptides give rate optima in the range pH 8.5-10, 1-2 units higher than found with dipeptides. The second amino acid residue influences not only the rate of reaction, but also the extent of formation of the Amadori rearrangement product, the ketoamine. In both di- and tri-peptides, the presence of His as the second amino acid residue (from the N-terminus) greatly accelerates the reaction.[294]

New analogues of muramyl dipeptide are listed below in Table 8.

TABLE 8 MDP Analogues

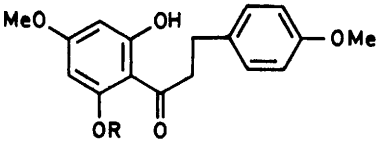
Peptide	Ref.
Two analogues containing a carbocyclic analogue of D-glucosamine	295
Conjugates of 1-thio-NAcMur dipeptide with lipid A subunit analogues	296
Conjugates of MDP and 6-deoxy-6-mycolyl-amino-α, α-trehalose	297
N-Ac-6-O-phosphonoMur-Ala-D-iGln-OMe	298
Analogues containing a masked thiol function at the C-terminus	299
Alternative synthesis of O-(2-acetamido-2-deoxy-β-D-Glcp)-(1-4)-N-AcnorMur-L-Abu-D-iGln-OH	300
Conjugate of N-Ac-Mur-Als-D-iGln-cysteamine with N^{α}-maleoyl-β-alanyl-[methoxinine^{15}]-human little-gastrin-1_{2-17}	301

4.3 Non-Carbohydrate Peptide Conjugates – Biotinyl-aminohexanoyl-[Ala^{1}Phe(4-N_3)^8]-angiotensin II has been prepared as a photo-activatable probe for angiotensin receptors. It binds well to angiotensin II receptors from rat liver membranes.[302] The 6-azacadeguomycin derivative (119) has been evaluated for its ability to potentiate T-cell responses to plant mitogens, but showed no increase in lymphocyte proliferation.[303] Several 2,3-and 3,4-dihydroxybenzoyl-dipeptides have been synthesised and their leucine aminopeptidase inhibiting abilities evaluated. The most active compound was 2,3-dihydroxybenzoyl-Ala-Phe-OH, IC_{50}2.5 μM.[304]

The synthetic lipopeptide analogues Pam-Cys-Ala-Gly-OH and Pam-Cys-Ser-Lys_4-OH, where Pam is (120), induce tumour cytotoxicity in murine bone marrow-derived macrophages comparable to the effects of lipo-polysaccharides from the outer membrane of E coli.[305] Some non-ionic surfactants (121) have been prepared in which the hydrophilic part is an oligopeptide and the hydrophilic end is an alkylperfluoro group. The sarcosine compound proved the most water soluble, allowing a greater lowering of surface tension.[306] Two dihydrochalcone peptide conjugates (122) have been prepared as part of a programme to find specific blockers of the membrane transport of small peptides in animal small intestines and kidney. No biological activities are indicated.[307] Novel LH-RH antagonists based on the structure of the antifungal drug ketoconazole have been reported. The most active of the series is (123), IC_{50} 0.48 μM.[308]

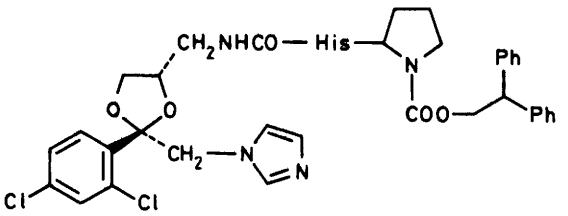
Peptide derivatives of aza-18-crown-6 (124) and 4,13-diaza-18-crown-6 (125) have been prepared. An X-ray examination of (124a) both in the free form and as its NaI and KI complexes was also carried out. Unlike the case of valinomycin, the amide rather than the ester CO is involved in binding. In type (124) compounds, the K(I) binding contacts are approximately an order of magnitude greater than those for Na(I), but in type (125) compounds the binding ability is lower and there is no selectivity for K(I).[309]

A number of conjugates reported involve the thiol group of cysteine. The synthesis of peptides containing S-(N-alkyl-

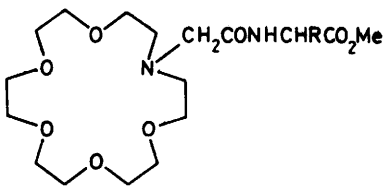


R = —Gly—Gly—OH or —CH_2CH_2CHCO—Gly—OH
(with NH_2)

(122)

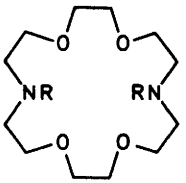


(123)



R = Me, CH_2Ph, CH_2CHMe_2, $CHMe_2$, and $CH_2CHMeEt$

(124)



R

a; GlyGlyOMe

b; GlyAlaOMe

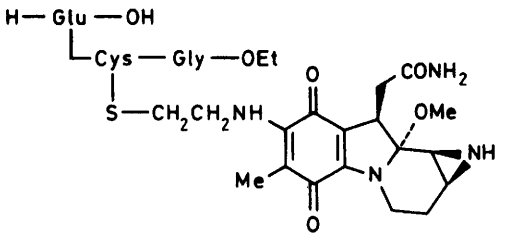
c; GlyPheOMe

d; GlyLeuOMe

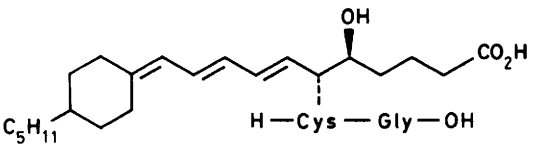
e; GlyIleOMe

f; GlyValOMe

(125)



(126)



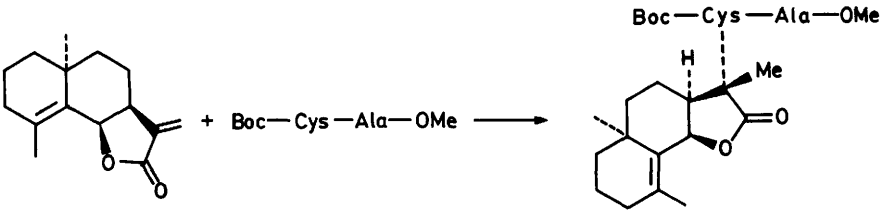
(127)

carbonyl)cysteine has been explored and S-(N-methylcarbamoyl)glutathione prepared. Carbamoylation of cysteine compounds is known to occur in the metabolism of N-alkylformamides in both rodents and humans.[310] A water soluble conjugate of glutathione with mitomycin C (126) has been found far more effective against sarcoma 180 and leukaemia P 388 than mitomycin C itself.[311] The conformationally restricted analogue of the natural peptide-leukotriene LTD_4 (127) has an affinity for guinea pig lung preparation two orders of magnitude lower than LTD_4, but has no antagonistic activity.[312]

Stereoselective addition of Boc-Cys-Ala-OMe to α-methylene-γ-butyrolactones has been studied. In phosphate buffer (pH7.4)-ethanol, for example, (-)-frullanolide gives (128) (Scheme 14). This is parallel to the observed high enantioselectivity of the allergic skin reaction in the guinea pig; almost no cross-reaction to the (-)-enantiomer was observed in (+)-enantiomer sensitised animals and vice versa.[313] A novel synthetic foot-and-mouth disease peptide vaccine consisting of virus $VPI_{125-134}$ antigenic determinant linked to the carboxyl terminus of tripalmitoyl-S-glyceryl-Cys-Ser-Ser-OH as a built in adjuvant has been prepared. This vaccine induces protection against homologous challenge and serotype-specific virus neutralising antibodies in guinea pigs after single administration without further adjuvants or carriers.[314]

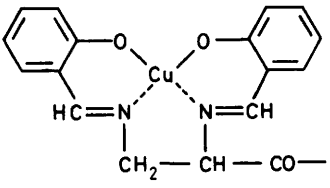
Some metallopeptide analogues of LH-RH have proved to have high biological activity. When platinum is co-ordinated to an N^{ε}-(D,L-2,3-diamino-3-propionyl)-D-lysine residue in Glp-His-Trp-Ser-Tyr-D-Lys(A_2pr)-Leu-Arg-Pro-Gly-NH_2, the LH-releasing potency is 50x that of native LH-RH. [Ac-D-Nal(2)^1,D-Phe(p-Cl)^2,D-Pal(3)^3, Arg^5, D-Lys(X)^6,D-Ala^{10}]-LH-RH, X being (129), causes 100% inhibition of ovulation at a dose of 3 μg in rats.[315]

Chlorambucil (Chl) and melphalan (Mel), the nitrogen mustard derivatives of 4-phenylbutyric acid and Phe, have been incorporated into other LH-RH analogues. Of the peptides prepared, [D-Mel^6]-LH-RH and [Ac-D-Nal(2),D-Phe(P-Cl)^2,D-Pal(3)^3 Arg^5,D-Mel^6,D-Ala^{10}]-LH-RH possessed the expected high agonist and antagonist activities respectively. Their in vitro effects

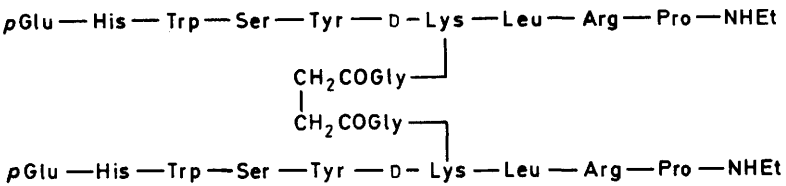


(128)

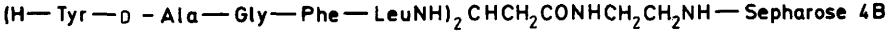
Scheme 14



(129)



(130)



(131)

make them suitable for the study of how alkylating analogues of LH-RH could interfere with intracellular events in certain cancer cells.[316]

Dimeric analogues (130) of des-Gly^{10}-[D-Lys^{6}]GnRH-NHEt have been made. All three displayed increased activity in receptor binding and in LH-release assays with respect to the parent monomer; when n=1 the highest activity in vitro and in the in vivo postcoital assay were observed.[317] A photoreactive analogue of bovine parathyroid hormone, [Nle^{8},Lys(N-ε-4-N_3-2-nitrophenyl)^{13}Nle^{18},Tyr^{34}]-$BPH_{1-34}NH_2$, has been prepared.[318]

Moving on to C-terminal conjugates, some N-(dipeptidyl)-S-acetylcysteamine and N,N-(dipeptidyl)cystamine salts have been evaluated as radioprotective agents. The most active compound was N-glycylglycine-S-acetylcysteamine trifluoroacetate.[319] As dansyl peptides are ideal substrates for fluorometric proteolytic enzyme assays, two general methods for labelling synthetic peptides with a C-terminal dansyl group using solid phase peptide synthesis have been developed.[320] Two enkephalin analogue molecules have been cross-linked and attached to Sepharose gel to give (131) for use in receptor affinity purification.[321]

Two models have been developed for synthetic hepatitis B vaccines. Two peptides comprising a determinant of hepatitis B surface antigen and residues 16-26 of the pre-S(2) region of the middle protein were incorporated as either monoepitope or diepitope multiple antigen peptides (MAP's). H-Lys-Ala-OCH_2-Pam resin was coupled with Boc-Lys(Boc)-OH then N-deprotected. This process was repeated, giving a branched peptide chain with eight free amino groups. These were coupled then to either $(TKPTDGN)_2$ or LQDPRVRGLYFPAGG (suitably protected) to give the vaccine models. MAP's containing both epitopes were made using H-Lys-Cys-Ala-OCH_2-Pam resin, which was built up as before. The two different branched epitopes were the coupled together via cystine bridges. The biological responses obtained with these materials show that the diepitope MAP model eliminates the need for a protein carrier and that the pre-S(2) peptide determinant serves as a T-helper cell epitope that enhances the immune response of the S-region and overcomes

the poor immunogenicity encountered with a single epitope of the S region.[322]

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5
β-Lactam Antibiotic Chemistry

BY C. H. FRYDRYCH

1. INTRODUCTION

The β-lactam ring continues to provide a synthetic challenge to a multitude of chemists in both academia and industry. The preparation of intermediates to carbapenems continues to attract considerable attention. Last year showed an explosion of interest in carbon analogues of cephalosporins.

I have retained all of the sections used by my predecessor, including the Appendix.

This year has seen the publication of the latest conference report "Recent Advances in the Chemistry of β-Lactam Antibiotics"[1] covering the excellent sessions at Cambridge in 1988. Two reviews on the synthesis of penems have appeared[2,3] together with a review of the synthesis of 1β-methylcarbapenem intermediates[4] and another covering all non-classical β-lactam antibiotics.[5] The preparation of monobactams by cycloaddition[6] has been reviewed, a further publication covers all aspects of their preparation.[7] Cycloaddition routes to other types of β-lactams have been reviewed.[8] Two comprehensive reviews of the ester enolate imine condensation route to β-lactams have appeared.[9,10] A review of the photolytic reactions of chromium carbene complexes[11] includes the preparation of various β-lactam derivatives.

2. NEW NATURAL PRODUCTS

No reports of new β-lactam containing natural products have been found in the 1989 literature.

3. BIOSYNTHESIS

The biosynthetic pathway to penicillins and cephalosporins continues to attract considerable attention. The investigation is aided by the availability of purer forms of the enzymes involved.[12]

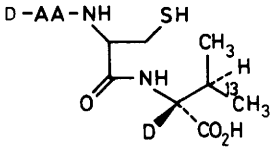
The use of a mutant form of *Cephalosporium acremonium* which has no β-lactam synthesising enzyme has allowed further elaboration of the biosynthesis of the tripeptide precursor

LLD-ACV.[13] The use of doubly-labelled ($^{2}H_{6}$ $^{18}O_{2}$) valine for intracellular synthesis of LLD-ACV by this mutant has allowed isolation of labelled tripeptide. Mass spectrometry then revealed that one and both valine oxygen atoms can be lost during its incorporation into the tripeptide. It has previously been shown that no further oxygen exchange occurs during conversion of LLD-ACV to isopenicillin N.

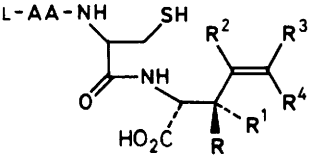
The availability of purer forms of the enzymes isopenicillin N synthetase (IPNS) and desacetoxycephalosporin C/desacetylcephalosporin C synthetase (DAOC/ DAC synthetase) has prompted a re-examination of the stereospecificity of valinyl methyl group incorporation into the previously reported products (see Volume 20).[14] Doubly labelled (valine 2-^{2}H, 4-^{13}C) unnatural substrate D,L,D-ACV (**1**) is converted by IPNS directly to penicillin N. Subsequent treatment with DAOC/DAC synthetase gave each of the expected ring-expansion products with greater than 95% stereospecificity. The latter enzymatic conversion has also been investigated using the labelled cofactors $^{18}O_{2}$ and C2-^{13}C-α-ketoglutarate.[15] The results indicate that ^{18}O is >98% incorporated into the succinate produced but only partially into the desacetylcephalosporin C which results.

Further unnatural substrates, modified in the valinyl portion of natural LLD-ACV, continue to expand the scope of enzyme mediated β-lactam synthesis and further the understanding of the biosynthetic pathway. In the course of detailed deuterium labelling studies with the allylglycine tripeptides (**2a-d**) (see also Volumes 19 and 21) a further product (**3**) has been isolated. Epoxide formation from an iron oxene intermediate to give (**4**) is proposed as a possible mechanism.[16] In a further modification, the 3-methylallyl peptides (**2e,f**) were prepared[17] in order to provide an insight into the stereochemical requirements of the cyclisation process. Incubation of (**2e**) with IPNS provided the 2α-vinyl penam (**5**) as the sole β-lactam product. In contrast (**2f**) gave rise to the cepham (**6**) and homoceph-3-em (**7**). Penam (**5**) is seen as arising *via* the normal desaturative mechanism by way of a conformationally restricted intermediate while (**6**) and (**7**) are formed by [2+2] and ene reactions respectively of a more conformationally mobile iron-oxene intermediate.

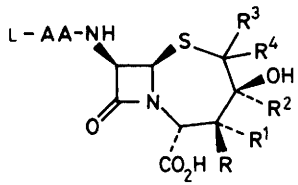
Further examples of the production of monocyclic γ-lactams by incubation of modified substrates with IPNS (see also Volume 21) have now appeared.[18] The tripeptide (**8**) in which the normal cysteine and valine have been replaced by homocysteine and cysteine respectively gave a single product upon incubation with IPNS. The compound was shown to be the bicyclic γ-lactam



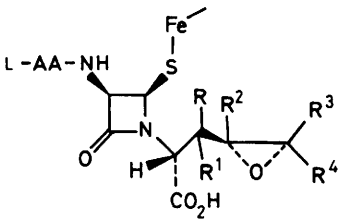
(1) D-AA=D-α-aminoadipyl



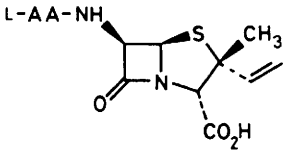
(2)**a**; R = R^1 = R^2 = R^3 = R^4 = H
b; R = R^1 = D, R^2 = R^3 = R^4 = H
c; R = R^1 = R^2 = R^3 = D, R^4 = H
d; R = R^1 = D, R^2 = R^3 = H, R^4 = D
e; R = H, R^1 = Me, R^2 = R^3 = R^4 = H
f; R = Me, R^1 = R^2 = R^3 = R^4 = H



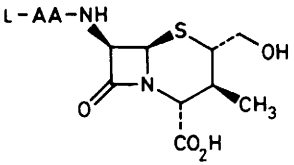
(3)



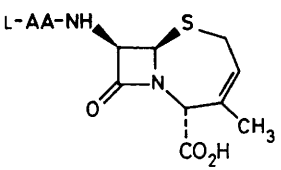
(4)



(5)



(6)



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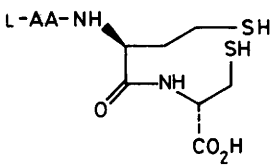

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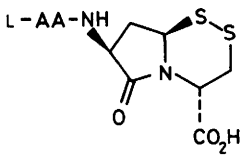
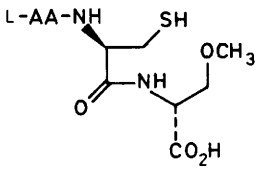
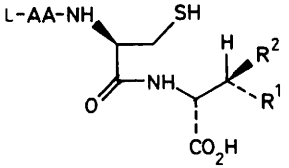


(9)



(10)



(11)**a**; R^1 = OMe, R^2 = CH=CH_2
b; R^1 = CH=CH_2, R^2 = OMe

disulphide (**9**). The mechanism proposed for its formation involves intramolecular trapping of an iron-bound monocyclic γ-lactam by the cysteine thiolate.

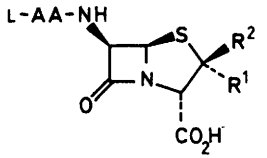
Continuing studies on the incorporation of a methoxyl group into the valinyl residue of LLD-ACV have involved the O-methyl serine peptide (**10**) and unsaturated methoxy peptides (**11 a,b**).[19] Incubation of (**10**) with IPNS gave a mixture of α- and β-methoxy penams (**12**). In contrast (**11a**) provided a 2-methoxy-2-vinyl penam of unstated stereochemistry (**13**) and the homoceph-3-em (**14**) while (**11b**) failed to give any β-lactam-containing products. The failure of (**11b**) to act as a substrate for IPNS is possibly due to extra hydrogen bonding of the methoxy substituent within the active site resulting in an unreactive conformation of the putative intermediate.

A report has appeared[20] detailing the synthesis of the 2-methoxy cysteine containing tripeptide (**15**) in which the aminoadipoyl moiety of L,L,D-ACV is also modified by replacement of a methylene by oxygen. Incubation of (**15**) with IPNS provided 6α-methoxy-10-oxaisopenicillin N (**16**). This result demonstrates a considerable steric and electronic tolerance by the enzyme IPNS to substitution at the cysteinyl 2-position.

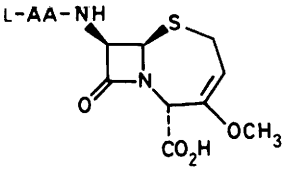
A mechanistic study on the enzyme clavaminate synthase (CS), now purified to homogeneity, has furthered our understanding of clavulanic acid biosynthesis.[21] Incubation of [2-^{2}H, 3-^{13}C] proclavaminic acid (**17**) with CS provided clavaminic acid (**18**) with no loss of deuterium label. The fate of the C-3' hydrogens was examined by conversion of [2-^{2}H, 3'-$^{2}H_2$] proclavaminic acid (**19**) into clavaminic acid (**20**) again with no significant loss of deuterium label. Finally, the heavy oxygen labelled [3-^{13}C, ^{18}O] proclavaminic acid (**21**) provided ^{18}O labelled clavaminic acid (**22**) with complete retention of label.

4. PENICILLINS AND CEPHALOSPORINS

Syntheses of these bicyclic systems and their parent penam and cepham rings will be detailed first. Reaction of 4-acetoxyazetidinone derivative (**23**) with an unsaturated thiol followed by N-protection and ozonolysis gave (**24**).[22] Condensation with benzyloxynitromethane, elimination, subsequent desilylation and ozonolysis resulted in cyclisation to the penam-3β-carboxylate (**25**). Epimerisation completed the synthesis of benzyl penicillanate. A full report has now appeared[23] (see also Volume 20) on the preparation of penams by condensation of thiol containing



(12) R^1, R^2 = H, OMe
(13) R^1, R^2 = OMe, $CH{=}CH_2$



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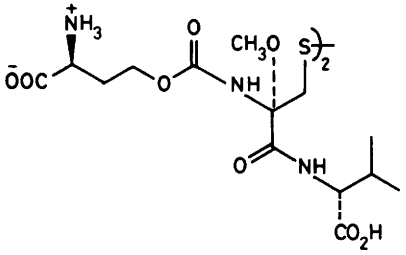

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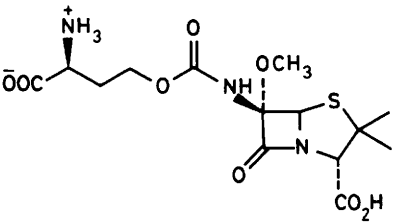

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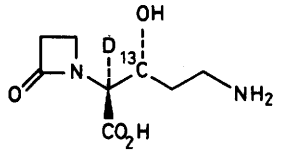

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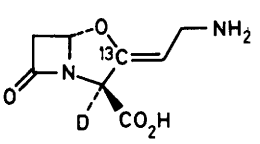

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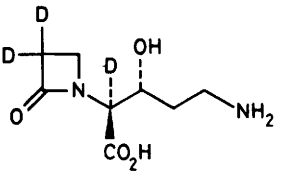

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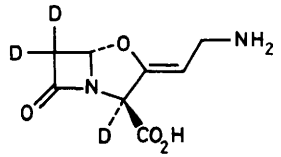

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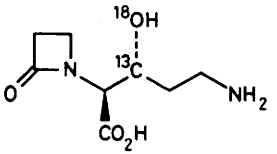

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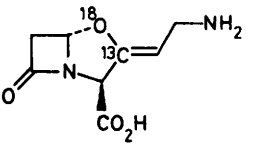

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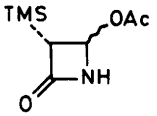
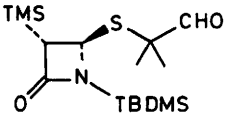
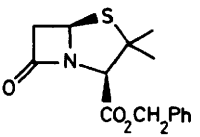


(23)
TMS = Me_3Si



(24)
TBDMS = Me_2Bu^tSi



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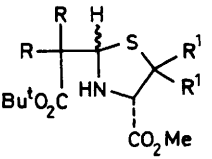
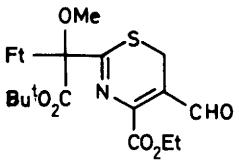


(26)

amino acids with various formylacetate synthons to give (**26**); subsequent cyclisation by the Mukaiyama-Ohno method has provided 2- (and 6-) substituted penam-3-carboxylates.

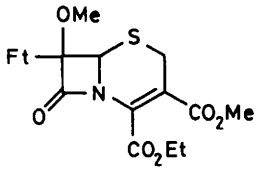
The annulation of thiazines (**27**), prepared by hetero Diels Alder reaction (see also Volume 21),[24] has resulted in the total synthesis of the cephamycin framework (**28**), the stereochemistry at C(7) was not determined. Publications (see also Section 9) have appeared detailing the β-lactamase inhibitory activity of 7-hydroxyethylcephem analogues.[25] The much-used carbapenem intermediate (**29**) was elaborated by C4-substitution and phosphorane introduction to (**30**), Wittig cyclisation and further manipulation at the cephem 3-position provided a series of potent inhibitors (**31**). A radical-based route to Δ2 cephems involved treatment of chloro derivative (**32**) with tributyltin hydride;[26] only endo cyclisation onto the acetylene was observed providing (**33**).

There are a smaller number of papers on penicillin transformations this year. The preparation of 2β-substituted penams and penicillin to cephalosporin conversions continue to feature highly. A report details the incorporation of a fluorine containing substituent at the penicillin 6α-position.[27] Reaction of 6α-hydroxymethyl derivative (**34a**) with diethylamino-sulphur trifluoride (DAST) provided (**34b**), the formyl derivative (**34c**) provided the fluorohydrin (**34d**). None of the difluoromethyl analogue (**34e**) was observed even after prolonged treatment with excess DAST. A report on the preparation of penicillin sulphoxides details the use of a hydrogen peroxide-formic acid-polyphosphoric acid system.[28] The actual oxidant is believed to be performic acid. The desulphurisation of penicillins with triphenyltin hydride occurs *via* thiostannane (**35a**),[29] a homolytic chain mechanism was proposed, 4-unsubstituted derivatives (**35b**) were obtained in high yields. An alternative procedure for halogenative cyclisation of disulphides (**36**) to 2β-halomethyl penams involves a two-phase system.[30] Addition of sodium nitrite to an aqueous hydrohalic acid-dichloromethane mixture provides (**37**); the active agent is thought to be a nitrosyl halide. A report[31] compares the reactions of 6-acylamino, 6-phthalimido and 6-diacylamino (**38 a,b,c**) azetidinone disulphides under halogenative cyclisation conditions. The diacylamino derivatives are in general less reactive but similar products are formed in all cases. A full report has now appeared[32] detailing the factors which influence the relative rates of β-elimination and C6-epimerisation of penam sulphones (**39**) when treated with base. Careful choice of 3α-substituent, either acyl or ester, increases the acidity of the 3β-hydrogen allowing



(27)

Ft = Phthalimido



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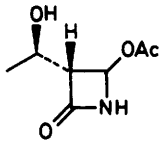

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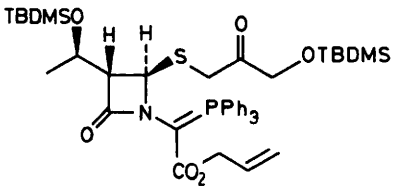

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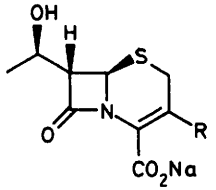
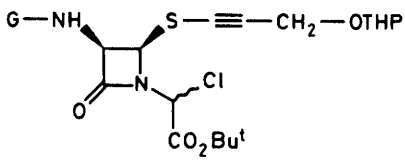
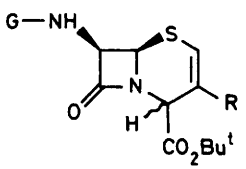


(31)

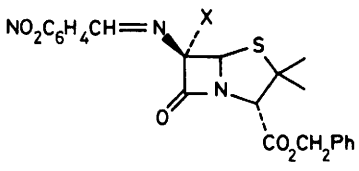


(32)

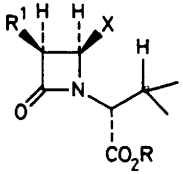
G = $PhCH_2CO$, THP= Tetrahydropyranyl



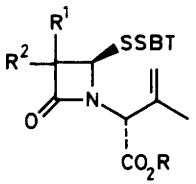
(33)



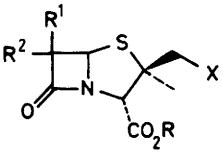
(34) **a**; X = CH_2OH
b; X = CH_2F
c; X = CHO
d; X = CH(OH)F
e; X = CHF_2



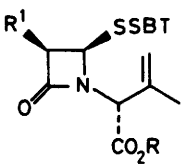
(35) **a**; X = $SSnPh_3$
b; X = H



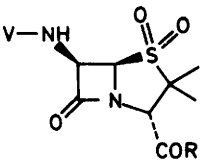
(36) BT =



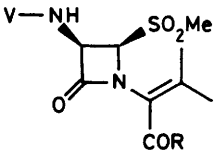
(37) X = Cl, Br



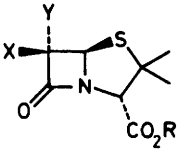
(38) **a**; $R^1 = R^2CONH$
b; R^1 = Ft
c; $R^1 = (R^2CO)_2N$



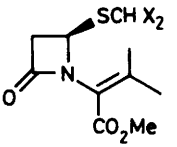
(39) V = $PhOCH_2CO$



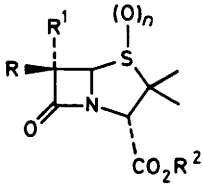
(40)



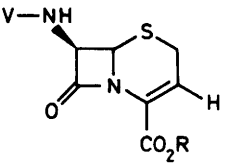
(41) **a**; X = Y = Hal
b; X = Hal, Y = H
c; X = H, Y = Hal
Hal = Cl or Br



(42) X = Cl, Br



(43) **a**; R = H, $R^1 = CH_2CH{=}CH_2$, $n = 0$
b; R = H, $R^1 = CH_2CH{=}CH_2$, $n = 2$
c; $R = CH_2CH{=}CH_2$, R^1 = H, $n = 0$



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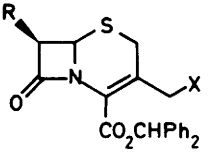
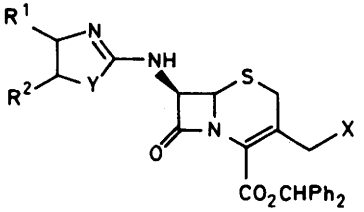
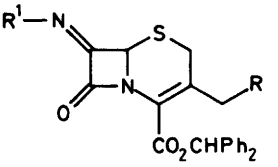


(45) **a**; $R = -\overset{+}{N}{\equiv}\overset{-}{C}$
b; $R = -N{=}CBr_2$



(46)
$R^1 = R^2$ = H
$R^1R^2 = C_6H_4$



(47) **a**; R^1 = H
b; R^1 = MeS

efficient preparation of 3,4-*cis* azetidinone sulphinates (**40**).

A number of publications have appeared concerning 6-halo- and 6,6-dihalo-penams. The preparation and use of a new organotin hydride, trineophyltin hydride,[33] allows efficient reductive dehalogenation of 6,6-dihalo derivatives (**41a**) to 6β-halo compounds (**41b**). The high diastereoselectivity observed is ascribed to the steric bulk of the reagent. The same authors[34] have developed a complementary synthesis of 6α-halopenams (**41c**). Reaction of a 6,6-dihalo derivative with either hydrogen and catalytic Wilkinson's catalyst, or methanol and stoichiometric Wilkinson's catalyst, provides a mixture of 6-halopenams in which the α-isomer predominates. In a further report[35] they have shown that catalytic hydrogenation with 5% $Pd/CaCO_3$ or 5% Rh/Al_2O_3 also gives predominantly 6α-halopenams (**41c**). When methanol is present the reactions are vastly accelerated and methanol not molecular hydrogen acts as the hydrogen source. The reductive removal of bromine from 6-bromo and 6,6-dibromopenicillanates at sulphide, sulphoxide and sulphone oxidation levels has been achieved[36] using aluminium metal and catalytic lead bromide in methanol containing hydrobromic acid.

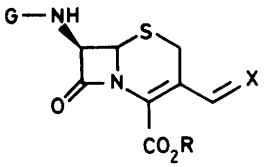
The reaction of a number of 6-substituted penicillanates with Seyferth reagents ($PhHgCX_3$, X = Cl, Br) has been reported[37] as giving the corresponding 4-dihalomethylthioazetidinones (**42**), isolated after triethylamine mediated isomerisation. The novel 6-allylpenicillanates (**43a-c**) have been prepared;[38] reaction of 6α-bromopenicillanate and the corresponding sulphone gave the 6α-allyl derivatives (**43a**) and (**43b**) respectively, under similar conditions 6,6-dibromopenicillanate provided an α-allyl-β-bromo derivative which yielded 6β-allyl analogue (**43c**) after treatment with tributyltin hydride.

Moving on to cephalosporin chemistry, a route to 3-unsubstituted derivatives involves reaction of an azetidinone disulphide like (**38a**) with allyl bromide.[39] The resulting diolefin was ozonolysed and further treatment with trimethyl phosphite provided (**44**). At the C(7)-position, reaction of the isocyanide (**45a**) with bromine provides the dibromo derivative (**45b**),[40] reaction with dinucleophiles then provides various C(7)-linked aminoheterocycles (**46**). An improved procedure for epimerisation of 7α-aminocephalosporanates and their oxa-analogues involves sodium cyanoborohydride reduction of 7-imino derivatives (**47a**).[41] These are in turn available from the methylsulphenimines (**47b**) by acid catalysed methanolysis.

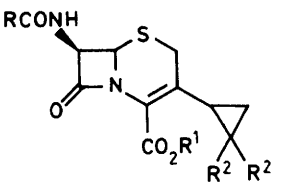
The search for novel, biologically advantageous C(3)-substituents continues apace. An

alternative synthesis of 3-alkenylcephems involves reaction of the 3-formyl compound (**48a**) with alkyl Grignard reagents.[42] Elimination *via* a mesylate of the resultant secondary alcohols provides 3-vinyl- and 3-(2-propenyl)cephems (**48b**) and (**48c**). Three reports have appeared on the reaction of 3-vinyl derivatives with diazoalkanes. Two publications[43,44] report the preparation of the 3-cyclopropyl derivative (**49a**) by the action of diazomethane in the presence of palladium acetate. A further report[45] describes the formation of pyrazoline and bispyrazoline derivatives (**50**) and (**51**) from reaction with excess diazomethane. Reaction with diphenyldiazomethane was sluggish and provided only a mixture of epimeric diphenylcyclopropane derivatives (**49b**).

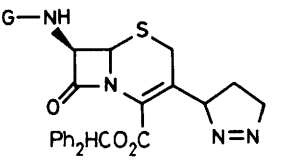
The C(3)-triflate (**52**) has been used to prepare a series of directly substituted derivatives,[46] lacking the normal C(10)-methylene group. Reaction with lithium halides, sodium thiolates, a sodium selenate and secondary amines all produced the corresponding C(3)-substituted Δ3-cephems. Tertiary amines also displaced the triflate but double bond isomerisation occurred giving Δ2-cephems. A detailed study[47] has resulted in the efficient preparation of the C(3)-ethylaminomethyl cephem (**53b**) from 7-aminocephalosporanic acid (7-ACA) (**53a**) using N-ethylbenzaldimine with trialkylsilyl triflate catalysis. The novel C(3)-spiro cephem (**54**) was isolated[48] from reaction of the corresponding C(3)-cyclohexenopyridinium cephem with a silylating agent and tertiary base. The cyclopenteno homologue gave only the expected mixture of Δ2 and Δ3 isomers. A study[49] on the bromination of C(3)-methylene cephem methyl esters at all sulphur oxidation levels revealed the formation of dibromocephams (**55**). Subsequent base mediated dehydrobromination gave the familiar C(3)-bromomethyl Δ3-cephems. 2-Methylene cephem derivatives (**56a,b**) were prepared[50] by Mannich reaction of the corresponding cephem β-sulphoxide and sulphone. The α-sulphoxide failed to react. Cycloaddition of (**56a,b**) with diazoalkanes provided the corresponding C(2)-spirocyclopropanes (**57a,b,c**). A series of C(2)-substituted cephems (**58a,b,c**) has been prepared[51] by acylation and alkylation of cephem β-sulphoxides, reduced antibacterial activity was ascribed to steric hindrance at the enzyme active site. The reaction of cephem esters with iodoarene dihalides has been reported.[52] Reaction of the reagent at sulphur is followed by participation of the C(7)-side-chain in ring-opening to give (**59**). In acetonitrile, fluoride ion acts as a base providing (**60**) while in dichloromethane nucleophilic attack occurs to give the 4-fluoroazetidinone (**61**). In contrast, reaction with an iodoarene dichloride results in isolation of isothiazole (**62**).



(48) **a**; X = O
b; X = CH_2
c; X = CHMe



(49) **a**; R^2 = H
b; R^2 = Ph



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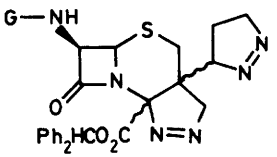

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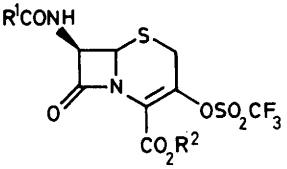

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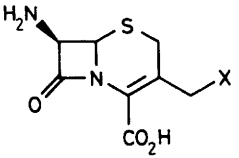
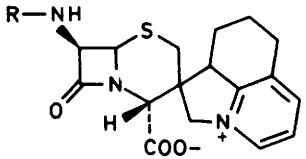
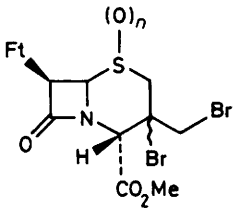


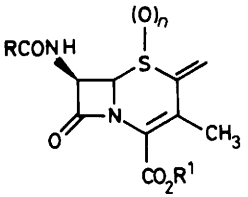
(53) **a**; X = $OCOCH_3$
b; X = NHEt



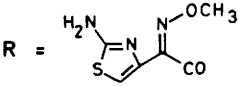
(54)

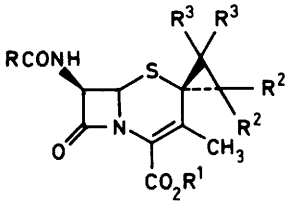


(55)
n = 0, 1, 2

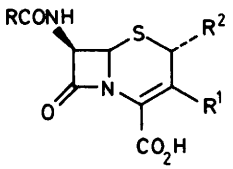


(56) **a**; *n* = 1, ß-isomer
b; *n* = 2

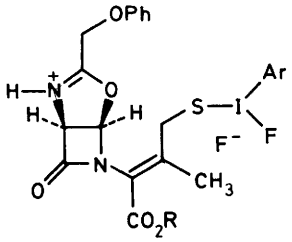




(57) **a**; R^2 = R^3 = H
b; R^2 = H, R^3 = Ph
c; R^2 = Ph, R^3 = H



(58) **a**; R^2 = CO_2H
b; R^2 = CH_2CO_2H
c; R^2 = $CH_2CH_2CO_2H$



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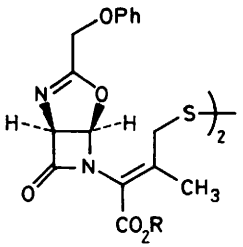

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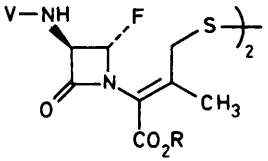

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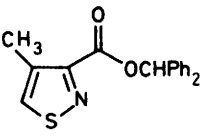
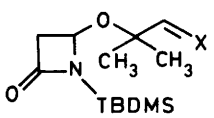
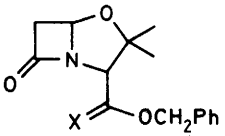


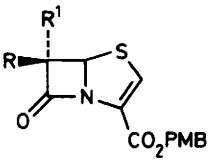
(62)



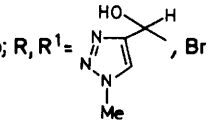
(63)a; X = CH_2
b; X = O
c; X = $C(NO_2)OCH_2Ph$



(64)a; X = NO_2^{2-}
b; X = O

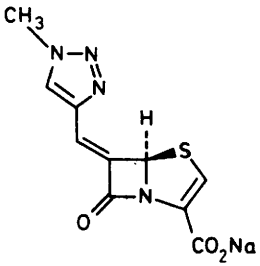


(65)a; R = H, R^1 = Br



b; R, R^1 =

PMB = 



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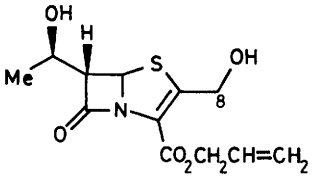

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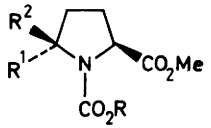
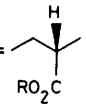
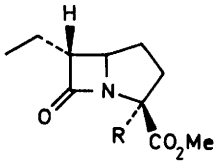
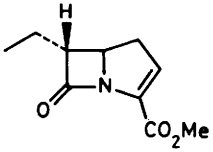


(68)a; R^1 = R^2 = H
b; R^1, R^2 = H, OMe
c; R^1 = H, R^2 =





(69)a; R = H
b; R = SePh



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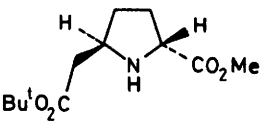


(71)

5. CLAVULANIC ACID AND OXAPENAMS

Displacement of the 4-acetoxyazetidinone derivative (**23**) with an allylic alcohol, followed by desilylation at C(3) and N-protection provided (**63a**). Ozonolysis to aldehyde (**63b**) was followed by reaction with (benzyloxy)nitromethane and mesylate mediated elimination to (**63c**). N-Deprotection and nucleophilic cyclisation provided (**64a**) which has ozonolysed to give the oxapenam-3α-carboxylate (**64b**) after treatment with base.

6. PENEMS

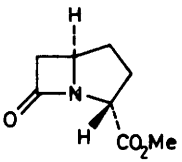
The synthesis of a novel 6-methylenepenem has been reported.[54] A 6α-bromoazetidinone disulphide like (**36**) (R^1 = Br, R^2 = H) derived from 6-APA, was converted to 6α-bromopenem (**65a**) via oxalimide cyclisation with trimethyl phosphite. Treatment of (**65a**) with base gave the C(3)-anion which was reacted with 1-methyl-1,2,3-triazole-4-carbaldehyde to give C(6)-disubstituted penem (**65b**). Acetylation, elimination and carboxylate deprotection provided the target 6-methylenepenem (**66**), a highly potent β-lactamase inhibitor. A publication[55] details the development of a viable synthesis of the 2-hydroxymethylpenem (**67**). Differential protection of the two hydroxyl groups with trimethylsilyl for secondary and ester for primary sites was combined with selective removal of the C(8)-ester protection using an enzyme.

7. CARBAPENEMS, CARBACEPHEMS AND RELATED SYSTEMS

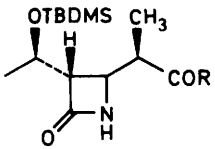
The scope of this section remains as defined in Volume 19; Section 8 should be consulted for the synthesis and chemistry of azetidinone precursors of carbapenems. The appearance of carbacephem in the title of this section reflects the increased interest in this area.

The synthesis of a bicyclic precursor to antibiotic PS-5 from (*S*)-proline has been reported.[56] Anodic oxidation of (**68a**) gave the 5-methoxy derivative (**68b**). Lewis acid mediated C(5)-alkylation with ketene silyl acetals of butyric esters provided all possible isomers of (**68c**), typically with 50% d.e. at C(5) and no selection at C(6). Separation of isomers was achieved by HPLC. Deprotection and cyclisation of the desired isomer provided the carbapenam 3β-carboxylate (**69a**). Introduction of a phenylseleno group gave (**69b**); oxidation and elimination then resulted in the carbapenem (**70**) in >98% optical purity. A similar cyclisation of the pyrrolidine

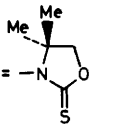
(**71**), derived from (*R*)-Glutamic acid, was used in the preparation of the (3*R*,5*R*)-carbapenem-3-carboxylic acid (**72**).[57] This material was found to be enantiomeric with a natural product isolated from *Serratia* and *Erwinia* species, which was therefore assigned (3*S*,5*S*) stereochemistry. (See Volume 20). This represents the first naturally occurring carbapenem or carbapenam to have (5*S*) ring junction geometry. Two publications[58,59] detail further examples of the C(4)-alkylation of 4-acetoxyazetidinone (**29**) with achiral enolates. In both cases the resulting 2-(azetidin-2-one-4-yl)propionic acid derivatives (**73a, b**) were used directly as activated acyl species for the preparation of 1β-methylcarbapenem derivatives. The carbapenem analogue of the penem β-lactamase inhibitor (**66**) (see Section 6) has been prepared.[60] The novel 6α-bromocarbapenem (**74**) was prepared by Wittig methodology, ultimately from 6-APA. The C(6)-substituent was introduced as previously described for the penem. The naturally occurring carbapenem OA-6129B2 (**75a**), having the less common (8*S*) configuration, has been used[61] in a synthesis of highly potent optically active (8*R*)-8-fluorocarbapenems. Reaction of the protected (**75b**) with diethylaminosulphur trifluoride (DAST) provided the desired fluoro analogue (**75c**) with complete inversion of stereochemistry in moderate yield. A substantial report[62] describes attempts to achieve oxidative rearrangement of Δ1-carbapenems to 1α-hydroxy-Δ2-carbapenems (**76**). It was anticipated that osmium (VIII) oxide would attack the exo face of the double bond providing 1α,2α-diol (**77a**). Subsequent dehydration would then give the desired (**76**). In fact, endo attack was observed and the resulting 1β,2β-diol (**77b**) underwent spontaneous intramolecular β-lactam ring opening to give the fused γ-lactone (**78**). A paper[63] has reported the total synthesis of 6α- and 6β-amido-1-methylcarbapenems. While derivatives bearing protecting groups at C(6) could be obtained as sodium salts for biological testing, no carbapenem with a recognised, biologically active, side-chain was isolated. The 1β-methyl group, while providing increased metabolic stability, does not appear to significantly enhance chemical stability in these compounds. A variation of the reaction of 4-acetoxyazetidinones with chiral enolates was used to prepare the 1β-methoxy derivative (**79**) in high chemical and optical yield.[64] Progression via Merck cyclisation provided the 1β-methoxycarbapenem (**80**). In the continuing search for potent carbapenems with increased chemical and metabolic stability, the introduction of a 5α-methyl group has been reported.[65] The synthesis began with cycloaddition of 3-methyl-3-butenyl acetate and chlorosulphonyl isocyanate (CSI) providing azetidinone (**81**). Standard carbapenem chemistry *via* Merck cyclisation, gave the



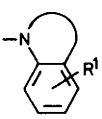
(72)

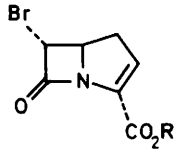


(73) **a**; R =

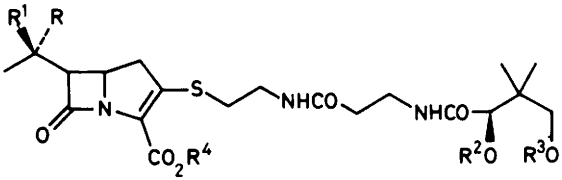


b; R =

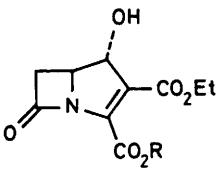




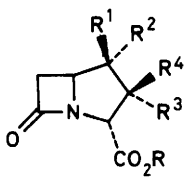
(74)



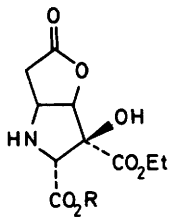
(75) **a**; $R^1 = R^2 = R^3 = H$, R = OH, R^4 = Na
b; R = OH, R^1 = H, $R^2R^3 = C(Me)_2$, R^4 = PNB
c; R = H, R^1 = F, $R^2R^3 = C(Me)_2$, R^4 = PNB



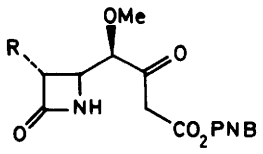
(76)



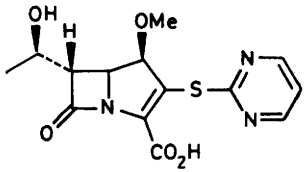
(77) **a**; R^1 = H, $R^2 = R^3$ = OH, $R^4 = CO_2Et$
b; $R^1 = R^4$ = OH, R^2 = H, $R^3 = CO_2Et$



(78)



(79) R =



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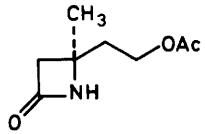
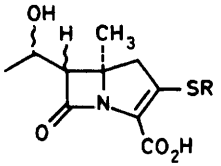


(81)

key 5α-methyl-2-oxocarbapenam intermediate; C(2)-functionalisation then provided a range of derivatives (**82**), all of which showed enhanced chemical and enzymatic stability but reduced antibacterial activity.

This year has seen a dramatic increase in the number of papers published on carbacephems. Many reports detail the manipulation of the C(3)-position. The increased chemical stability of the carba-system over the natural cephem has been put to good use producing stable carbacephem analogues of unstable cephems.

A synthesis[66] of a basic carbacepham unit from piperidyl substituted vinyl bromide (**83**) involved palladium mediated carbonylation to (**84**). After hydrogenation of the C(7)-methylene group, incorporation of a methoxycarbonyl group at C(4) was *via* electrochemical methoxylation to (**85a**), Lewis acid catalysed allylation, double bond isomerisation to (**85b**) and oxidation-esterification providing (**85c**). Vinyl bromides were also used[67] in the synthesis of carbacephems (**87**) by copper mediated N-C ring closure of (**86**). The preparation of 4-methyleneazetidinones was also reported (see Section 8). Three reports have appeared detailing the synthesis and chemistry of 1,2-dehydrocarbacephems. Two variants of the synthesis have the α,β-unsaturated aldehyde (**88**) as a common intermediate. In the first process,[68] addition of thiophenol to give (**89a**) is followed by Emmons-Horner cyclisation, oxidation to sulphoxide (**91a**) and elimination to give (**90**). A direct transformation of (**88**) to (**90**) upon treatment with trialkylamines is also detailed.[69] The proposed mechanism involves addition, cyclisation and elimination *via* intermediate (**91b**). The α-methyl saturated aldehyde (**89b**) was used to prepare 2α- and 2β-methylcarbacephems (**91c**). 1,2-Disubstituted carbacephems have been prepared[70] by functionalisation of the double bond of (**88**) to give α,β-disubstituted aldehydes (**89c**) followed by cyclisation to (**91d**). An alternative synthesis involving functionalisation of the 1,2-double bond of 1,2-dehydrocarbacephem (**90**) provided 1,2-diols, 1,2-dihalides and various halohydrins (**91d**). An enantioselective synthesis[71] of the carbacephem analogue (**92**) of the antibiotic Cefaclor uses the Evans chiral 4-aryl-2-oxazolidinone (**93**) in an asymmetric [2+2] cycloaddition providing (**94**). Hydrogenation of the double bond allowed purification of the corresponding (3*S*,4*R*) diastereoisomer to 100% d.e. in 60% overall yield from (**93**). Simultaneous removal of the auxiliary and the N-benzyl group by Birch reduction, followed by mono-acylation provided (**95a**). Ozonolysis to acid (**95b**), homologation to (**95c**) and rhodium catalysed cyclisation gave (**96a**). Conversion to the 3-chloro



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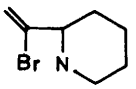

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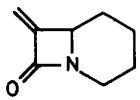

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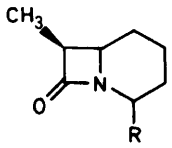
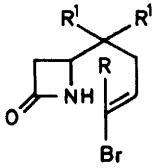


(85) **a**; R = OMe
b; R = CH=CHMe
c; R = CO_2Me



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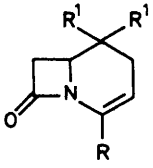

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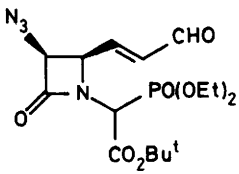

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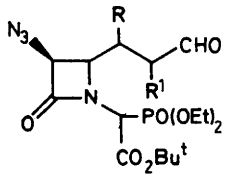
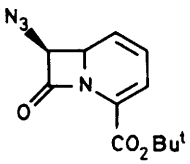


(89) **a**; R = SPh, R^1 = H
b; R = H, R^1 = Me
c; R, R^1 = Halogen, OH, OR



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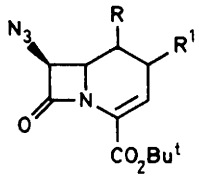
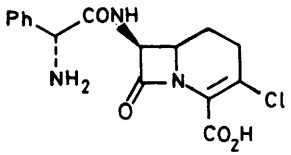
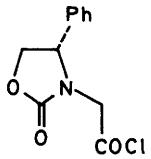


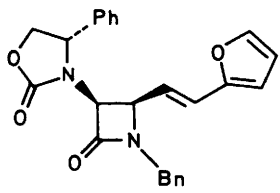
(91) **a**; R = PhSO, R^1 = H
b; R = $(NR^2_3)^+$, R^1 = H
c; R = H, R^1 = Me
d; R, R^1 = Halogen, OH, OR
e; R = R^1 = H



(92)

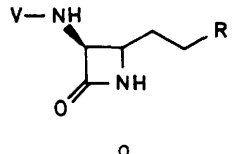


(93)



(94)

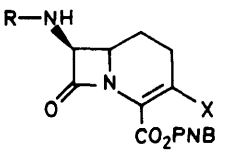
Bn = Benzyl



(95)a; R = 

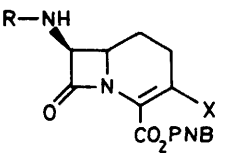
b; R = CO_2H

c; R = $C(O)C(N_2)CO_2PNB$



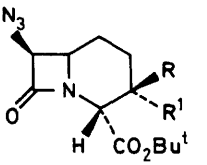
(96)a; R = V, X = OH

b; R = H, X = Cl



(97)a; R = H, X = OSO_2CH_3

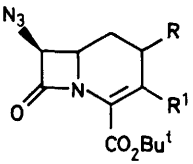
b; R = $PhCH(NH_2)CO$, X = SR



(98)a; R = H, R^1 = SPh

b; R = H, R^1 = SOPh

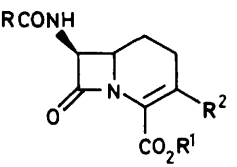
c; R = SOPh, R^1 = Cl



(99)a; R = H, R^1 = Cl

b; R = βCH_3, R^1 = Cl

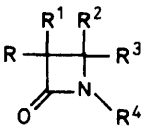
c; R = αCH_3, R^1 = SOPh



(100)a; R^2 = OSO_2CF_3

b; R^2 = CO_2R^3

c; R^2 = SO_2R^4



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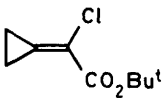

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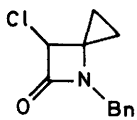

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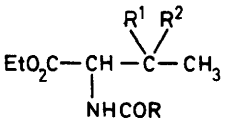
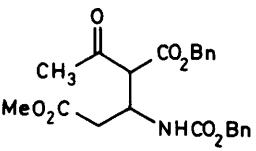


(104)a; R^1R^2 = O

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



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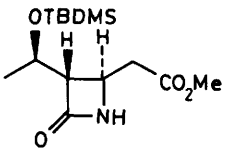

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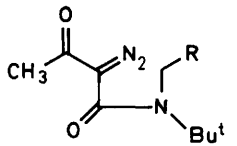
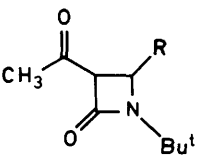


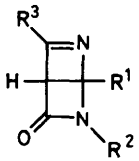
(107)a; R = CH_2CO_2Et

b; R = CO_2Et



(108)a; R = CH_2CO_2Et

b; R = CO_2Et



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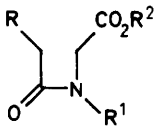
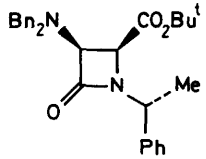


(110)a; R^1 = Ar

b; R^1 = CH(Me)Ph



(111)

derivative with $(PhO)_3PCl_2$ gave concomitant Delft cleavage to **(96b)**. Acylation with an N-protected phenylglycine derivative and deprotection provided **(92)**. An alternative synthesis of **(92)** was achieved from 3-unsubstituted carbacephem **(91e)**.[72] Addition of thiophenol to give **(98a)**, oxidation to **(98b)** and chlorination to **(98c)** was followed by sulphoxide elimination providing **(99a)**. Azide reduction and enantioselective phenylglycylation of the resulting amine using immobilised penicillin acylase provided **(92)**. The 2β-methyl analogue of **(92)** was prepared *via* **(99b)**. In the 2α-methyl series the stereochemistry of the chloro intermediate obtained was opposite to that in **(98c)** resulting in elimination of HCl and isolation of the 3-phenylsulphinyl derivative **(99c)**. A further report[73] details the formation of halohydrins by addition to the double bond of compounds like **(92)**. Treatment of base gives the epoxide which rearranges with catalytic acid to a 3-hydroxy derivative like **(96a)**. Formation of the mesylate and manipulation of the C(7)-amino function gives **(97a)**. Acylation and mesylate displacement provided a range of 3-thiosubstituted carbacephems **(97b)**. The related C(3)-triflates **(100a)**, having various C(7)-side chains have proved useful in the synthesis of a wide variety of 3-substituted carbacephems. Carbon-carbon bond formation was achieved by palladium catalysed coupling with organo-stannanes.[74] A range of substituted and unsubstituted carbon substituents was introduced including vinyl, substituted vinyl, propynyl and various substituted-methyl functions. Alkoxy-carbonylation with carbon monoxide and the appropriate alcohol, again with palladium catalysis, provided **(100b)**. Displacement of the triflate with sulphinates[75] provided a number of 3-sulphonyl analogues **(100c)**. In a further report[76] incorporation of quaternary ammonium substituents is detailed, resulting from displacement of triflate with aromatic tertiary bases. A number of 3-cyclopropyl carbacephems, and their cephem analogues were prepared by reaction of 3-vinyl derivatives with diazomethane and catalytic palladium acetate (see Section 4).[43]

8. AZETIDINONES

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

Reactions in which one bond is formed

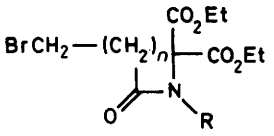
1-2 bond forming reactions.- This section includes 'two-step' [2+2] additions where an intermediate β-amino-acid or -ester was isolated. New reagents for the cyclisation of β-amino acids

continue to be sought and the scope of existing reagents broadened. Azetidinones of type (**101**) have been prepared by cyclisation using di-2-pyridyl sulphite[77] and 1-methanesulphonyloxy-6-trifluoromethylbenzotriazole.[78] A number of β-thiolesters were cyclised using copper (I) triflate and calcium carbonate in refluxing toluene or dioxan.[79] Further details of the diastereoselective addition of enolate anions to N-methoxycarbonylimines and base-induced cyclisation to β-lactams have appeared.[80] The synthesis of N-benzyloxyazetidinones is reported[81] from N-benzyloxyimines *via* reaction with ketene silyl acetals and base-induced cyclisation to compounds like (**101**, R^4 = OBn). A direct synthesis was also mentioned from lithium enolates of esters and N-benzyloxyimines. A synthesis of a C(4)-spirocyclopropylazetidinone[82] begins with Michael addition of benzylamine to vinylcyclopropane acid (**102**); subsequent deprotection and cyclisation with 2-chloro-1-methylpyridinium iodide gives (**103**). Two publications report the synthesis of azetidinones from acetoacetates. In the first,[83] nitrosation of ethyl acetoacetate is followed by reduction and N-acylation to give (**104a**). Schiff base formation and reduction provides α-acylamino-β-aminoesters (**104b**) which were cyclised to 3-acylaminoazetidinones and further processed to various *cis*-4-substituted monobactams. An alternative approach[84] involves palladium mediated carboacylation of an enamide with benzyl acetoacetate. Carbonylation of the intermediate palladium species in methanol gave (**105**). Manipulation of the acetyl group and cyclisation with pyridinium tosylate gave azetidinone (**106**), a well known intermediate to carbapenem antibiotics. A full report of the synthesis of β-amino acids *via* 1,3-dipolar cycloadditions of nitrones has now appeared. Subsequent cyclisations provided intermediates to 1β-methylcarbapenems.[85]

3,4 bond-forming reactions.- An investigation of the relative importance of conformational and electronic effects in the C-H insertion reactions of diazoacetoacetamides has appeared.[86] Conformational preferences dominated allowing insertion both α or β to an ester function; thus (**107a**) provided (**108a**) by β-insertion while (**107b**) gave (**108b**) by insertion α to the ester. A full report of the photochemical reactions of pyrimidin-4-ones and the chemistry of the resultant 'Dewar pyrimidinones' (**109**) has now appeared.[87] The oxidative coupling of dianions derived from amides (**110a**) has been reported.[88] Reaction with N-iodosuccinimide provided mainly *cis* β-lactams while use of copper acetate resulted in lower selectivity with the *trans* isomers

predominating. Reaction of the dianion of the chiral amide (**110b**) with N-iodosuccinimide resulted in isolation of all four possible 3,4-isomers in 58% overall yield. The (3*S*,4*S*)-isomer (**111**) made up 90% of the product mixture. The electrochemical cyclisation of ϖ-bromoalkanamides (**112**, n = 0) to β-lactams has now been extended to γ- and δ-lactams from (**112**, n = 1 and 2).[89]

1,4 bond-forming reactions.- These reactions almost invariably involve nucleophilic attack of N(1) at C(4) with displacement of a suitable leaving group. Most reports concentrate on the preparation of the appropriate β-substituted amide (**113**). A variation on this procedure[90] involves the silicon mediated Pummerer-type rearrangement of (**114**) upon reaction with a ketene silyl acetal under zinc iodide catalysis. This process was seen as biomimetic, involving a sulphonium intermediate (**115**) similar to that proposed in reaction of the Arnstein peptide with isopenicillin N synthetase. A second report[91] extends the scope of this cyclisation. An asymmetric reduction of β-ketoamides to β-hydroxyamides using Baker's yeast provided[92] the necessary precursor for mesylation and base mediated cyclisation. Oxidation and rearrangement of phenylseleno derivative (**116**) provided the α-methylene-β-hydroxyamide which was cyclised *via* the corresponding mesylate to give α-methylene-β-lactams (**117**).[93] A full report has now appeared on the conversion of tartaric acid to α,β-dihydroxyhydroxamate (**118a**). The hydroxyl groups could be selectively silylated to give (**118b**) or (**118c**) depending on the quantity of imidazole added.[94] The assignment of structures (**118b**) or (**118c**) to the two monosilyl derivatives was reversed from that in the original publication. While the α-silyloxy-β-hydroxy derivative gave the expected α-silyloxy-β-lactam (**119**) *via* mesylate or Mitsunobu cyclisation, the α-hydroxy-β-silyloxy derivative also provided (**119**) in modest yield. No firm mechanism was given for this unusual transformation. Condensation of the ketene silyl acetal of ethyl trimethylsilyl acetate with a chiral aldehyde gave 99:1 selectivity for the anti aldol product. Removal of the silyl group (necessary to achieve the selectivity) and cyclisation *via* Mitsunobu chemistry provided (**120**), useful in the synthesis of 1β-methylcarbapenems.[95] The chiral norephedrine derived epoxide (**121**) was opened with lithium dimethylcuprate to give exclusively the anti product. Hydroxamate formation, replacement of the chiral auxiliary by a dithiolane moiety and Mitsunobu cyclisation provided (**122a**)[96] which could be transformed to hydroxymethyl derivative (**122b**).



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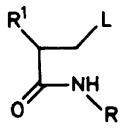
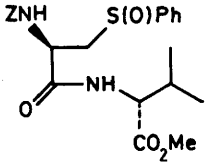
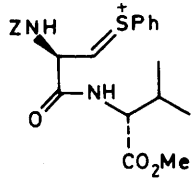


(113) L = leaving group



(114)
Z = $PhCH_2O_2C$



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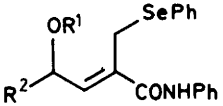

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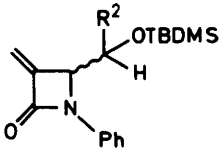

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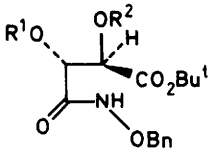
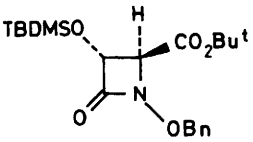


(118)**a**; $R^1 = R^2 = H$
b; R^1 = TBDMS, $R^2 = H$
c; $R^1 = H$, R^2 = TBDMS



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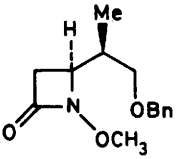
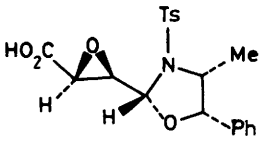


(120)



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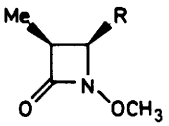
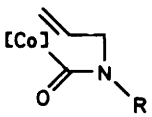
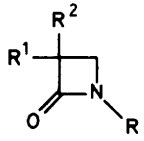


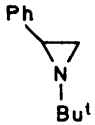
(122)**a**; R = CHO
b; R = CH_2OH



(123)



(124)**a**; $R^1 = H$, $R^2 = CH_2OH$
b; $R^1R^2 = CH_2$



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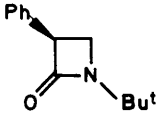
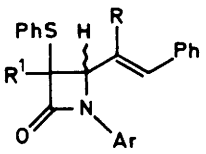


(126)



(127)

2,3 bond-forming reactions.- A single report of this type of ring closure, equally applicable for γ- and δ-lactams, has appeared. The carbamylcobalt salophen complex **(123)** undergoes homolytic cleavage upon irradiation to give a carbamyl radical which inserts into the double bond. After trapping, the intermediate β-lactam can be converted to 3-hydroxymethyl **(124a)** or 3-methylene **(124b)** azetidinones.[97]

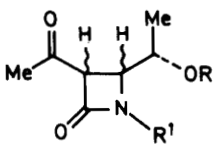
Reactions in which two bonds are formed

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

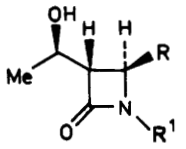
[3+1] additions : 1-2 and 2-3 bond formation.- A full account of the carbonylation-ring expansion of aziridines to β-lactams under rhodium catalysis has now appeared.[98] It has been demonstrated that insertion into chiral aziridines occurs with retention of configuration, and with no loss of optical purity. In a further development, addition of (L)-menthol to the carbonylation of a racemic aziridine resulted in kinetic resolution, thus racemic N-t-butyl derivative **(125)** gave the (3*S*)-β-lactam **(126)** and recovered (2*R*)-aziridine.

[2+2] additions

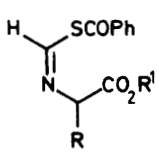
1-2 and 3-4 bond formation.- As in previous volumes detailed mention of ring formation of this type will only be made where new chemical features are apparent; see also the Appendix. The use of alkylphenylthioketenes as monoalkyl ketene equivalents involved their cycloaddition with imines to give 3-phenylthio derivatives **(127)**.[99] Treatment with tributyltin hydride then resulted in desulphurisation to 3-alkyl β-lactams which could be transformed to 4-acetoxy derivatives suitable for carbapenem synthesis. The cycloaddition of diketene with a chiral imine derived from (*S*)-ethyl lactate[100] provides 3-acetyl-4-(1-hydroxyethyl)azetidinones **(128)**. A detailed study of reaction conditions resulted in a high yield of the desired (3*S*,4*S*) isomer. Selective reduction provided the 3-((1*R*)-1-hydroxyethyl) derivative **(129a)**. Manipulation of the C(4)-substituent was possible to give a 4-acetoxy compound. Alternatively, elimination, hydroboration and oxidation provided the 4-carboxymethyl analogue **(129b)**. *S*-Acylation of secondary thioformamides under kinetic conditions resulted in *S*-acylthioimidates **(130)** which underwent cycloaddition[101] with



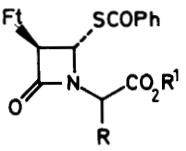
(128)



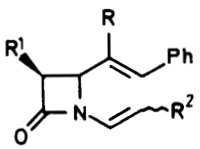
(129) **a**; R = $CH(OR^2)CH_3$
b; R = CH_2CO_2H



(130)



(131)



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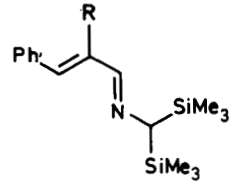

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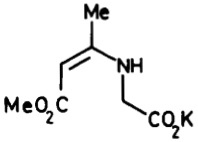

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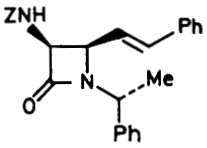

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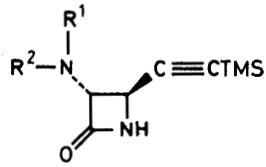

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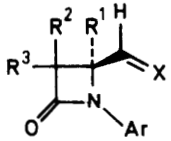
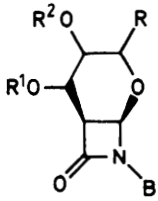


(137) **a**; X = NAr
b; X = O



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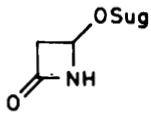
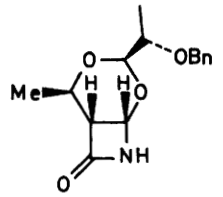


(139) Sug = Sugar derivative



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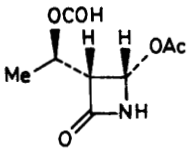

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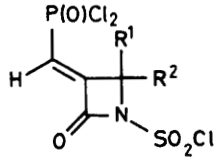

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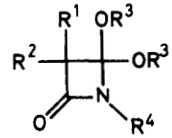

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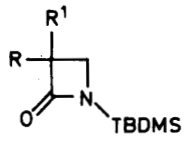
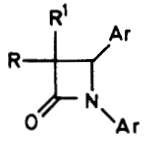
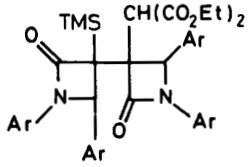


(144) **a**; R = R^1 = H
b; R = OH, R^1 = H
c; R = OTMS, R^1 = $CHXR_2$



(145) **a**; R = R^1 = H
b; R = TMS, R^1 = H
c; $RR^1 = C(CO_2Et)_2$



(146)

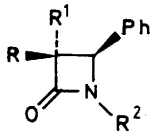
phthalimidoketene to give 4-acylthioazetidinones (**131**). The normal S→N transacylation was discouraged by the low reaction temperature employed. The synthesis of N-vinyl azetidinones (**132**) from bis(trimethylsilyl)methylimines (**133**) was achieved by two routes.[102] In the first imines (**133**) were converted to N-vinylimines by fluoride-ion induced Peterson olefination; cycloaddition with a phenoxyketene equivalent then provided (**132**). Alternatively direct cycloaddition of (**133**) was followed by olefination to (**132**). Activation of the Dane salt (**134**) and cycloaddition with an (*R*)-α-methylbenzylamine derived imine was followed by direct acylation of the C(3)-amine to give a mixture of the two possible *cis* β-lactams from which the desired (3*S*,4*R*) isomer (**135**) could be isolated by a single crystallisation in good yield.[103] The cycloaddition of various N-alkyl-N-arylaminoketenes with imines was reported as giving predominantly *cis* β-lactams.[104] A mechanism involving a dipolar intermediate was proposed whereby its structure and hence the *cis*/*trans* nature of the β-lactam product is determined by electronic and steric effects.

Moving now to the ester enolate plus imine variant, the synthesis of enolisable N-trimethylsilyl imines from bis(trimethylsilyl)formamide and organolithium reagents and their reaction with lithium enolates provided either pure *cis* or *cis*/*trans* mixtures of β-lactams.[105] The use of stannous enolates of α-sulphur substituted esters allowed cycloaddition with various imines, including enolisable imines, giving C(3)-alkylthio (arylthio) azetidinones.[106] Two reports discussed the cycloaddition of N,N-disubstituted glycine ester enolates with imines. Reaction of N,N-dibenzyl glycine lithium enolates[107] gave mainly *cis* β-lactams using stoichiometric base or *trans* isomers with excess base. In a separate report the formation and cycloaddition of zinc enolates[108] provided mixtures of *cis* and *trans* β-lactams. The *trans* compounds (**136**) were obtained exclusively from reactions of zinc enolates with an N-(trimethylsilyl)imine. Reaction of N,N'-diaryl-α-diimines with lithium ester enolates gave 4-iminoazetidinones (**137a**), hydrolysis then provided 4-formylazetidinones (**137b**).[109] A further communication[110] and a full account[111] on the use of the Reformatsky reaction in β-lactam synthesis have now appeared. 3-Alkyl β-lactams could be prepared by the action of zinc dust on suitable α-bromo esters and reaction with imines. Special activation of zinc with chlorotrimethylsilane was required for the efficient synthesis of 3-unsubstituted β-lactams from ethyl bromoacetate.

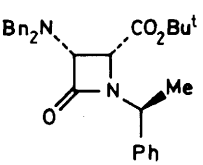
1-4 and 2-3 bond formation.- Further examples of the synthesis of fused bicyclic (and tricyclic) azetidinones **(138)** by cycloaddition of glycals with isocyanates have been published.[112] A full account of the formation and chemistry of these compounds has also appeared.[113] The same authors have prepared chiral β-lactams **(139)** by the addition of sugar vinyl ethers to *p*-toluenesulphonyl isocyanate.[114] The cycloaddition of chlorosulphonyl isocyanate (CSI) to 4*H*-1, 3-dioxins provided bicyclic azetidinones **(140)**.[115] Further reactions involving debenzylation, oxidation and Baeyer Villager rearrangement provided the formate **(141)**, synthetically equivalent to the better known silyl protected carbapenem intermediate. The cycloaddition of allenylphosphonic acid derivatives with CSI provided 3-(dichlorophosphonylmethylene) azetidinones **(142)** in good yield.[116] 4,4-Dialkoxyazetidinones **(143)** were obtained from the cycloaddition of ketene acetals with aryl isocyanates.[117] Facile β-lactam ring opening under various conditions showed these derivatives to be versatile intermediates to a variety of acyclic amides.

Chemistry of azetidinones

The papers in this section will be dealt with, as far as is possible, according to the azetidinone position at which the chemistry occurs. The C(3) anion of simple azetidinone **(144a)** underwent electrophilic oxygenation with MoOPH to give **(144b)**; silylation and formation of the corresponding C(3) anion allowed reaction with alkylating agents, aldehydes and Michael acceptors to give **(144c)**.[118] The related C(3) anion of **(145a)** reacts with trimethylsilyl chloride at -78°C to give **(145b)**, anion formation and reaction with a ketomalonate provided 3-methylene azetidinone **(145c)**.[119] In the presence of excess base a second anion attack, on **(145c)**, resulted in dimer **(146)**. Electroreduction of 3-bromo and 3,3-dichloroazetidinones **(147a,b)** in the presence of acetic anhydride provided[120] *trans* 3-acetyl derivatives **(147c)** together with normal electroreduction products. The chiral (3*R*,4*R*) azetidinone **(148)**, obtained by 3,4 bond formation *via* an acylic dianion (*vide supra*), underwent epimerisation at C(3) upon treatment with trifluoroacetic acid.[121] The resulting (3*S*,4*R*) compound was converted to a known intermediate for the synthesis of monobactams. A further crop of publications has appeared dealing with the thienamycin type substituent, namely (1*R*)-1-hydroxyethyl, at C(3). Nucleophilic epoxide ring opening[122] on **(149)** to give an iodohydrin and reductive dehalogenation to the desired **(150)** was possible in one-pot using sodium iodide-tributyltin hydride. Reduction of the (3*S*,4*S*) isomer of 3-acetyl compound



(147)a; R = H, R^1 = Br
b; R = R^1 = Cl
c; R = H or Cl,
R^1 = $COCH_3$



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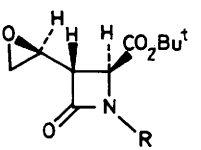
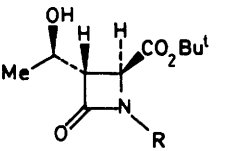


(149)



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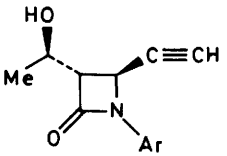

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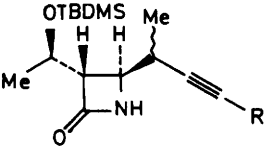
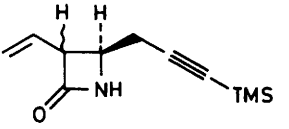


(152)a; R = H
b; R = TMS, β-methyl



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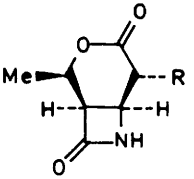
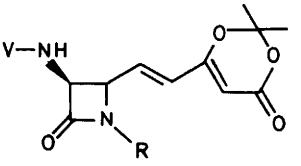


(154)a; R = H
b; R = Me



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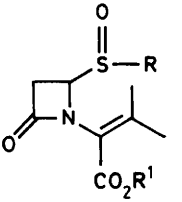
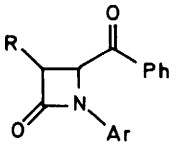
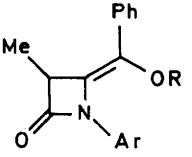


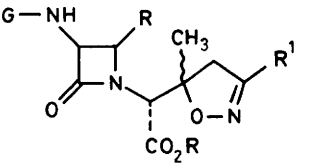
(156)a; R = OH
b; R = OR^2



(157)a; R = Ph
b; R = Me



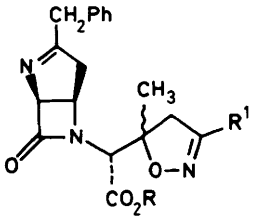
(158)



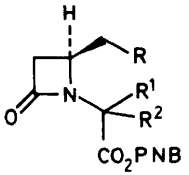
(159)a; R = SSBT
b; R = SBT

BT = Benzothiazolyl

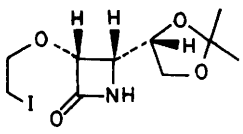
(128) by ionic hydrosilation[123] resulted in an 8:1 mixture of the two possible alcohols with the desired (1'*R*) stereochemistry predominating. Increasing the quantity of $BF_3.OEt_2$ from 0.08 to 0.35eq. gave a 17:1 mixture and simultaneously removed the azetidinone nitrogen protecting group thus avoiding a tedious deprotection reaction. Two reports have appeared detailing the use of microorganisms to produce a 3-((1*R*)-1-hydroxyethyl)azetidinone. The first investigates a number of species capable of C(3)-epimerisation and reduction of a 3-acetyl substituent, only one species was able to produce the desired stereochemistry, and with modest selectivity.[124] The second[125] reports the enantioselective hydrolysis of esters of the (*R*) or (*S*) side-chain hydroxyl isomers in (dl) azetidinones. The organism *Bacillus subtilis* was found to give the (1'*R*3*S*,4*R*) compound **(151)**, suitable for carbapenem synthesis, with 95% ee. Finally, a serendipitous separation of the epimers of **(152a)** was discovered[126] upon trimethylsilylation of the terminal acetylene. The β-methyl derivative gave the expected **(152b)** while the α-methyl epimer underwent elimination to give 3-vinyl compounds **(153)**. Methylation of the bicyclic azetidinone **(154a)** gave exclusively the α-methyl derivative **(154b)**.[127] Lactone ring-opening, C(3)-epimerisation and oxidation provided the known 1β-methylcarbapenem intermediate (**73**, R = OH). Turning now to chemistry at C(4), 4-carbon homologation of a 4-formylazetidinone derivative[128] with a β-ketoester derived phosphorane gave an intermediate **(155)** which proved useful in the synthesis of carbacephems. Ring opening of a penam sulphone provided sulphinic acid **(156a)**. Treatment with thionyl chloride and an alcohol gave the diastereomeric sulphinates **(156b)**.[129] A theoretical and practical study of the 4-benzoylazetidinones **(157a,b)** indicated that for **(157a)** the C(3) enolate is more stable resulting in deuteration and alkylation at this position. Calculations predicted that the C(3) and C(4) enolates of **(157b)** would be of equal stability. In practice[130] alkylation occurred on oxygen to give the 4-methylene enol ether **(158)**. The same authors have studied[131] the stereochemical outcome of the reduction of 4-acyl-β-lactams to 4-(α-hydroxyalkyl) derivatives with sodium borohydride. Mechanisms accounting for the observed results were proposed. Reaction of the oxazoline-azetidinone disulphides **(159a)** with triethyl phosphite under anhydrous conditions gave rise to two products.[132] The first **(160)** is formed by displacement of the C(4)-substituent by the enol form of the 3-acylamino group. The second **(159b)** is the result of desulphurisation with inversion at C(4). Reaction of the azetidinone phosphoranes **(161a)** with acetic anhydride-DMSO gave a mixture of acetoxy derivative **(161b)** and methylene compound **(161c)**.[133]



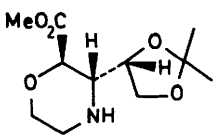
(160)



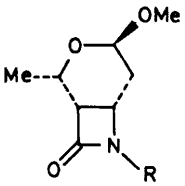
(161) **a**; R^1R^2 = PPh_3
b; R^1 = OAc, R^2 = H
c; R^1R^2 = CH_2



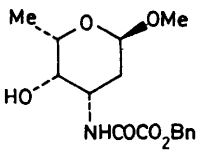
(162)



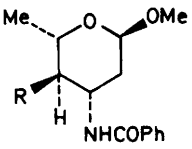
(163)



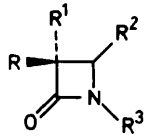
(164) **a**; R = H
b; R = $COCO_2Bn$
c; R = COPh



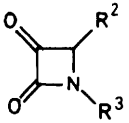
(165)



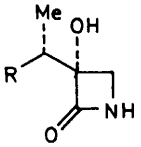
(166) **a**; R = CO_2Me
b; R = OH



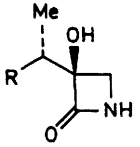
(167) **a**; R,R^1 = PhS, H
b; R,R^1 = PhS, Cl
c; R,R^1 = PhS, OMe



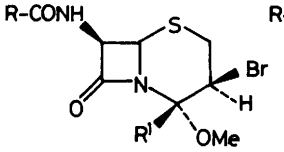
(168)



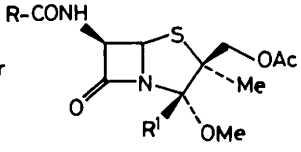
(169) **a**; R = Bn_2N
b; R = NH_2



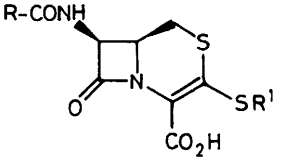
(170) **a**; R = Bn_2N
b; R = NH_2



(171) **a**; R^1 = CH_2OH
b; R^1 = CO_2H



(172) **a**; R^1 = CO_2R^2
b; R^1 = CO_2H



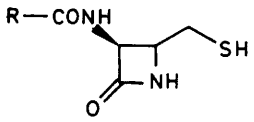
(173)

Further uses of azetidinones

Rearrangement of β-lactam (**162**) upon treatment with sodium cyanide in methanol gave the optically active morpholines (**163**).[134] Three further examples of racemic morpholines were reported. The β-lactam-pyran hybrid (**164a**) was prepared by [2+2] cycloaddition and subsequent transformations in four steps from (*S*)-ethyl β-hydroxybutyrate. Conversion to the imide (**164b**) followed by treatment with buffered *m*-chloroperbenzoic acid provided daunosamine derivative (**165**). Alternatively, treatment of imide (**164c**) with DBU in refluxing methanol gave ester (**166a**), hydrolysis and carboxyinversion reaction gave N-benzoylacosaminide (**166b**).[135]

9. MAJOR STRUCTURAL VARIANTS

As usual, systems retaining a β-lactam ring will be dealt with first. The order will be monocycles, four-six fused systems (cephem analogues) and finally other bicyclic systems. Treatment of the 3-phenylthioazetidinone (**167a**) with sulphuryl chloride gave the highly reactive 3-chloro-3-phenylthio compounds (**167b**). Stereospecific replacement of chloro by methoxy gave (**167c**). Hydrolysis of (**167b**) with moist silica gel and catalytic zinc chloride gave azetidine-2,3-diones (**168**).[136] The 3-hydroxy-β-lactams (**169a**) and (**170a**) were synthesised by Grignard reagent mediated ring closure of the corresponding β-amino esters. Hydrogenolysis gave (**169b**) and (**170b**), designed as potential inhibitors of a D-alanine:D-alanine ligase enzyme.[137] Both compounds were found to be inactive. Oxidation of the known 4β-hydroxymethylcepham (**171a**) with pyridinium dichromate gave the corresponding 4β-carboxycepham (**171b**). Esterification and treatment with silver acetate resulted in substitution of bromine by acetate with ring contraction providing penam (**172a**) which was deprotected to acid (**172b**). Both the penam (**172b**) and cepham (**171b**), having their carboxylic acid functions in the "wrong" configuration, showed only very low levels of antibacterial activity.[138] A series of 2-isocephems (**173**) was prepared[139] by the previously reported reaction of 3-halopyruvates, in this case with sulphur substituents at C(3), with 4-mercaptomethylazetidinone (**174**). Dehydration of the intermediate isocepham was achieved with P_2I_4 in pyridine, literature methods having failed. An overview of recently published chemistry on 1-oxa nuclear analogues of cephems and penems has appeared[140] as part of a conference report. A series of three papers describes a new approach to 1-oxacephalos-



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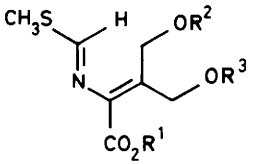
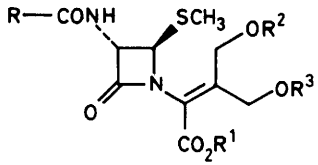
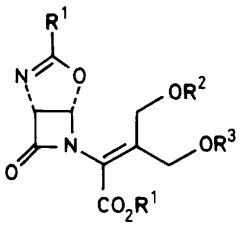


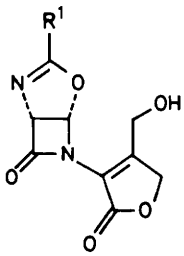
(175)**a**; $R^2R^3 = CMe_2$
b; $R^2 = Bu^t$, $R = CON(Pr^i)_2$



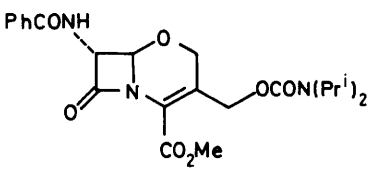
(176)**a**; $R^2R^3 = CMe_2$
b; $R^2 = Bu^t$, $R^3 = CON(Pr^i)_2$



(177)**a**; $R^2R^3 = CMe_2$
b; $R^2 = Bu^t$, $R^3 = CON(Pr^i)_2$
c; $R^2 = R^3 = H$
d; $R^2 = H$, $R^3 = CON(Pr^i)_2$



(178)



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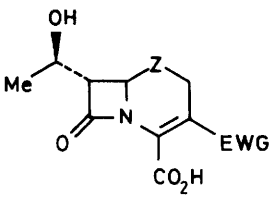
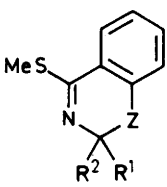
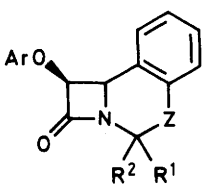


(180)**a**; Z = S
b; Z = O

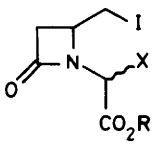
EWG = Electron withdrawing group



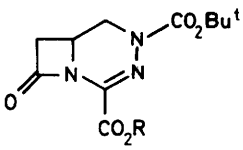
(181)**a**; Z = O
b; Z = N



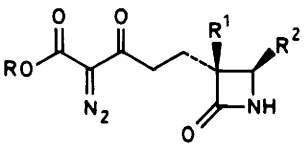
(182)**a**; Z = O
b; Z = N



(183)**a**; X = Cl
b; X = $NHNHCO_2Bu^t$



(184)**a**; R = CH_2Ph
b; R = Na

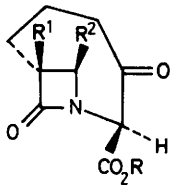


(185)**a**; $R^1 = H$, $R^2 = Me$
b; $R^1 = Me$, $R^2 = Pr^i$

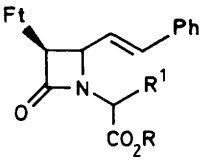
porins.[141-3] The imines (**175a,b**) were prepared by condensation of the appropriate ketone with a 2-isocyanoacetate; dehydration, addition of hydrogen sulphide to give a thiazoline and base-catalysed ring-opening/ methylation to (**175a,b**). [2+2] Cycloaddition of (**175a**) with azidoacetyl chloride then gave 4-methylthioazetidinone (**176a**) after azide reduction and amine acylation. Chlorinolysis of (**176a**) provided the oxazolino-azetidinone (**177a**). Deprotection with mild acid provided bis(hydroxymethyl)derivative (**177c**) which lactonised rapidly to give (**178**). Synthesis of the differentially protected azetidinone (**176b**) allowed selective deprotection of a single hydroxyl group providing (**177d**). Cycloacetalisation then gave the 6,7-*trans* 1-oxacephem (**179**). A series of 7α-(1-hydroxyethyl) cephems[144] (**180a**) and the corresponding 1-oxacephems[145] (**180b**) bearing electron-withdrawing substituents at C(3) were prepared from 4-acetoxyazetidinone (**29**). Substitution at C(4), phosphorane incorporation, Wittig cyclisation and manipulation of an intermediate 3-formyl substituent. Antibacterial activity was poor in both series, while β-lactamase inhibition was significant for both, slightly better in cephems than in 1-oxacephems. The [2+2] cycloaddition of aryloxyketenes with dihydrobenzoxazines (**181a**) and dihydroquinazolines (**181b**) provided a series of 3-oxa- (**182a**) and 3-aza-1-dethia-cephams (**182b**) after Raney nickel desulphurisation.[146] In the latter case β-lactams were obtained only from derivatives (**181b**) in which R^1,$R^2 \neq$ H. The azetidinone chloroacetate (**183a**) was reacted with t-butyl carbazate to give the 2-t-butoxycarbonylhydrazinoacetate (**183b**). Reaction with silver (I) oxide resulted in oxidative cyclisation to 2,3-diaza-1-dethia-cephem derivative (**184a**). Deprotection of the C(4)-ester gave the antibacterially inactive sodium salt (**184b**).[147] The rhodium (II) catalysed intramolecular carbene insertion of diazo derivatives (**185a,b**) into the β-lactam N-H bond gave the novel 1,3-bridged anti-Bredt β-lactams (**186a,b**).[148] Compound (**186b**) in which $R^1 \neq$ H and R^2 is bulky has increased stability over mono-substituted (**186a**). Azetidinones of type (**187**) were used to prepare the bicyclic derivatives (**188**), (**189**) and (**190**).[149] [2+2] Cycloaddition of a benzodiazepine with methoxyketene gave the four-seven fused systems (**191**) and (**192**).[150]

Moving on now to non-β-lactam analogues, the β-sultams[151] (**193a**) were prepared by reaction of a substituted methanesulphonyl chloride with imines. A further report[152] describes the reactions of the dianion of parent β-sultam (**193b**) with electrophiles to give 2-substituted and 2,4-disubstituted products.

Reaction of (D)-(-)-penicillamine with glycolaldehyde gave a 4:1 mixture of diastereo-



(186) **a**; $R^1 = H$, $R^2 = Me$
b; $R^1 = Me$, $R^2 = Pr^i$



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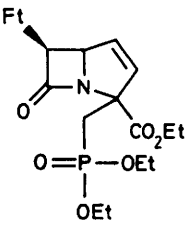
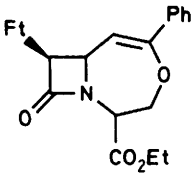


(188)



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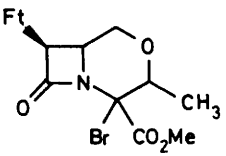
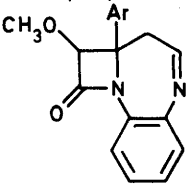
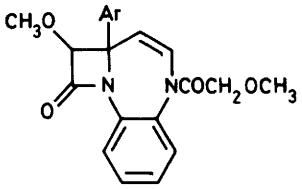


(190)



(191)



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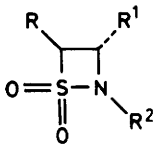
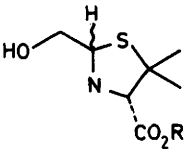


(193) **a**; R = CO_2Me, R^1, R^2 = various
b; R = $R^1 = R^2 = H$



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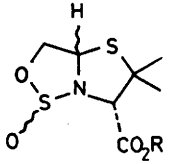
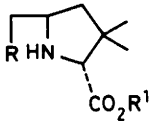
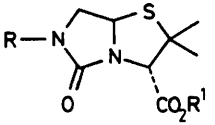


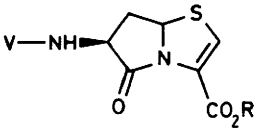
(195)



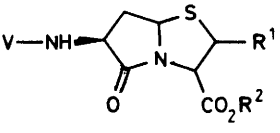
(196) **a**; R = CONHOH
b; R = NCO



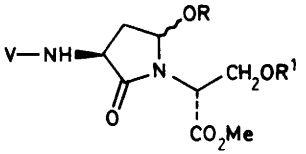
(197) **a**; R = H
b; R = R^2CO



(198) **a**; R = PNB
b; R = Na



(199) **a**; $R^1 = H$
b; R^1 = OCOPh

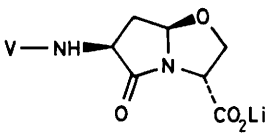


(200) **a**; R = H, R^1 = Bn
b; R = Me, R^1 = Bn
c; R = Me, $R^1 = H$

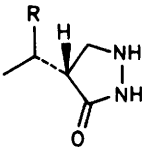
isomers of **(194)** favouring the (5*R*) isomer. After protection of the acid as an ester, reaction with thionyl chloride gave three of the four possible isomers of cyclic sulphamidite **(195)**.[153] Deprotection of the ester gave the tetrabutylammonium carboxylate which decomposed spontaneously upon dissolution in D_2O. β-Lactam ring-opening of 6-unsubstituted penam-3-carboxylate esters with hyroxylamine gave hydroxamates **(196a)**. Lossen rearrangement to isocyanate **(196b)** and ring closure provided 7-unsubstituted bicyclic imidazolidinones **(197a)**. Acylation of N(7) with various side-chains from β-lactam antibiotics gave a series of derivatives **(197b)**. Deprotection of the C(3) ester provided acids which were devoid of antibacterial activity.[154] A full account has now appeared (see also Volume 19) of the synthesis of γ-lactam analogue **(198b)** of 6-acylamino-penems. The bicyclic thiazolidine **(199a)**, derived from (D)-cysteine methyl ester and aspartic acid semi-aldehyde was benzoylated at C(2) to give **(199b)**. Syn-elimination of benzoic acid then provided **(198a)**. Ester deprotection gave the final compound **(198b)** which had weak antibacterial activity.[155] The hydroxy lactams **(200a)**, which co-exist with the open amino-aldehyde form, were prepared from (L)-phenoxyacetyl allylglycine and (D)-O-benzyl serine. Treatment with acidic methanol provided methoxy lactams **(200b)**. Removal of the benzyl protecting group gave the separable alcohols **(200c)**. Cyclisation and ester deprotection gave the γ-lactam analogue **(201)** of oxapenams.[156] The compound was antibacterially inactive. The synthesis of bicyclic pyrazolidinones as antibacterial agents has continued with the preparation of analogues of the carbapenems PS-5 and thienamycin.[157] The necessary monocyclic pyrazolidinones **(202a,b)** were readily prepared by condensation of anhydrous hydrazine with the appropriately substituted acrylate. Progression by previously reported procedures gave compounds **(203a,b)** which were significantly less antibacterially active than their acylamino substituted counterparts. The 4-methyl-3-thiosemicarbazones of 4-oxopentanoic acid **(204a)** and 5-oxohexanoic acid **(204b)** were reacted with acetic anhydride to give the bicyclic γ- and δ-lactams **(205a)** and **(205b)** which were devoid of antibacterial activity.[158]

10. MECHANISTIC STUDIES, MODE OF ACTION AND DEGRADATION

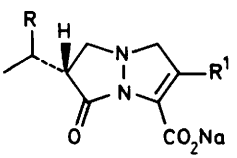
This section will encompass general mechanistic studies, interactions of β-lactams with enzymes, molecular graphics and mechanisms and products of degradation of β-lactams especially antibiotics.



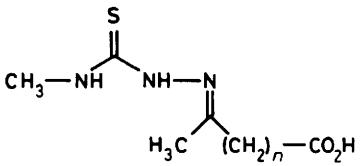
(201)



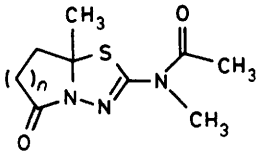
(202) **a**; R = H
b; R = OH



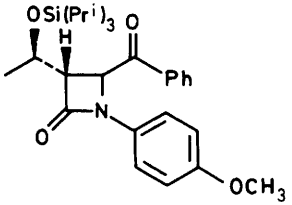
(203) **a**; R = H
b; R = OH
R^1 = CO_2Na, COMe, CN



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



(205) **a**; *n* = 1
b; *n* = 2



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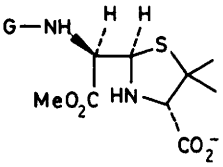

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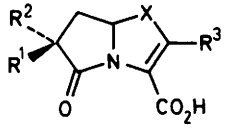
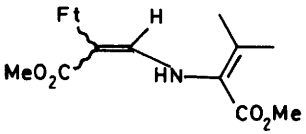
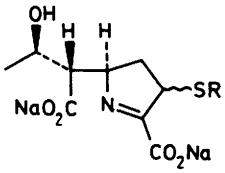


(208) **a**; X = S
b; X = CH_2



(209)



(210)

Low temperature Fourier transform infra-red spectroscopy was used in a kinetic study of the formation of azetidinone (**206**) by [2+2] cycloaddition. The data indicated that reaction occurred exclusively through a ketene intermediate and not *via* imine acylation.[159] Hydrolysis of β-lactam esters to acids by the pig liver esterase enzyme is reported for the methyl esters of a carbapenem, a Δ2-cephem, and a C(3)-methyl-Δ3-cephem.[160]

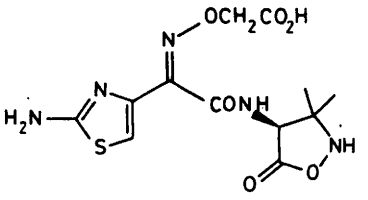
A study of conformational changes during the interaction of a β-lactamase enzyme with penicillin sulphones revealed a structural dependency. The binding of sulphones with large hydrophobic C(6)-substituents resulted in significant changes of enzyme conformation. It is proposed that this results in displacement of the catalytic sites with respect to the acyl-enzyme bond preventing deacylation and hence giving permanent inhibition. The C(6)-unsubstituted sulphone caused no conformational changes upon binding, its β-lactamase inhibitory action depends upon chemical factors for a slow breakdown of the acyl-enzyme complex.[161]

The interaction of a penicillin ester, a cephalosporin ester and a cephalosporin lactone with the β-lactamase I enzyme from *Bacillus cereus* has been quantified. The penicillin and the lactone are substrates, despite the lack of ionisable carboxyl function; the cephalosporin ester is not a substrate. None of the compounds were substrates for the β-lactamase II enzyme of the same organism.[162] The penicilloate methyl ester (**207**) is a reversible competitive inhibitor of the above *B.cereus* β-lactamase I enzyme. The ester binds more strongly than the corresponding free acid suggesting its proximity to an anionic function in the enzyme active site. The corresponding trifluoroethyl ester does not bind, perhaps suggesting a strict steric requirement at this position.[163] X-Ray diffraction studies of complexes of a number of β-lactams with a *Streptomyces* R61 transpeptidase-carboxypeptidase revealed that the enzyme's reactive serine had opened the β-lactam ring of each compound. The known half-lives of the various complexes could be correlated with the distance of the antibiotic's acid function from complementary groups on the enzyme.[164]

A full account has appeared on molecular modelling of γ-lactam analogues (**208a,b**) of penems and carbapenems. Calculations indicated that compounds (**208a,b**) could be accomodated in the same active sites of penicillin-binding proteins as their β-lactam counterparts. Synthesis of the compounds, some of which have been reported elsewhere, allowed determination of low levels of antibacterial activity.[165] Molecular mechanics calculations using both MM2 and AMBER force

fields provided geometries for a number of β-lactam antibiotics which agreed well with those given by X-ray crystal structures.[166] The same techniques were then used to obtain geometries for several bicyclic γ- and δ-lactams for which X-ray structures are not available.[167]

Moving on to studies on the degradation of β-lactams, treatment of a 7β-phthalimido-cephem sulphone methyl ester with triethylamine resulted in clean C(7)-epimerisation. In the presence of methanol, collapse of the bicyclic system with loss of SO_2 results in the formation of isomeric enamines (**209**).[168] A study of the dehydropeptidase I enzyme catalysed hydrolysis of carbapenems revealed that the principal product was a mixture of 1-pyrrolines (**210**) epimeric at C(3). Nonenzymatic acid-catalysed hydrolysis gave a 2-pyrroline which isomerised to (**210**) upon neutralisation.[169] A report details the desulphation of a monobactam antibiotic, Tigemonam, to give an N-hydroxyazetidinone which undergoes rearrangement to the isoxazolidin-5-one (**211**). The structure was confirmed by independent synthesis.[170] A kinetic study of the copper catalysed hydrolysis of benzylpenicillin suggests a mechanism involving a copper complex where both β-lactam and hydroxide ion are bound, hydrolysis being an intra-complex process.[171] Detailed studies of the hydrolysis of the cephem Cefodizime (**212**) have appeared. Under acidic conditions[172] the hydrolytic loss of the C(3)-mercaptothiazole unit and lactonisation of the resulting 3-hydroxymethyl-cephem are the initial processes. Subsequently β-lactam cleavage occurs together with some decarboxylation. In the final stages of decomposition Z→E isomerisation of the methoxyimino group occurs. Under neutral or alkaline conditions[173] degradation involves mainly C(7)-epimerisation and Δ2-cephem formation. β-Lactam cleavage and loss of C(3)-substituent occur to a much lesser extent. Studies on the aminolysis of clavulanic acid have revealed[174] that aminoalcohols and their alcohol-masked counterparts are less efficient than primary aliphatic amines. A further publication[175] reports on the catalytic effect of various metal ions on the hydrolysis of clavulanic acid. The copper (II)-clavulanate complex, involving co-ordination of β-lactam nitrogen and carboxylate group, is hydrolysed by hydroxide ion some 10^6 faster than clavulanate alone.



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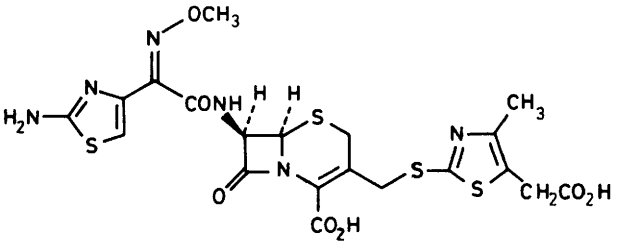

(212)

APPENDIX TO CHAPTER 5 : β-LACTAM ANTIBIOTICS PREPARED FOR STRUCTURE-ACTIVITY RELATIONSHIP STUDIES AND MISCELLANEOUS β-LACTAMS

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

β-Lactam	Reference
6-(4-Substituted benzyloxybenzoyl)penicillins	176
Aminothiazole penicillins	177
Carbenicillin derivatives	178
6-Azino and 6-Hydrazino penicillins	179, 180
Piperazine-2,3-dione substituted penicillins and cephalosporins	181, 182
Poly-p-methacryloylaminophenoxy penicillin	183
Aminothiazole penicillins and sulphoxides	184
6α-(Substituted-formamido) penicillins	185
6-(β-Aminooxypropionyl) penicillins	186
2β-(Substituted-methyl) penams	187
Radiolabelled penicillin for binding assay	188
Oxidation of 2β-heteroarylthiomethyl penams	189
Orally active cephalosporin esters	190, 191
3-Dithiohydantion cephalosporins	192
7-Propenoamido cephalosporins	193
7-Aminoimidazole cephalosporins	194
Cephalosporin sulphone esters as elastase inhibitors	195
7-Aminothiazole cephalosporins	194
7-(O-Aminoacylmandelamido) cephalosporin pro-drugs	197
7-[2-(Substituted benzylthio)alkamido] cephalosporins	198
7-(6,8-Disubstituted coumarin) cephalosporins	199
7-Hydrazo cephalosporins	200

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6
Metal Complexes of Amino Acids and Peptides

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1 Introduction

This chapter deals with the synthesis, structures, reactions and applications of metal-amino acid and metal-peptide complexes and covers material published in 1989. A number of relevant reviews have been published. These cover topics such as luminescence studies of lanthanide(III) complexes with amino acids and other ligands, [1,2] ligand (including amino acid and peptide type ligands) design for selective complexation of metal ions in aqueous solution,[3] spectroscopic studies of metalloproteins and metalloenzymes,[4] zinc metallothionein in mammalian brains,[5] the preparation and metal ion ($Cu^{+/2+}$, Cd^{2+}) binding of fragments of a metallothionein,[6] structural aspects of metal-γ-glutamyl-peptide complexes in metal detoxification,[7] the accumulation of cadmium and zinc in plants including the role of Cd-binding peptides,[8] the endogenous formation, isolation and identification of phytochelatins, the peptides which bind toxic heavy metals in plants hence contributing to their protective mechanisms,[9] genotoxicity and metabolism of chromium compounds,[10] cysteine containing oligopeptide model complexes of iron sulfur proteins,[11] the use of $CuCl_2$ as a racemization suppressant in peptide coupling reactions,[12] the separation of peptides and proteins by immobilised metal ion affinity chromatography,[13,14] and the use of angiotensin converting enzyme inhibitors, α-adrenoceptor antagonists and calcium channel antagonists in the treatment of hypertension.[15]

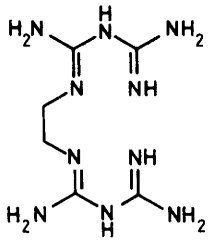
2 Amino Acid Complexes

2.1 Synthesis and Crystal Structures.- A large number of new metal-amino acid complexes have been synthesised and characterised and crystal structures have been determined in a number of cases.

The first ternary chromium(III)-histidine or cysteine-nucleotide complexes with purine and pyrimidine bases have been prepared and characterised by a variety of physical techniques.[16,16a] Fluoromanganate(III) complexes of formula $M[MnF_4(Gly)_2].3H_2O$ where M = NH_4, Na, K have been synthesised by the reaction of MnO(OH) in 48% HF with glycine in water in the presence of M_2CO_3.[17] The magnetic moments and spectra (electronic and i.r.) of these complexes are reported and monodentate, amino group coordination of glycine to the metal is postulated. Complex formation between Co(II), Ni(II) and Cu(II) and the amino acids cysteine, cystine and methionine has been studied and a variety of solid complexes characterised.[18] The reaction of ferrihydrite, $5Fe_2O_3.9H_2O$ with cysteine at pH 6-8 produces haematite, goethite α-FeO(OH) or lepidocrocite, γ-FeO(OH).[19] The compound formed depends on the cysteine:iron ratio and the nature of the buffering medium.

The synthesis, X-ray structure and electronic spectum of trans-$[Co(en)_2(Boc\text{-}L\text{-}Val)Cl]BF_4$ prepared from trans-$[Co(en)_2Cl(OH)]^+$ and an active ester of Boc-L-valine has been described.[20] This complex is an intermediate in metal mediated peptide synthesis, and the crystal structure establishes that the coordinated Cl and O atoms are trans and the protected amino acid acts as a monodentate ligand via the carboxylate oxygen. A number of cobalt(III) complexes of the type $[Co(AA)(en\text{-}bigH)_2]^{2+}$ where AA = D,L-Ala, β-Ala, L-Leu, DL-Val and L-His; $(en\text{-}bigH)_2$ = N,N-ethylenedibiguanide (1) have been prepared and characterised by conductivity as well as i.r. and visible spectroscopy.[21] In the case of $[Co(His)(en\text{-}bigH)_2]^{2+}$, the amino acid of histidine behaves as a bidentate N,N donor rather than as an N,O donor. The complexes trans(N)-$K[Co(AA)_2ox]$, trans-$K[Co(L\text{-}Val)CO_3]3.5H_2O$ and trans-$[Co(en)_2Sar]I_2$ where AA = Gly, Sar, D,L-Ala, L = Ala and ox = oxalate, have been synthesised and screened for bacterial activity against E. coli B.[22] The complex $K[Co(Sar)_2ox]$ showed the greatest activity.

The synthesis and stereochemistry of the sulphur-bridged complexes $[Co^{III}{}_3(L\text{-}Cys\text{-}N,S)_n(aet)_{6-n}]^{3-n}$ (n=0-6; aet = 2-aminoethanethiolate) has been described.[23] The relationship between optical activity of aminocarboxylatocobalt(III) complexes and aminocarboxylato chelate ring conformation has been studied



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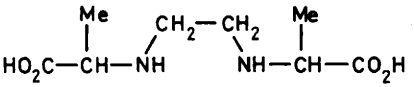

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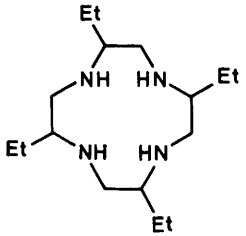

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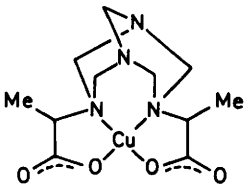

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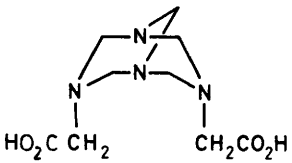

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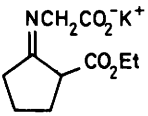

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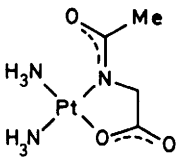


(7)

and it was found that the diastereoisomers of cis(NO_2), trans(NH_2)-(R-alaninato)(1,3-diaminopropane)dinitrocobalt(III) exhibit almost symmetrical c.d. spectra, indicating a weak vicinal and conformational contribution of the R-alaninato ligand to the optical activity of the diastereoisomers.[24] The crystal structures establish that in both diastereoisomers, the R-alaninato chelate ring adopts a strained envelope conformation, with the side chain methyl group in an equatorial position.

Preparation of the cobalt(III) complex $[Co(SS\text{-}eddp)Cl_2]^-$ where SS-eddp is ethylenediamine-N,N'-di-S-α- propionic acid (2) by air oxidation of a basic solution of the ligand and cobalt(II) chloride gave only the Δ-s-cis isomer.[25] Chloro-aqua and diaqua complexes were also characterised. Reaction of the s-cis-$[Co(SS\text{-}eddp)Cl_2]^-$ complex with amino acids gives the s-cis-$[Co(SS\text{-}eddp)(AA)]^{n+}$ complexes (AA = L-Ala, S-Me-L-Cys or L-Cys). The L-Ala and S-Me-L-Cys complexes were obtained exclusively as the s-cis meridional isomers. The S-Me-L-Cys ligand is coordinated to cobalt(III) via the nitrogen and oxygen donors, while cysteine is bound through the nitrogen and sulphur atoms.

The complex $[CoBr(L)OH_2]Br_2$ where L=(2R,5R,8R,11R)-2,5,8,11-tetraethyl-1,4,7,10-tetraazacyclododecane,(3) reacts with racemic serine and α-methyl serine to give pairs of diastereoisomeric complexes.[26] One isomer of each pair crystallised preferentially as an appropriate salt. The less soluble diastereoisomer contained the coordinated (R)-amino acid. X-ray crystallography establishes that the OH group of the (R)-amino acids is involved in intramolecular hydrogen bonding to one of the sec-NH atoms in the macrocyclic ligand, while the (S)-amino acids interact with a cocrystallised water molecule and with an anion. These different hydrogen bond interactions lead to different solubilities for the diastereoisomers. Chiral differentiations of α-amino acids by coordination compounds had previously been shown to depend on a three point attachment. Thus Dunlop et al. in 1966 reported the stereoselective interaction of amino acids and metal complexes. Crystallography on (+)-$[Co(S\text{-}Glu)(en)_2]ClO_4$ obtained from the reaction of (±)$[Co(CO_3)(en)_2]ClO_4$ and (S)-Glu established that the polar sidarm of the (S)-Glu interacts with an N-H group of an en chelate ring.

The reaction of bis(L-alaninato)copper(II), $Cu(L\text{-}Ala)_2$ with formaldehyde and ammonia at pH 8.5 gives the copper(II) complex (4) the structure of which was confirmed by X-ray crystallography.[27] It has previously been shown that at pH 8.5 bis(glycinato)metal(II) complexes react with formaldehyde and ammonia to give the metal(II) complexes of (5).[28]

Eighteen complexes of copper(II) of the general formula $CuL_2.nH_2O$ (n = 1-4) with N-protected amino acids (HL) have been prepared and characterised by spectroscopic methods and magnetic susceptibility measurements.[29] The complexes are of three types; (a) binuclear species having bridging bidentate coordination via the carboxylate group, (b) mononuclear complexes with symmetrical chelating bidentate coordination or bridging bidentate coordination (no Cu-Cu interaction) and (c) mononuclear complexes with highly unsymmetrical chelating bidentate or non-chelating unidentate coordination via the carboxylate group. The complex $[Cu(Bz\text{-}Ala)_2H_2O]_2$ has a typical copper(II) acetate monohydrate type structure. The crystal structure of the blue, orthorhombic modification of bis(L-N,N-dimethylisoleucinato)aquacopper(II) has also been solved by X-ray diffraction and refined to R=0.047.[30] The coordination geometry around the copper atom is a tetrahedrally distorted pyramid with N-Cu-O angles varying from 83.4° to 97.6°.

The interaction of Cu(II) with N-tosyl-DL-asparagine and N-tosyl-L-glutamine in aqueous solution has been investigated and solid complexes isolated.[31] Complexes characterised were $CuL_2.xH_2O.yMeOH$ (L=tosyl-α-aminoacidate monoanion), $Cu(LH_{-1}).2H_2O$ and $K_2[Cu(LH_{-1})_2]$. Potentiometric and spectrophotometric measurements on the complexes are also described. A number of bis(chelates) of copper(II), palladium(II) and platinum(II) with N-glycosides derived from glucose and α-amino acids have been synthesised.[32]

Spectroscopic techniques (^1H, ^{13}C n.m.r. and i.r.) have been used to compare the mode of coordination of Cu(II) in complexes with DL-asparagine (Asn) and DL-aspartic acid (Asp) in solution and in the solid state.[33] At pH 5 only the carboxylate group of Asn is involved in coordination, whereas at pH 9 both the

carboxylate and amino groups are involved. In the solid complex Cu(Asn), coordination occurs via the carboxyl, amine and amide groups, but in $Cu(Asn)(OH)(OH_2)_2$ only the carboxyl and amino groups are involved. The reaction of CuO with L-ornithine in basic boiling water gives bis(1-pyrroline-2-carboxylate)copper(II) octahydrate.[34] The new ligand is formed by oxidation of L-ornithine to the corresponding α-keto acid followed by cyclisation. The crystal structure of the copper(II) complex is described.

Reaction of glycine with ethyl-α-ketocyclopentylcarboxylate gives a Schiff base which has been isolated as the potassium salt, $K(Rgly).H_2O$ (6).[35] This salt has been used to prepare the copper complex of the deprotonated Schiff base. The copper(II) complex is believed to be pentacoordinate and square pyramidal.

The complex $Zn(L\text{-}Leu)_2$ has been prepared.[36] The two L-leucine molecules act as bidentate ligands towards Zn(II) forming a distorted N_2O_2 tetrahedron. The carboxyl oxygen of a third amino acid molecule completes pentagonal coordination around Zn(II). The $^{63}Cu(II)$-doped complex has also been investigated by e.s.r. spectroscopy.

Reaction of Mo_2^{4+} with racemic valine or leucine in water followed by addition of the appropriate counter ion gives the complexes $[Mo_2(D\text{-}Val)_2(L\text{-}Val)_2](ZnCl_4)_2.4H_2O$ and $[Mo_2(D\text{-}Leu)_2(L\text{-}Leu)_2](Tos)_2Cl_2.2H_2O$ (Tos = p-toluenesulphonate).[37] The complex $[Mo_2(D\text{-}Val)(L\text{-}Val)(NCS)_4].1.5H_2O$ was prepared by adding NCS^- to a solution of Mo_2^{4+} and DL-valine. The structures of all three complexes were determined by X-ray crystallography. In the first two complexes the dimeric unit resides on a crystallographic centre of symmetry and the four ligands are coordinated to the Mo_2^{4+} unit in the cyclic order of DDLL. In the latter complex the molecule has a cisoid arrangement of the amino acid ligands and the four NCS^- ligands. A number of complexes of the type $[(diene)RuCl(AA)]_n$ (diene = norbornadiene = nbd, 1,5-cyclooctadiene = cod; AA = Gly, Ala, Val) have been synthesised and the crystal structure of $[(cod)RuCl(Phe)]_4$ shows that the amino acid forms symmetrical carboxylate bridges between

two ruthenium atoms.[38] These complexes react with nitrogen bases, B, to give the monomers (diene)RuCl(AA)B which contain N,O bidentate amino acid ligands. Complexes of the type (diene)-$Ru(AA)_2$ have also been synthesised and the crystal structure of Δ-(nbd)Ru(L-Phe)$_2$ has been determined.

N-tosylated α-alanine and β-alanine bind Cd^{2+} acting initially as simple carboxylate donors at low pH.[39] As the pH is raised they act as bidentate ligands through the carboxylate oxygen and the deprotonated amide nitrogen atoms. Cadmium-113 n.m.r. and potentiometric measurements establish that the thermodynamic stability of the complexes is almost unaffected by the chelate ring size. The crystal structure of $[Cd(Tos-\beta-Ala)_2(H_2O)_4]$ establishes that the amino acid derivative binds *via* the unidentate carboxylate group and the structure consists of discrete units in which the Cd^{2+} exhibits a nearly regular octahedral geometry.

Divalent calcium plays an important role in living organisms, and many Ca^{2+}-related processes occur by means of calcium-protein interactions. Because of the similarity of rare earth coordination chemistry to that of calcium (size, preference for oxygen donors, variability in coordination number, lack of strong directionality) X-ray studies of lanthanide-amino acid complexes may give valuable information about structural characteristics of calcium binding sites in proteins. The holmium complex $Ho(L-Asp)Cl_2.6H_2O$ has been prepared and its crystal structure determined.[40] The Ho^{3+} cations are eight-coordinate *via* five water ligands and three carboxylate oxygens of three different aspartate molecules. The crystal structure consists of parallel polymeric chains of holmium-L-aspartate-hydrate. The chloride anions and the sixth non-coordinating water molecule reside in cavities between the chains, and stabilise the crystal packing by forming hydrogen bonds. The crystal structure of $Pr_2(L-Glu)_2(ClO_4)_4.11H_2O$ has also been determined.[41] The two crystallographically independent Pr(III) ions are bridged by four carboxylate groups. The pair of metal ions are coordinated by a total of sixteen oxygen atoms, two of which are coordinated to both cations, making the coordination number equal to nine for each. One of the ligand oxygen atoms is provided by a perchlorate

group. In the crystalline state, the L-glutamic acid residues interlink the lathanide ion pairs to form infinite layers perpendicular to the long c axis.

The isomorphic crystals of dysprosium(III) and holmium(III) complexes with proline, $[Ln(C_5H_9NO_2)_2(H_2O)_5]Cl_3$, have also been prepared and characterised by X-ray crystallography.[42] The structures of these complexes differ significantly from that of $[Nd(Pro)_3(H_2O)_2](ClO_4)_3$ with different bonding modes for the proline ligands. The holmium and dysprosium structures contain one-dimensional polymers with the chains lying along the y-axis. N-benzoylglycine hydrazide, Bz-GlyNHNH$_2$, reacts with trivalent lanthanide metal ions giving complexes of the type $[Ln(Bz\text{-}GlyNHNH_2)_2Cl(H_2O)_2]Cl_2.nH_2O$ where Ln = La^{III}, Pr^{III}, Nd^{III}, Sm^{III}, Eu^{III}, Gd^{III}, Tb^{III}, Dy^{III} or Y^{III} and n = 1 or 2.[43] The i.r. and ^{1}H n.m.r. data suggest bidentate coordination of the amino acid derivative in all of the complexes.

Cis-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ reacts with N-acetylglycine to give initially the O-coordinated amino acid complex cis-$[Pt(NH_3)_2(Ac\text{-}Gly)H_2O]^+$ which then undergoes chelate ring closure to give N,O coordinated $[Pt(NH_3)_2(Ac\text{-}Gly)]^+$.[44] Deprotonation results in precipitation of $[Pt(NH_3)_2(Ac\text{-}Gly)].2H_2O$. These compounds represent the first reported complexes in which acetylglycine acts as an N,O-chelating ligand (7). Acid dissociation constants for deprotonation have been determined by n.m.r. in H_2O (2.6±0.1) and D_2O (3.4±0.3). In strongly basic solution the N-coordinated amino acid complex cis-$[Pt(NH_3)_2(Ac\text{-}Gly)(OH)]^-$ is formed, while in strongly acidic solution the acetylglycine ligand is completely dissociated. Complexes of Pt(II) with a variety of amide containing ligands for example 2-(acetylamino)benzoic acid and 2-(benzoylamino)benzoic acid have been prepared and characterised.[45]

2.2 Equilibrium Studies.- A variety of reports on the formation constants of metal complexes of amino acids and their derivatives have been published and these are summarised in the Table.[46-63] Although copper(II) complexes continue to be widely studied, measurements are now being reported for Be(II)[46,58]

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

cations	ligand	method	Ref
Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}	tetracycline/ glycine	potentiometry, 25°C, I = 0.1M $NaNO_3$	46
Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}	2-amino-3-phosphonopropionic acid. 3-amino-3-phosphonopropionic acid. 2-amino-4-phosphonobutanoic acid	potentiometry, 25°C, I = 0.2M KCl	47
Cu^{2+}	L-Ala, L-Leu, L-Val	potentiometry, 25°C in dioxane-water mixtures, I = 0.1M $NaNO_3$	48
Cd^{2+}	3-aminopropane-1-thiol	potentiometry, 25°C in 10%(v/v) MeOH-H_2O, I = 3M $NaClO_4$	49
Cu^{2+}	L-Asp, L-Glu	potentiometry, 25°C I = 1.0M $NaClO_4$	50
Cu^{2+}	Tos-Asn, Tos-Gln	potentiometry, spectrophotometry	31
VO^{2+}	L-Ser, L-Thr	potentiometry, e.s.r.,visible and c.d. spectra	51

cations	ligand	method	Ref
Ni^{2+}, Co^{2+}	Gly, Ala, Val/imidazole	potentiometry 37°C, I = 0.15M KNO_3	52
Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}	aminopolycarboxylic acids or bipy or phen/ acetohydroxamic acid	potentiometry, 25°C, I = 0.1M KNO_3	53
H^+, Co^{2+}, Ni^{2+}, Cu^{2+}	2-amino-N-hydroxypropanamide	potentiometry, 25°C, I = 0.5M KCl	54
Cu^{2+}, Ni^{2+}	δ-hydroxylysine	potentiometry, 25°C, I = 0.15M $NaNO_2$	55
Cd^{2+}	Asp, Glu	potentiometry, 25°C, I = 1.0M $NaClO_4$	56
Cd^{2+}	Ser	potentiometry, 25°C, I = 3.0M $NaClO_4$	57
Cd^{2+}	Tos-Ala, Tos-β-Ala	^{113}Cd n.m.r. and potentiometry	39
Be^{2+}	DL-Asp, Met	potentiometry, 25°C, I = 0.5M $NaClO_4$	58

cations	ligand	method	Ref
UO_2^{2+}, Th^{4+}	Asp	potentiometry, 25°C, I = 1.0M $NaClO_4$	59
VO^{2+}	L-Cys and D-Pen	potentiometry, 25°C, I = 2.25M $NaNO_3$	60
Cu^{2+}	ternary complexes involving Trp, Tyr, Phe or L-Dopa	potentiometry, 25°C, I = 0.2M	61
Cu^{2+}	ternary complexes involving bis(imidazole-2-yl) methane and bis(imidazole-2-yl) nitromethane with bidentate and tridentate amino acids	potentiometry, 35°C, I = 0.2M	62
Co^{2+}, Ni^{2+}, Zn^{2+}	salicyloylamino acids (Sal-Gly, Sal-Ala, Sal-Met); ML, ML_2^{2-}, $ML(OH)^-$ species	potentiometry, 25°C, I = 0.2M	63

Cd(II),[49,56,57] VO^{2+},[51,60] UO_2^{2+},[59] and Th(IV),[59] and there is continuing interest in the area of mixed ligand complexes involving amino acids.

Equilibria in solutions containing Cu(II) and potentially terdentate amino acids have been investigated by e.s.r. spectroscopy. The amino groups of R,S-2,3-diaminopropionic acid and R,S-2,4-diaminobutyric acid which are protonated in acid solution bind equatorially to copper with increasing pH with the exception of the 2:1 complex of the latter ligand in which the fourth nitrogen atom binds axially at pH>8. In the 2:1 complex of S-asparagine above pH 9 the first deprotonated amide nitrogen is bound axially and the second equatorially. In the 2:1 complex of S-histidine the imidazole nitrogens enter the equatorial plane when deprotonated.[64]

2.3 Reactions in Solution.- Temperature jump techniques have been used to study the kinetics of complexation of L-histidine with the oxomolybdate anion.[65] Reactions were carried out in the pH range 5.0-7.0 at I = 0.1M ($NaClO_4$) and 25°C. The polymerisation of molybdenum(VI) in the same pH range has also been studied as has molybdenum(VI)-imidazole complexation in an attempt to assess the possible participation of the imidazole nitrogen of histidine in coordination to molybdenum(VI).

Trace metals such as copper and iron are known to catalyse the oxidation of thiols by molecular oxygen. The kinetics of the Cu(II), Fe(II) and Fe(III) catalysed oxidation of cysteine by dioxygen have been studied.[66] The reaction proceeds in two steps,

$$2RSH + O_2 \longrightarrow RSSR + H_2O_2 \qquad (i)$$
$$2\ RSH + H_2O_2 \longrightarrow RSSR + 2H_2O \qquad (ii)$$

the first of which follows Michaelis-Menten kinetics with respect to RSH, and probably O_2, and is, partly at least, second order with respect to Cu. The rate of reaction (ii) is first order with respect to RSH and H_2O_2 and is enhanced by Fe(II) and Fe(III) but not at all by Cu(II). The present study indicates a possible role for binuclear complexes (Cu + Cu or Cu + Fe) in the reaction.

Previously it had been shown that Ni(II) retarded the rate of racemization of L-alanine in contrast to a number of other metal ions such as Cu(II), Cr(III), Co(III), Pd(II) and Pt(II) all of which accelerated it. This study was extended to L-serine and L-threonine.[67] The metal ions Zn(II), Cu(II), Co(II) and Pd(II) were found to enhance the rate of racemisation of L-serine by between three and forty-fold while Ni(II) retarded it. All the metal ions accelerated racemisation in L-threonine with the exception of Ni(II) and Zn(II) both of which retarded it. The retarding effect of nickel(II) increased with increasing concentration of the metal ion suggesting that nickel(II) salts might have applications as racemisation suppressants in peptide synthesis. The unique behaviour of nickel(II) has been investigated using the correlation between pH profiles of electronic spectra and rates of racemisation in the case of the Ni(II)/alanine system.[68]

The acid dissociation and dimerisation reactions of platinum(II) complexes of the general type $[Pt(AA)DMSO(Me_2SO)(H_2O)]^+$ (AA = Gly, Sar, N,N-Me_2-Gly) have been investigated by potentiometric and n.m.r. techniques.[69] The dimerisation reaction can be summarised by the equation

$$Pt\text{-}OH + Pt\text{-}OH_2^+ \underset{k_{-d}}{\overset{k_d}{\rightleftharpoons}} Pt\text{-}OH\text{-}Pt^+ + H_2O$$

where $Pt\text{-}OH_2^+$ and Pt-OH denote the aqua species and its deprotonated conjugate base and $Pt\text{-}OH\text{-}Pt^+$ denotes the M-OH bridged dimer. Equilibrium constants (K_d) and rate constants (k_d and k_{-d}) for the dimerisation reaction over a range of temperatures are reported.

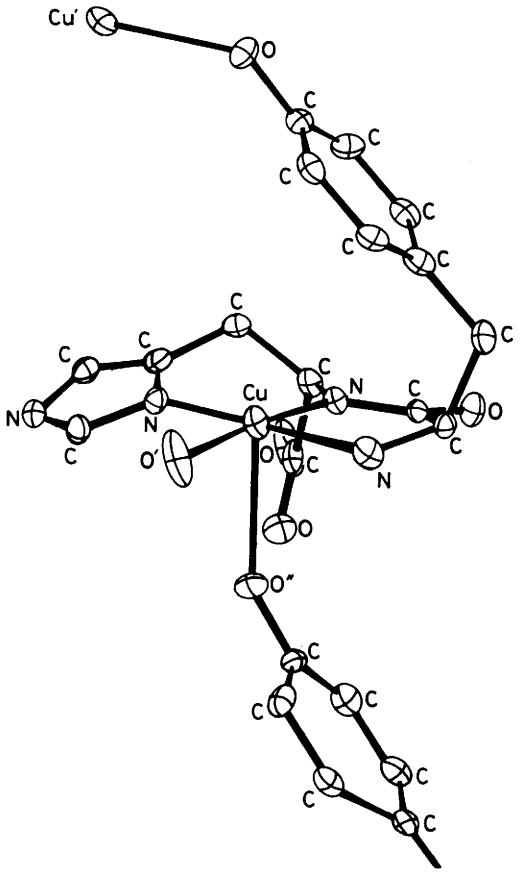
3 Peptide Complexes

The chemistry of metal peptide complexes is the subject of an increasing number of articles with many of the compounds described having direct or indirect implications for bioactive systems.

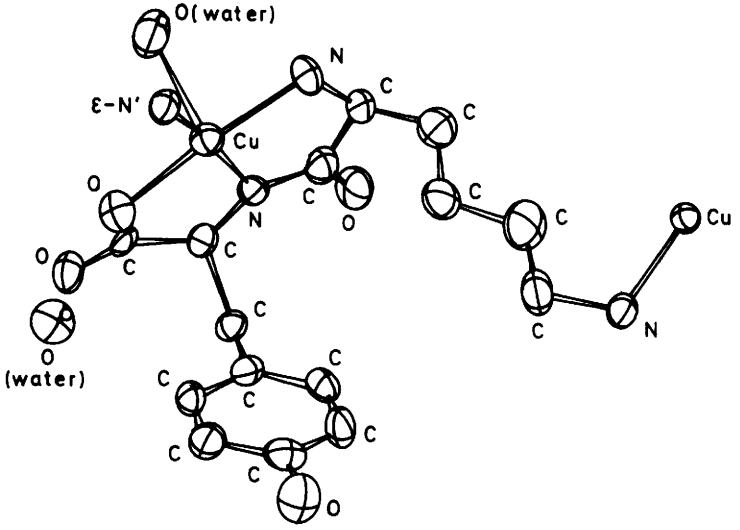
3.1 Crystal Structures, synthesis.- The crystal structure of Cu(Tyr-His) (8) a model for a tyrosinase substrate complex has been determined by X-ray crystallography.[70] This complex which was isolated at pH 7 contains copper(II) in a square pyramidal ligand field with three nitrogen atoms of one ligand and the carboxylate oxygen of a neighbouring ligand in the base plane. A phenolic oxygen (unionized) from another neighbouring ligand occupies the apex of the square pyramid. The structure of the complex Cu(L-Lys-L-Tyr).$2H_2O$ has also been determined by crystallography.[71] This complex is polymeric (9) with each copper in a distorted square pyramidal environment having NH_2, N^- and COO^- donors of one peptide ligand as well as the ϵ-NH_2 group of a neighbouring ligand in the square plane and a water ligand in the apical position.

Reaction between $[Ni(NH_3)_6]Cl_2$ and the peptide analogue N-(2-mercaptopropionyl)glycine (10) in aqueous dimethylformamide affords a trinuclear complex which was obtained in crystalline form as $(NH_4)_3[Ni_3(C_5H_6NO_3S)_3].3MeOH.0.5Et_2O$ from $MeOH/Et_2O$.[72] This complex contains a novel Ni_3S_3 trigonal antiprismatic core (11). The geometry around each nickel is square planar with the thiolate S, amido N and carboxylate O occupying three coordination sites and a bridging thiolate S from a neighbouring ligand in the fourth position. While the trimeric structure is retained in aqueous methanol and DMF solutions the addition of ligands such as CN^-, pyridine or imidazole gives the monomers $[Ni(C_5H_6NO_3S)L]^{-/2-}$. All the complexes undergo irreversible oxidation to unstable nickel(III) species in the potential range 0.3 to 0.4 V vs SCE.

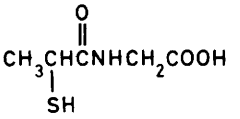
Some copper(II) and nickel(II) complexes of N-acetyl, N-chloroacetyl, N-trifluroacetyl, N-formyl and N-benzoyl peptides have been synthesised and characterised by spectroscopic, conductimetric and magnetic susceptibility measurements.[73,74] The following complexes were characterised; $Ni(For\text{-}Phe\text{-}Gly)_2L_x.nH_2O$, $Ni(Bz\text{-}Ala\text{-}Gly)_2L_x.nH_2O$, $M(R\text{-}Phe\text{-}Gly)_2.nH_2O$ and $M(R\text{-}Phe\text{-}Gly)_2L_2.nH_2O$ (M = Cu or Ni; R = CH_3CO^-, CH_2ClCO^-, CF_3CO^-; L = imidazole, 1-methylimidazole, 1,2-dimethylimidazole; x = 0,2). In all of these complexes bidentate carboxylate coordination of the peptide to the metal occurs.



(8)



(9)



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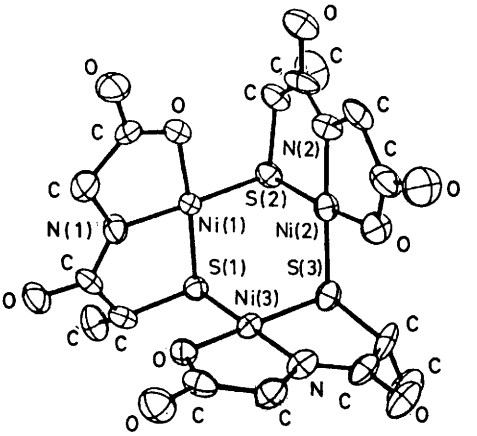
O
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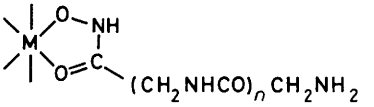
(11)


M
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NH
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(CH2NHCO)n CH2NH2

(12)

The dipeptide complexes trans-$[Pd(Gly\text{-}AA)_2 nucl_2]Cl_2$ (AA = Gly, L-Ala, L-Val, L-Leu; nucl = inosine, guanosine) have been isolated and characterised by microanalytical, conductimetric and spectroscopic methods.[75] ^1H n.m.r. spectroscopy of D_2O and DMSO solutions of these complexes give evidence for the existence of a number of conformational isomers.

The complexes $Ni(Gly_2\text{-}NHO)_2.H_2O$, $Ni(Gly_3\text{-}NHO)_2.H_2O$, $Fe(Gly_2\text{-}NHO)_3$ and $Fe(Gly_3\text{-}NHO)_3$ which contain hydroxamic acids of di- and tri-glycine (Gly_2-NHOH, Gly_3-NHOH) have been synthesised and microanalytical data, magnetic moments, i.r. and electronic spectra have been reported.[76] The complexes appear to contain octahedrally coordinated metal ions with the hydroxamate ligands coordinated through the C=O and NHO^- groups,(12).

3.2 Stability Constants, Species in Solution.- Potentiometric and spectrophotometric methods have been used to study the interaction of metal ions with the biologically active peptides angiotensin II,[77] (13) Arg^8-vasopressin (14) and Arg^8-vasotocin (15)[78] as well as with the synthetic analogues (16)-(19). Above pH 8 Ni(II) coordinates to angiotensin II via four nitrogen donors starting from the N-terminal Asp residue. While coordination of the imidazole of His may be present below pH 8 it is absent at high pH. The high resolution n.m.r. spectrum of angiotensin II and its Ni(II) complex at pH 11 have been recorded and side chain resonances assigned. Complexes of Cu(II) with vasopressin and vasotocin are the most stable copper peptide complexes with 4N coordination yet reported. This stability is lost however when Glu is replaced by a residue of opposite chirality such as D-Val.

The interaction of copper(II) with peptides containing the Phe-Phe submit has been studied by potentiometric and spectroscopic methods (e.s.r., uv-visible and c.d.).[79] The peptides studied were Phe-Phe-Ser-Asp-Lys, Phe-Phe and Phe-Phe-Phe and evidence for axial coordination of the $\beta\text{-}CO_2^-$ group of the Asp residue in the first peptide has been obtained. The copper(II) complexes of the dipeptides Gly-Asp, Gly-Asn, Gly-Ser, Gly-Thr, Gly-Phe, Ser-Gly, Thr-Gly, Phe-Gly, Gly-Gly were studied by

Asp — Arg — Val — Tyr — Ile — His — Pro — Phe

(13)

Cys — Tyr — Phe — Gln — Asn — Cys — Pro — Arg — Gly — NH_2

(14)

Cys — Tyr — Ile — Gln — Asn — Cys — Pro — Arg — Gly — NH_2

(15)

Asp — Arg — Val — Tyr

(16)

MeCO — Tyr — Ile — His

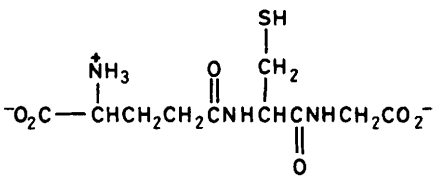
(17)

Cys — Tyr — Ile — D-Val — Asn — Cys — Pro — Arg — Gly — NH_2

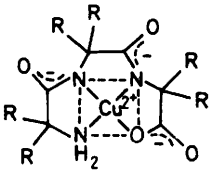
(18)

Cys — Tyr — Phe — D-Val — Asn — Cys — Pro — Arg — Gly — NH_2

(19)



(20)



(21)

potentiometric and spectrophotometric methods at high pH and at ligand to metal ratios up to 50:1.[80] The influence of the side chain donor groups and the aromatic rings on the competitive formation of $Cu(AH_{-1})OH$ and $Cu(AH_{-1})_2$ was evaluated. Complexes of copper(II) with opioid peptides were detected by fast atom bombardment mass spectrometry and formation constants for the Leu^5-, Met^5- and [D-Ala^2, Met^5]- enkephalin complexes were determined by potentiometry.[81] It was concluded that at physiological pH all brain enkephalins could be bound by endogenous copper provided the copper(II) concentration exceeded 10^{-7}M. Complexes of Gly-Gly, Gly-Val, and Gly-Tyr with Cu(II) and Zn(II) were studied by micropotentiometric titrations under physiological conditions.[82]

Complex formation between Zn^{2+} and LH-RH, the hypothalamic neurohormone p-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-GlyNH_2, and some of its analogues in aqueous solution were investigated by potentiometric and n.m.r. methods.[83] The suggested mode of binding of the metal ion to the hormone is through the imidazole N and the O of the His-Trp peptide linkage. The fact that Zn^{2+} can displace Ca^{2+} from its binding sites thus disrupting various cellular process, is due to longer Zn^{-} complex lifetimes and higher thermodynamic stabilities, a combination which compensates for the high $[Ca^{2+}]/Zn^{2+}$ concentration ratios.[84] Conformational-activity studies of thymulin a metallo nonapeptide, by one and two dimensional n.m.r. techniques show that Zn^{2+} which is known to induce the bioactivity of the peptide confers a specific, physiological conformation on the molecule.[85] The binding of Zn(II) to a 25 residue synthetic peptide corresponding to the zinc finger of the protein Xfin has also been studied by n.m.r. spectroscopy.[86] The peptide adopts a compact, folded conformation containing helical and hairpin-like regions. The affinity of a 30 residue 'zinc finger' peptide for Co(II) and Zn(II) has been determined by spectrophotometric titrations.[87] The reduced affinity for cobalt(II) is rationalised on the basis of loss of crystal field stabilisation energy in going from an octahedral environment in $[Co(H_2O)_6]^{2+}$ to a tetrahedral environment in the peptide complex.

A 56 residue RNA-binding protein from murine leukaemia virus was synthesised and found to bind Cd(II), Co(II) and Zn(II) via

the peptide sequence -Cys-X_2-Cys-X_4-His-X_4-Cys-.[88,89] The d-d spectrum of the cobalt(II)-protein complex is typical of tetrahedral cobalt(II) and the electronic spectra of both the Co(II) and Cd(II) complexes contain metal to thiolate charge transfer bands. The ^{113}Cd n.m.r. spectrum of the Cd(II) complex is reported. ^{113}Cd n.m.r. spectra are also reported for the 1:1 Cd^{2+} adduct with an 18 residue synthetic peptide comprising the amino acid sequence of the first finger region of nucleic acid binding protein from HIV-1, the causative agent of AIDS.[90] The spectra are consistent with 3 Cys, 1 His coordination to Cd(II). ^{1}H n.m.r. spectra (500 MHz) are also reported for the 1:1 Cd(II) and Zn(II) adducts.

The peptides Ac-Asp-Val-Asp-Ala, Ac-Tyr-Val-Asp-Ala and Ac-Asp-Gly-Tyr-Val-Asp-Ala have been studied as models for the calcium binding sites in several proteins.[91] Stability constants of a number of complexes have been determined and on the basis of induced paramagnetic shifts it was concluded that Pr^{3+} binds to the first peptide through the terminal carboxylate group at pH 2.4 and to both Asp residues at pH 7. In the Tyr- containing peptides both the Ala and Asp residues are involved in metal binding at pH 5.5. Similar results were obtained using i.r. spectroscopy for the Cu(II), Ni(II) and Fe(II) complexes. The binding of the synthetic hexapeptide Tyr-Asn-Arg-Gly-Asp-Ser which mimics the 570-575 sequence in the fibrinogen α chain to the platelet glycoprotein IIb - IIIa complex was found to depend on a number of factors including divalent cations.[92] For the synthetic peptide to bind two cation sites on the glycoprotein, one specific for Ca^{2+} and the other for Ca^{2+} or Mg^{2+} must be occupied. In order to test the hypothesis that the non linear expansions observed when Ca^{2+} is sequentially added to intracellular calcium proteins depends on the coordination event at specific binding sites, three peptides which mimic the metal binding loop of calmodulin have been synthesised and the volume changes on the binding of lathanide ions to these were measured.[93]

Stoichiometries and stability constants of proton and copper(II) complexes of monophosphonodipeptides, $NH_2CHRCONHCHR'PO_3H_2$ and of diphosphonodipeptides, $H_2O_3PCHRCONHCHR'PO_3H$ have been determined potentiometrically.[94]

Many analogues of these dipeptides show significant antibacterial properties, act as enzyme regulators or display antitumour activity. While the phosphonate substitutions do not significantly alter the basic bonding mode of the peptides deprotonation and concomitant coordination of the peptide group becomes less favoured.

Complex formation between copper(II) and Gly-Gly (HL) has been studied spectrophotometrically over a wide range of ligand concentrations (0.005-1.0M) and pH (0.5-13).[95] The complexes CuL^+, $Cu(HL)^{2+}$, $Cu(LH_{-1})$, $Cu(LH_{-1})OH^-$, $Cu(LH_{-1})L^-$, $Cu(LH_{-1})_2^{2-}$ and $Cu_2(LH_{-1})_2OH^-$ have been identified and their formation constants determined. In $Cu(HL)^{2+}$ the peptide is present as a monodentate carboxylato-bonded ligand while in $Cu(LH_{-1})_2^{2-}$ both ligands act as 2N bidentate donors. The lability of five peptide complexes of the type $Cu(LH_{-1})L^-$ have been investigated by the n.m.r. relaxation of water protons. The ligand exchange rates decrease with the size of the peptide side chain and a high trans effect is shown by the deprotonated peptide group. Copper(II) complexes of glycyl containing di- and tri-peptides (HL) with non-coordinating side chains were investigated by e.s.r. spectroscopy of fluid or frozen aqueous solutions containing 1:1, 2:1 and 50:1 ligand to metal ratios in the pH range 6-13.[96] At high ligand to metal ratios low temperatures promote the formation of the complexes $Cu(LH_{-1})L^-$ at pH 9 and $Cu(LH_{-1})_2^{2-}$ at pH 13 in the case of the X-Gly dipeptides.

Formation constants have been determined for complexes of $[Cu(dien)]^{2+}$ with amino acids (Gly, Ala, 2-Ala, Val, Ser, Thr, Met) and peptides (Gly-Val, Gly-Leu, Gly-Gly-Gly).[97] In the Cu(dien)peptide complexes coordination of deprotonated peptide groups to the metal is not observed. Binary and ternary complexes of copper(II) with Gly-Tyr and amino acid esters (GlyOEt, AlaOMe, AlaOEt, SerOMe, SerOEt, HisOMe, LeuOEt and 2-AbaOEt) were investigated by potentiometric and spectrophotometric methods.[98] Base hydrolysis of the amino acid esters in these complexes was also studied. Formation constants of a number of ternary Cu(II)-ATP-glycyl peptide complexes have been determined by potentiometric methods.[99]

Complex formation of Zn(II) and Cd(II) with cysteine, glutathione (GSH) and N-acetylcysteinemethylamide (ASH) has been studied by differential pulse polarography.[100] While Cd(II) forms 1:1 and 1:2 complexes with Cys 1:2 and 1:4 complexes were obtained with GSH and ASH. Stability constants and species distribution curves are reported for complexes of Ni(II) and Fe(III) with hydroxamic acids of di- and tri-glycine.[76]

A number of investigations on diastereoselectivity and cation selectivity by cyclic peptides ligands have been carried out. Cyclo(D-Leu-L-Pro)$_4$ was found to selectively complex with Ba^{2+} and the stability constant of the complex was similar to that formed by the diastereomer cyclo(L-Leu-L-Pro)$_4$.[101] However the rate of complex formation is much faster in the case of the first peptide and this has been ascribed to peptide bond isomerisation accompanying complex formation in the case of the L,L peptide. Cation transport across a $CHCl_3$ liquid membrane is similar for both diastereomers. Diastereoselectivity of Li^+, Na^+ and K^+ ions towards the cyclohexadepsipeptide cyclo(Val-Lac) (Lac = lactic acid) has been observed.[102] While the 'all L' diastereomer shows no affinity for these cations the L-Val-D-Lac diastereomer complexes appreciably and the implications of this for selective biological ion transport and for ion mediated biological catalysis are discussed. The cyclic hexapeptides cyclo(L-Asp-L-Phe-L-Pro)$_2$, cyclo(L-Aad-L-Phe-L-Pro)$_2$, cyclo(D-Asp-D-Phe-L-Pro)$_2$, cyclo(L-Leu-L-Phe-L-Pro)$_2$ and cyclo(L-Acm-Cys-L-Phe-L-Pro)$_2$ have been synthesised and their conformations, metal complexation and interaction with lipid membranes investigated.[103,104] While cyclo(D-Asp-D-Phe-L-Pro)$_2$, cyclo(L-Leu-L-Phe-L-Pro)$_2$ and cyclo(L-Acm-Cys-L-Phe-L-Pro)$_2$ form complexes with Ba^{2+} and Ca^{2+}, cyclo(L-Asp-L-Phe-L-Pro)$_2$ and cyclo(L-Aad-L-Phe-L-Pro)$_2$ do not. The complexing abilities of the cyclic peptides cyclo-ε-(Z-Lys)$_n$ (n=3-6) towards alkali, alkaline earth and some transition metal cations have been examined by ionization cyclotron photometry and atomic absorption spectrometry.[105] The cyclic peptides cyclo(GluOBzl-GluOMe), cyclo(Glu-GluOMe), cyclo(Glu-Glu), cyclo(GluOMe-Pro)$_2$ and cyclo(Glu-Pro)$_2$ have been synthesised and possible interactions between carboxy side chains in the free peptides and their metal complexes investigated.[106] The last two peptides form complexes with Ca^{2+} and Ba^{2+} without participation of side chains.

3.3 Reactions in Solution.- The involvement of alkaline earth and d-block metal ions in peptide synthesis from amino acids in aqueous solutions containing high concentrations of NaCl at 85°C has been investigated and only copper(II) was found to be catalytically active.[107] The implications of these results for prebiotic peptide synthesis in simple systems containing water, amino acids and mineral salts is discussed.

The synthesis of phytochelatins, the heavy metal binding peptides of plants proceeds by the enzyme catalysed transfer of a γ-Glu-Cys residue of glutathione to a growing chain of $[Glu(-Cys)]_n$-Gly oligomers.[108] The enzyme (phytochelatin synthase) is best activated by Cd^{2+} but also by Ag^{+}, Bi^{3+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Hg^{2+} and Au^{+}. A free radical carboxylation of glycyl residues in peptides and proteins to form aminomalonic acid residues is reported and the in vivo formation of these residues as calcium binding sites in distrophic tissue discussed.[109]

Results of investigations into the mechanisms for the carcinogenicity and mutagenicity of chromate have been reported. It appears that CrO_4^{2-} can readily be transported across cell membranes by anion carriers and it is then immobilised by reduction. Reducing intracellular constituents such as glutathione (GSH, **20**) may therefore have a bearing on the toxicity of chromate. The reduction of CrO_4^{2-} by GSH at pH 7.0 gives a green Cr(V) intermediate which subsequently decays to a purple chromium (III) species.[110] The kinetics and mechanisms of these reactions have been investigated and a chromium (III) complex of formula $Na_2[Cr(GSH)_2].2H_2O.0.7NaCl$ has been isolated.[111] Spectroscopic studies indicate that this complex contains N_3O_3 coordination to chromium. Confirmation for the reaction between Cr(VI) and glutathione in intact erythrocytes has been obtained using ^1H spin echo n.m.r. spectroscopy.[112]

The interaction of $[Pt(H_2O)_2en]^{2+}$ with the tetrapeptide Me_3CO_2C-Cys(Me)-Ser-Ala-Cys(Me)-NH_2, a model for metallothionein, has been studied by one and two dimensional ^1H n.m.r.[113] In aqueous solution Pt(II) forms a 1:1 complex involving the two 5-Me groups of the Cys residues. The reactions of cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ with amino acids and peptides have been

studied by ^{195}Pt, ^{15}N, ^{1}H and ^{13}C n.m.r. spectroscopy.[114] While acetylcysteine, homocysteine and glutathione form binuclear complexes having a Pt_2S_2 four membered ring cysteine gives also small quantities of cis-$Pt(NH_3)_2[SCH_2CH(NH_3)CO_2H]_2^{2+}$ and $[Pt(NH_3)_2(Cys\text{-}N,S)]^+$. Penicilliamine gives initially $[Pt(NH_3)_2]_2penH^{3+}$ in which the metal atoms are bridged by both S and O. On standing this isomerises to a species which contains an S atom bridge and in which one of the Pt atoms is bonded to O, the other to N.

Copper(II) transfer between Asp-Ala-His, a tripeptide sequence of albumin, and His was investigated by differential pulse polarography.[115] The reduction of copper(II) complexes of His and β-Ala-His at Hg electrodes has been studied by d.c. cyclic normal pulse and reverse pulse voltammetry.[116] The electrochemical reduction of some nickel(II)-dipeptide complexes has also been reported.[117]

The interaction of Sc^{3+} with the peptides Val-Ala, Glu-Val and Ala-Val-Leu in aqueous solution has been studied by ^{45}Sc n.m.r. spectroscopy.[118] The interaction of trace levels of vanadate, V(V), and vanadyl, V(IV), with buffers, chelating agents, enzyme substrates, cofactors, amino acids, peptides and proteins was examined by enzymatic methods.[119] It is concluded that biological activities may be affected by vanadium concentrations as low as 10^{-5}-10^{-7} M.

In the presence of catalytic amounts of Cu^{2+} ascorbate was found to cause site specific damage to peptides and proteins with the loss of histidine residues.[120,121] Electron transfer from chiral dihydroxysubstrates such as L-ascorbic acid, L-adrenaline and L-dopamine to iron(III) in $[Fe(tetpy)OH_2]^+$, where tetpy is 2,2',2'',2'''-tetrapyridyl, anchored to sodium poly(L- or D- glutamate) occurs stereoselectively with the terpy ligand acting as the electron transfer agent.[122]

The displacement of the tripeptides Gly-Ala-Gly, Gly-Gly-Ala, Gly-Aib-Gly, and Aib_3 (Aib = α-aminoisobutyric acid) from the complexes $Cu(H_{-2}L)^-$ (21) by triethylenetetramine (trien) follow second order kinetics with the values of k_2 decreasing greatly (e.g. $6.5x10^5$ from the Gly-Ala-Gly to the Aib_3 complex) with the

number of methyl groups in the second and third residues.[123,124] The values of k_2 are also pH-dependent and with the exception of the Aib_3 complex these reach maximum values at pH 10.7 due to a combination of the increased reactivity of trien and trien-H^+ over trien-H_2^{2+} and of $Cu(H_{-2}L)^-$ over $Cu(H_{-2}L)OH^{2-}$. Similar peptide displacement reactions were investigated for complexes of Ala-Gly-Ala, Gly-Ala-Ala, Ala-Ala-Ala, Gly-Gly-Aib, Ala-Ala-Aib, Aib-Aib-Gly and Aib-Aib-Ala. Methyl groups in the second or third residue decrease k_2 to a much greater extent than methyl groups on the first residue. Stopped flow spectrophotometry was used to study general acid catalysis by 2,6-lutidine buffers in the protonation and acid dissociation of the complexes $[Cu(H_{-2}Gly_3)]^-$, $Cu(H_{-1}Gly_3)$, $[Cu(H_{-2}Gly_4)]^-$, $CuH_{-1}Gly_4$ and $[Ni(H_{-2}Gly_3)]^-$.[125] Because of steric hindrance and hence limited coordinating power general acid catalysis was found to be very weak and this combined with the wide buffering range (pH 3.1-7.4) makes these buffers attractive alternatives in studies involving reactions of metal complexes.

A novel method based on the influence of ^{61}Ni on the room temperature e.p.r. spectra of Ni(III) complexes is used for the measurement of the electron transfer self exchange rate constant of $Ni^{III/II}(H_{-2}\ Aib_3)$.[126] The exchange rate decreases when pyridine replaces one of the aquo ligands in the nickel(III) complex. A number of histidine containing oligopeptide complexes of nickel(II) have been shown to possess superoxide dismutase activity and the involvement of an Ni(III)/(II) redox couple in the dismutation was confirmed by e.s.r. data.[127] These complexes also disproportionate H_2O_2 through the possible formation of an active oxene species such as NiO^{2+}. The decomposition of H_2O_2 in the presence of nickel(II) oligopeptide complexes leads to the production of hydroxyl radicals with catalytic activity following the ligand sequence Gly_4 > Gly_5 >Gly_3 > Gly_2 > Gly-His.[128]

The mechanism of formation of cobalt(III) complexes with the dipeptides Gly-Gly, Gly-Asp, Gly-Thr, Gly-Tyr and Gly-Pro by peroxide oxidation of cobalt(II) complexes was studied by electronic spectroscopy.[129] Oxygenation and deoxygenation kinetics for Co(II)/(2,2'-bipyridyl)/dipeptide complexes have been investigated by stopped flow spectrophotometry.[130] The cleavage

of N-terminal amino acids from peptides by a solid state polymeric reagent based on cellulose into which triethylenetetramine and cobalt(III) had been incorporated is reported.[131]

The use of silver trifluoromethanesulphonate for the quantitative removal of S-acetamidomethyl groups from Cys residues in peptides is described.[132] The quantitative estimation of removal of protecting groups from peptides by reduction with sodium in liquid ammonia has been demonstrated using protected oxytocein and a number of its analogues.[133]

The absorption properties of over 60 biologically active peptides on TSK 5PW gel onto which Cu(II), Ni(II) and Zn(II) were immobilised by iminodiacetic acid coupling were evaluated by h.p.l.c.[134] Iminodiacetate-derivatized polymers loaded with Cu(II) have also been used for the h.p.l.c. separation of synthetic peptide hormones, proteolytic digests and specific bioactive protein fragments.[135] The influence of different displacer salts on the retention properties of proteins separated by gradient anion exchange chromatography has been investigated.[136] The separation of angiotensins, cationic heptapeptides and model histidine derivatives by capillary zone electrophoresis is reported to be enhanced in the presence of Zn^{2+}.[137] The preparation of nickel chelate resins and their use in the chromatography of peptides and proteins is described.[138] The use of Cu(II)-polyamine complexes for the isotachophoresis of amino acids and peptides is also reported.[139]

3.4 Miscellaneous.- The gas phase interaction of alkali metal cations and small peptides has been studied by fast atom bombardment combined with tandem mass spectrometry.[140] These ions bind to and promote the hydrolysis of the C-terminal amino acid residues in much the same way as carboxypeptidase A does in solution. The mechanism for the formation of other fragment ions such as metalated immonium ions is described. One hundred and eight cyclic peptides for the treatment of hypercalcemia have been synthesised and forty of these at levels of 6.25 mg/kg produced a reduction of calcium levels in blood.[141] A zinc binding peptide of the $(\gamma$-glutamyl-cysteinyl$)_n$glycine type and of MW 4.5 kDa which

is associated with the occurrence of citrus blight has been isolated and purified.[142] Monoclonal antibodies capable of specific hydrolysis of Gly-Phe peptide bonds in the presence of triethylenetetramine complexes of Zn(II), Ga(III), Fe(III), In(III), Cu(II), Ni(II), Lu(III), Mg(II) or Mn(II) as cofactors have been induced.[143] The formation of ethyl carbamate during the distillation of ethanolic solutions of bovine serum albumin or wheat gluten involves Cu(II)-peptide complexes.[144] The formation and stability of poly(N^{ϵ},N^{ϵ},N^{ϵ}-trimethyl-L-lysine) in the presence of different concentrations of $NaClO_4$ is reported.[145] A cadmium adsorbent for wastewater treatment which contains thiolate groups and peptide linkages has been developed.[146]

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