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Amino Acids and Peptides

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Amino Acids and Peptides

Volume 23

A Review of the Literature Published during 1990

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Preface

This is the tenth volume in this series which it has been my privilege to co-ordinate, and I trust I will be forgiven for stepping down. The need for literature digests in the area is increasing all the time: the annual output on most topics has more than doubled since volume 1 (1968 literature). Unfortunately, this also makes the Reporters' work the more daunting, and I would like to record with special warmth how grateful I am to those who have soldiered on year after year so willingly and authoritatively. Dr John Davies has been one of these stalwarts (his help has been especially valuable in this my last year, during which he has agreed to contribute not one but two chapters), and I am glad to be able to hand over the operation to him.

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

By G.C.Barrett

1 Introduction

This year's literature on the chemistry and biochemistry of amino acids provides further proof of the ever-increasing rate of accumulation of new knowledge of these compounds. This expansion calls for increasing constraints on space allocated for the areas reviewed in this Chapter, which, as in earlier Volumes of this Specialist Periodical Report, emphasises papers covering the occurrence, chemistry and analysis of amino acids. Further narrowing is imposed within this context, only partial coverage being possible from what is judged to be routine literature. Biological areas such as the natural distribution and metabolism of well-known amino acids, for example, are not covered.

Patent literature is almost wholly excluded (but this is easily reached, mostly through Sections 16 and 34 of Chemical Abstracts). The Chapter is organised into a sequence of sections as used in all previous Volumes of this Specialist Periodical Report. Major Journals and Chemical Abstracts (to Volume 114, issue 11) have been scanned for the material to be reviewed.

2 Textbooks and Reviews

Textbook coverage of amino acids within plant biochemistry[1] and biosynthesis[2] has appeared, as has a review of the taste properties (particularly sweetness) of amino acids.[3] A clinical use for assay of 3-methylhistidine in urine, as a marker for skeletal muscle protein degradation, is discussed in a review of this amino acid.[4] Reviews of γ-carboxyglutamic acid[5] and selenocysteine[6] have appeared, in the latter case giving the background to the claimed discovery of the gene for its tRNA. Cyclopropane-based amino acids ("2,3- and 3,4-methano-amino acids") have been reviewed.[7] Numerous other reviews of aspects of amino

acid science have been published during the year under review, and references are located in the relevant sections of this Chapter.

A five-year retrospective survey on amino acids science[9] has been published in the first issue of a new Journal "Amino Acids" (Springer Verlag, Vienna and New York) whose well-justified launch includes in its first Volume, abstracts of papers that were presented at the Second International Congress on Amino Acids and Analogues, Vienna, August 1991.

3 Naturally Occurring Amino Acids

3.1 Isolation of Amino Acids from Natural Sources.- Isolation of amino acids has a simple requirement, to be sustained by proper practice, that the integrity of the amino acid in the extract is preserved. The well-known problem - losses of certain amino acids during protein hydrolysis - has been controlled in many cases by improvements in protocols. Classical 6M-hydrochloric acid hydrolysis procedures can give good recovery of tryptophan if tryptamine is included in the hydrolysis cocktail,[9] or if 3% phenol is added.[10] However, comparisons with standards show that more than 20% destruction of tryptophan must still be expected even when using these additives, though there is some improvement in the recovery of methionine and carboxymethylcysteine in these methods. Microwave irradiation of hydrolysis mixtures helps,[11] and vapour phase hydrolysis (7M-hydrochloric acid containing 10% trifluoroacetic acid, 20% thioglycollic acid, and indole)[12] can give up to 75% recovery of tryptophan.

An extraordinary physical property - adsorption of the $N^{\alpha}N^{\epsilon}$-bis(naphthalene-2,3-dicarboxaldehyde) derivative of lysine on to glass - is not shared by the N^{α}-mono-tagged amino acid.[13] Thus, reductive alkylation of proteins (N^{ϵ}-amino groups → NN-dimethylamino) is recommended before acid hydrolysis, to avoid this "loss" of lysine residues in this increasingly popular derivatization method through this unexpected way.

Methanesulphonic acid (115°, 22h)[14] continues to gain adherents for acid hydrolysis of proteins.

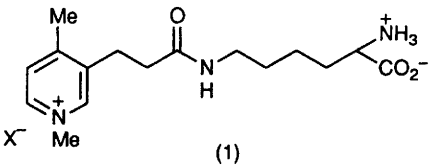
Care taken in preparative h.p.l.c. operations in processing aqueous extracts from fossil bones are described.[15] Errors due to contamination are minimized if all collagen analyses are based on a single bone sample. An aqueous two-phase system (water - aqueous polyethyleneglycol) has been advocated for isolation of amino acids from fermentation broth.[16]

3.2 New Natural Amino Acids.- Derivatives of protein amino acids that owe their exceptional biological activity to the overall structure of the derivative, with the amino acid moiety being merely the passive "carrier" of the derivatizing group, are not unusual. Amphikeumin (1) is an example of this class; it is a synomone, since it mediates partner-recognition between sea anemones and anemone-fish (and the fact that these words end in "-mone" is purely coincidental - synomone and pheromone, for example, have the same etymological base).[17] The range of extraordinary natural thioamides present in roots of radish (takuan) has grown, one of the new ones being the tryptophan derivative (2), presumed to be formed from L-tryptophan and 4-methylthiobut-3-enyl isothiocyanate.[18] The vinyl sulphide =CH-SMe in place of the tryptophanyl moiety[19] and the corresponding vinyl ether[20] are further examples.

A more complex heterocyclic system, though with equally suggestive biosynthetic origins, is represented in L-lupinic acid (3), isolated from the racemic amide through use of the aminopeptidase from Pseudomonas putida.[21]

A new antifungal antibiotic (4) has had all its structural features verified through X-ray analysis of its N-(N-phenylthiocarbamoyl-L-phenylalanyl) derivative.[22] "Pyrrolams" (5) and (6) are new simple pyrrolizidine alkaloids (from Streptomyces olivaceus) that can be recognized as cyclized proline homologues [but the absolute configuration in one case is (R), which might imply that proline itself is not on the biosynthetic pathway].[23] Amino alcohols are near relatives of amino acids, and as such, deserve brief mention in this section of this Chapter; xestoaminols A - C [B is (2S)-aminotetradeca-11,13-dien-(3R)-ol, and A and C are its dihydro- and tetrahydro-derivatives, respectively] have been isolated from a Fijian sponge Xestospongia sp.,[24] and are positional isomers of compounds reported from similar sources in 1989.

3.3 New Amino Acids from Hydrolyzates.- The meaning intended to be conveyed by the title of this section, is the discovery of new groupings in larger structures that would, in principle, be released as a new amino acid by hydrolysis (in principle rather than necessarily in practice). A new penta-functional crosslinking amino acid, allodesmosine, has been identified in bovine ligamentum nuchae elastin. It is a pyridinium salt like its well-known near-relative crosslinking amino acid, desmosine, and arises by further processing of the reduced aldol condensation product of two allysine, and one lysine, residues in the protein.[25] Pulcherosine (7) is a new trifunctional crosslinking amino acid from the fertilization envelope of the sea urchin embryo.[26] It occurs alongside the other major tyrosine-derived crosslinks, di-



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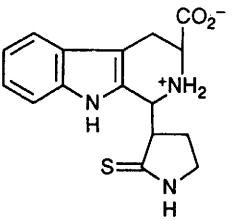

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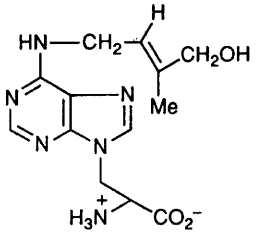

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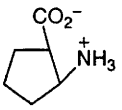

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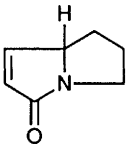

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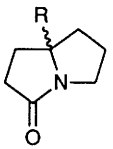
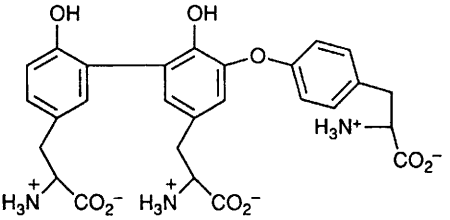


(6) R = OH, OMe, OCHMe, Et

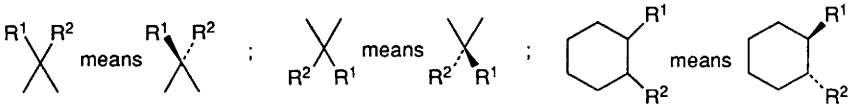


(7)

Three-dimensional features at chiral centres of structures depicted throughout this Chapter follow the convention:

(a) horizontally-ranged atoms, and their bonds, and ring atoms are understood to be in the plane of the paper;

(b) atoms and groups attached to these are ABOVE the page if ranged LEFTWARDS and BELOW the page if ranged RIGHTWARDS



tyrosine and tri-tyrosine. β-Aminoglutaric acid ("β-Glu") is a constituent of marine methanogenic bacteria.[27]

4 Chemical Synthesis and Resolution of Amino Acids

4.1 General Methods for the Synthesis of α-Amino Acids.- The reworking of a promising reaction through time, until it becomes established to be more generally applicable, is recorded in several papers relevant to this Section. Also, the well-known general methods are shown to continue to hold their own through further examples of non-routine character, many of these examples being mentioned elsewhere in this Chapter - particularly in the next section 'Asymmetric Synthesis'.

An α-halogenoglycine in a protected form is a useful synthon for α-amino acid synthesis, nucleophilic substitution by alkynyltin reagents $Bu_3SnC{\equiv}CR$ giving βγ-alkynylglycines.[28] The free alkynyl amino acids formed by deprotection were found in this study to be very labile but trapping experiments demonstrated that they had indeed been formed. N-Benzoyl-α-bromoglycine methyl ester readily undergoes nucleophilic substitution by side-chain functional groups in protected cysteines, serines, and threonines to give novel "cross-linking amino acids"[29] (by which is meant, compounds with the potential for synthesizing peptides as models for cross-linked proteins). The N,O- and N,S-acetal structures formed in this way are relatively easily hydrolyzed, though the cysteine derivatives seem to show stability sufficient for some applications. N-Acetyl bromoglycine methyl ester has been used for a synthesis of L-2-amino-4-methoxy-*cis*-but-3-enoic acid by reaction with $MeO.CH{=}CHLi$.[30] An alternative diethyl acetamidomalonate synthesis was reported later by the same workers [via the dimethylacetal of $HCO.CH_2.C(CO_2Et)_2NHAc \rightarrow$ (E)-$MeOCH{=}CH.CH(NH_2)CO_2H$, or $\rightarrow MeOCH(OCOMe).CH_2.CH(NH_2)CO_2H \rightarrow$ (Z)-$MeOCH{=}CH.CH(NH_2)CO_2H$].[31]

The *equivalent* α-acetoxyglycines, e.g. $Ph_2C{=}NCH(OAc).CO_2R$, on condensation with malonate anions give protected β-carboxyaspartates.[32] α-Keto-acid methyl esters can be condensed with benzyl carbamate to give protected αβ-unsaturated α-amino acids[33] available also through Wittig condensation of aldehydes with α-phosphono-glycines [e.g. $RCHO + ZNHCH(PO_3Et_2).CH(NH_2)CO_2Me$] or from base-catalyzed eliminations from β-halogeno- or β-acetoxy-α-amino acids. An alternative amination procedure is illustrated in the condensation of diethyl azodicarboxylate with lithium dienolates; full details in support of the preliminary communication of this work (Vol.22, p.7) stress the

importance of choice of catalyst, tin salts giving α-amination products while germanium salts yield γ-amino acids.[34]

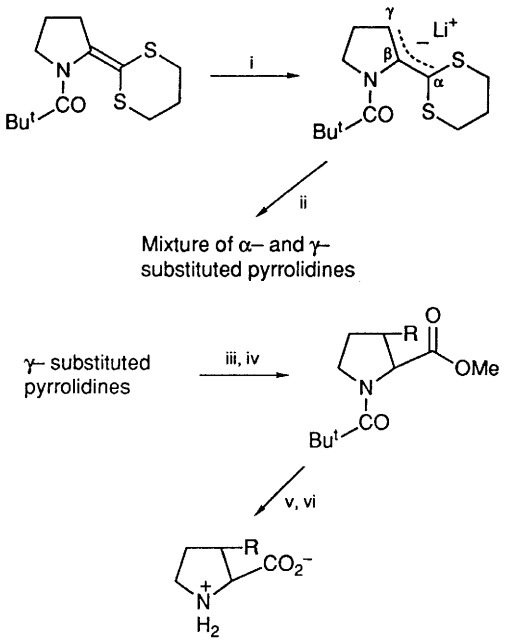
Oxalic acid mono-amide, $H_2N.CO.CO_2H$, should be an α-cationic glycine equivalent suitable for Wittig olefination, and the preparation of a suitably protected form of it has been described, starting from oxalyl chloride, through reaction with t-butanol and collidine - benzophenone imine.[35]

Further details (see Vol.22, p.7) are available[36] of the preparation of α-acylamino nitriles from Mannich-type condensation of benzotriazole with an aldehyde and an amide to give the substituted benzotriazole $R^1CONH.CHR^2.Bt$ which gives the α-acylaminonitrile with an alkali metal cyanide. Conditions are used that should permit a variety of functions within the aldehyde component to survive the reaction and subsequent hydrolysis of the nitrile to an α-amino acid. The same intermediate is involved in a preparation of α-substituted acyl aminals when NH_2 is used in place of cyanide.[37]

The Ugi four-component condensation has been used in an extraordinary "high-pressure mode" in which highly-hindered amino acids are constructed in the form of their N-(Z-L-valyl) derivatives [Z-L-Val-OH + $Ph.CH_2.NH_2$ + R^1_2CO + $CN.CH_2.CO_2R^2$ → $Z\text{-}L\text{-}Val.N(CH_2Ph).CR^1_2.CO\text{-}Gly\text{-}OR^2$].[38]

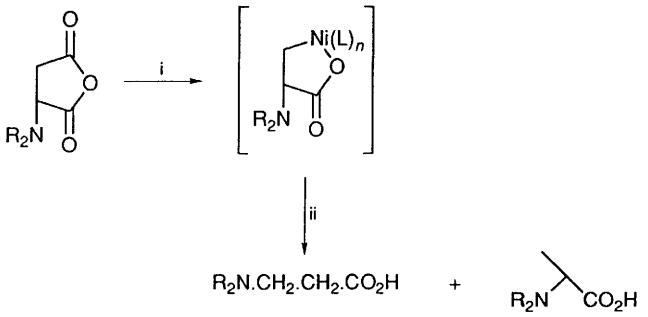
Alkylation of diethyl acetamidomalonate, using N-ferrocenylmethyl trimethylammonium iodide and NaOEt (reflux 45h to give N-acetyl β-ferrocenylalanine ethyl ester after work-up),[39] or using long-chain halogenoalkanes,[40] illustrate standard malonate applications. Improved routes to cis- and trans-3-substituted prolines[41] (condensation of diethyl acetamidomalonate with an αβ-unsaturated aldehyde, and routine elaboration of the resulting 3-substituted 5-hydroxyproline) have been described. A similar approach provides 4-hydroxyproline[42] and proline itself in a route involving reduction of the Michael adduct and cyclization of the derived toluene-p-sulphonate.[43] A new 3-substituted proline synthesis (Scheme 1) depends on the propensity of ketene dithioacetals for carbanion formation[44] and has been developed further for its potential in asymmetric synthesis (next Section, 4.2).

Similar alkylation procedures underpin other general methods, for example the phase-transfer catalyzed alkylation of $Ph_2C=N.CHR.CN$ with variously-substituted benzyl bromides followed by routine work-up.[45] A chiral phase transfer catalyst has been used with little success (as far as enantiomeric discrimination is concerned) in catalyzed alkylation of $Ph_2C=N.CH_2.CO_2Et$.[46] The other type of Schiff base, e.g. $R^1N=CH.CO_2R^2$, gives C-alkylation products with Reformatzky reagents RZnBr.[47] A different alkylation principle is involved in the conversion of the isocyanide $CN.C(CO_2Et)=CMe_2$ into 1-amino-2,2-dimethylcyclopropane carboxylic acid using trimethylsulphonium iodide and sodium hydride.[48]



Reagents: i, LDA, –78°C; ii, RX; iii, BF_3–Et_2O, then aq. K_2CO_3; iv, NaOMe; v, aq. NaOH; vi, aq. TFA, reflux 2h

Scheme 1



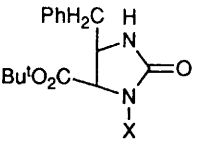
Reagents: i, Ni(cyclo-octadienyl)L_2/THF/heat; ii, H_3O^+

Scheme 2

Exploitation of side-chain functionalized amino acids as synthons for preparing other amino acids has continued to develop into useful general methods in some cases, and many new examples could be created from efficient reactions performed on amino acid side-chains (see Section 6.3). N-Benzyloxycarbonyl-L-vinylglycine methyl ester, for which there are now reliable methods of synthesis not anticipated in the early days, is open to use in this way,[49] [CH_2=CH.CH(NHZ)CO_2Me → R'CH_2CHR²CH(NHZ)CO_2Me] and so, also, are N-protected aspartic and glutamic anhydrides, proposed as synthons for alanines from an observation that oxidative addition and decarbonylation processes result from heating in THF with nickel complexes (Scheme 2).[50] Alkylation of the protected aspartic acid β-ester enolate[51] and their condensation with aldehydes so as to give βγ-unsaturated α-amino acids,[52] is fully described. A route from a protected L-aspartic acid to 2,3-diamino-4-phenylbutanoic acid via Curtius degradation of (8) involves benzylation of the β-carbanion with benzyl bromide, a process that is said to show higher diastereoselectivity than some analogous processes.[53] Organocuprates react with DL-4-iodo-2-(t-butyloxcarbonylamino)-butanoates to give heterocyclic side-chain analogues, while the corresponding use of chiral imines (9) leads to a satisfactory excess of the L-enantiomer.[54]

The Strecker synthesis, applied to 1-amino-2,2-dialkylcyclopropane-carboxylic acids, depends on the survival of the halogeno-alkyl moiety at the stage of preparation of the α-aminonitrile from the aldehyde $ClCH_2$.CR'R².CHO.[55,56] An analogous route involves cyclopropane ring-closure of an α-chloro-imine ClCR'R².C(=NR)R³.[57] A one-carbon homologation of aldehydes using (phenylthio)nitromethane is analogous to the Strecker synthesis but is claimed to be superior, especially for sensitive multifunctional synthesis targets such as the glycosylamino acid, polyoxin C (Scheme 3).[58] A quite different route to this compound uses the "penaldic acid equivalent", viz. 5-formyl N-butoxycarbonyl 2,2-dimethyl oxazolidinone (from L-serine) as protected amino acid moiety on which the glycoside moiety is constructed.[59]

Bucherer-Bergs synthesis of 1-aminocyclohex-2-ene-1,3-dicarboxylic acid from the corresponding cyclohexenone has been reported,[60] and this hydantoin alkylation route has also been used in a large-scale synthesis of phenylalanine (hydantoin is condensed with PhCHO).[61] No "General Methods" section on amino acids would be complete without mention of the azlactone synthesis, in which alkylation of 2-phenyloxazolin-5(4H)-one, generated in situ from hippuric acid, has led to "the 1- and 2-naphthol analogues of tyrosine", i.e. β-(4- and 6-hydroxy-1-naphthyl)alanines.[62]



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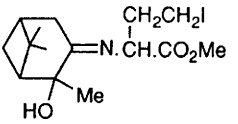

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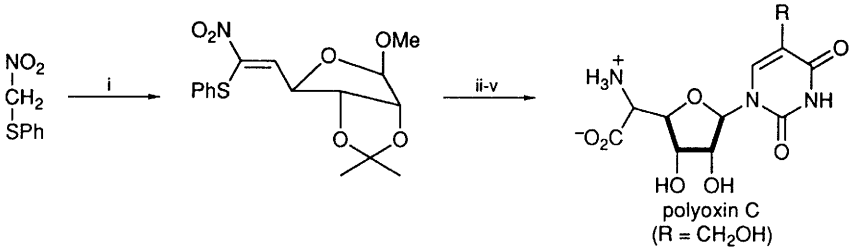
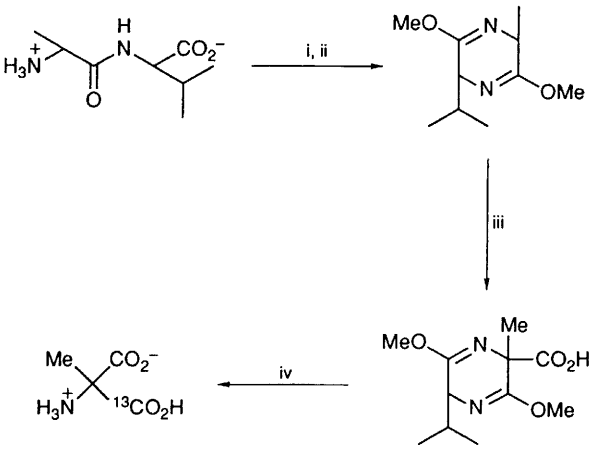


Reagents: i, Corresponding ribose-aldehyde; ii, KOTMS then MeOH [$NO_2C(SPh)=CH- \rightarrow HO\ (COSPh)CH-$]; iii, Tf_2O; $COSPh \rightarrow CO_2Me$; iv, NaN_3; v, $-OMe \rightarrow$ uracil

Scheme 3



Reagents: All standard (see Vol. 22, p. 7) *e.g.*, i, ii, cyclization, $Me_3O^+BF_4^-$; iii, alkylation; iv, hydrolysis

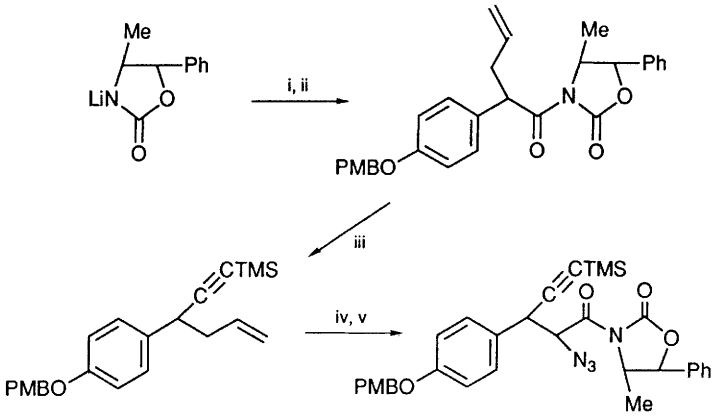
Scheme 4

4.2 Asymmetric Synthesis of α-Amino Acids.- Following on the 'General Methods' approach of the preceding Section, there are many well-developed general asymmetric synthesis routes to α-amino acids. These include direct extensions of some of those methods mentioned in the preceding Section - e.g. the Strecker synthesis of cyanohydrins catalyzed by the dioxopiperazine derived from L-phenylalanyl-L-histidine[63] - while other methods are more distantly related. Some of these have become fully explored, as seems to be the case with the Schöllkopf bis-lactim ether approach (exemplified in Scheme 4 for a synthesis, from the bis-lactim ether derived from L-alanyl-L-valine, of (2R)- and (2S)-[1-^{13}C]-2-amino-2-methylmalonic acid)[64] and they require less space this year since they have been illustrated often in this Section in preceding Volumes.

Good yields of homochiral α-amino acid esters are routinely formed by photolysis of chiral chromium aminocarbene complexes (formed from a tertiary amide and $Na_2Cr(CO)_5$ with TMSCl) in solution in an appropriate alkanol.[65] Homochiral β-lactams are formed similarly through reaction of these complexes with imines.[66] The topic continues to be well-reviewed[67,68] (see also Vol.22, p.8).

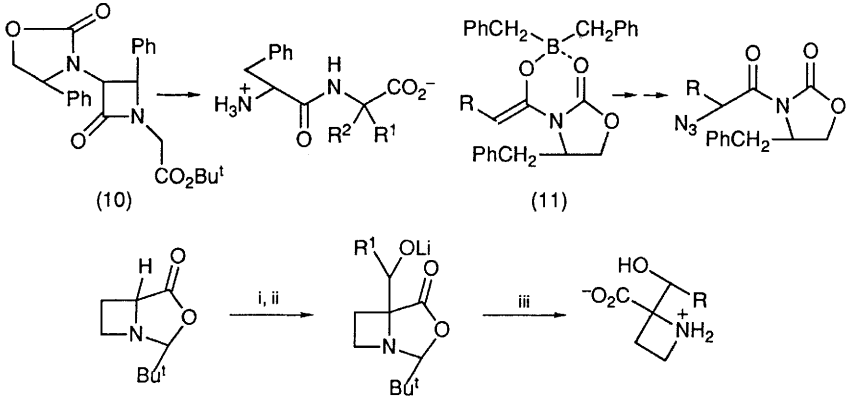
Chiral saturated heterocycles have occupied a firm niche in this Section, as vehicles for asymmetric synthesis of α-amino acids. Evans' methodology based on lithiated (4R,5S)-4-methyl-5-phenyloxazolidinone has been used for a synthesis of (+)-(2S,3S)-ethynyltyrosine (Scheme 5)[69] and an analogous oxazolidinone underpins the asymmetric double alkylation of the glycine derivative (10) en route to homochiral N-(L-phenylalanyl)amino acids.[70] L-Serine gives the same chiral heterocyclic system carrying a 4-methoxycarbonyl grouping, christened a nucleophilic L-alaninol synthon since conversion into the Wittig reagent and condensation with aldehydes [$CO_2Me \rightarrow -CH_2P^+Ph_3\ I^- \rightarrow HOCH_2CH(NHBoc)CH{=}CHR$ as a result of ring-opening] occurs readily and with high stereoselectivity.[71] Bromination (N-bromosuccinimide) of dibenzylboron enolates (11) derived from N-alkanoyl 4-benzyloxazolidin-2-ones, followed by electrophilic azidation (tetramethylguanidinium azide) gives (R)- or (S)-α-azidoalkanoic acids.[72] The more convenient potassium enolate reacting with 2,4,6-tri-isopropylphenylsulphonyl azide is better than 90% diastereoselective (but dependent on the nature of the acylating grouping).

The alternative chiral oxazolidinone (a cyclic acetal) continues to be studied (cf. Vol.22, p. 12),[73] this year in a bicyclic form (Scheme 6) in which the focus of interest is the racemization that accompanies alkylation of the exocyclic enolate by electrophiles. A methylene derivative (12) of Seebach's choice of oxazolidinone is susceptible to diastereoselective free radical addition leading to β-extended alanines.[74] A route from L-cysteine to the (2R)-thiazoline (13; R =



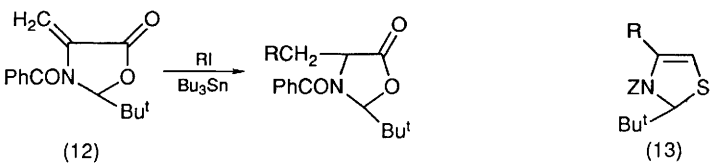
Reagents: i, 4-(4′-$MeOC_6H_4$–)$OC_6H_4CH_2CO_2H$, 0°C; ii, NaHDMS, then allyl bromide;
iii, $LiAlH_4$, then successively $COCl_2$/DMSO, Et_3N, CBr_4/PPh_3, Bu^tLi, TMSCl;
iv, O_3, then $NaClO_2$;
v, Bu^tOCOCl, Li salt of (4*S*, 5*R*)-4-methyl-5-phenyloxazolidinone, $ArSO_2N_3$

Scheme 5



Reagents: i, $LiNR_2$; ii, R^1CHO; iii, H_2O, H_3O^+

Scheme 6



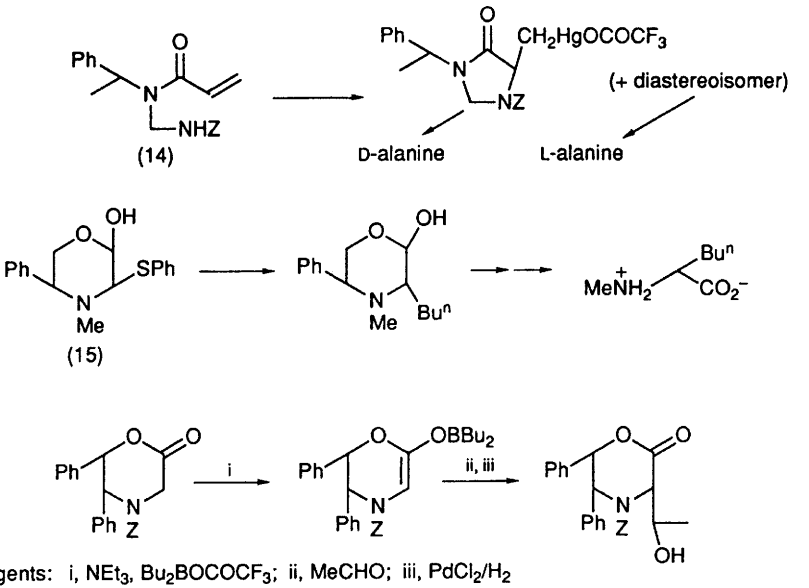
CO_2R), useful in this context (see Vol. 22, p.10 for uses of the equivalent oxazoline) has been described.[75]

The analogous imidazolinones have also been used in asymmetric synthesis of amino acids, illustrated further for $Hg(OCOCF_3)_2$ cyclization of the chiral amidal (14) formed from 1,3,5-tri-(S)-phenylethylhexahydrotriazine and acryloyl chloride.[76]

Six-membered chiral glycine-cation equivalents have been supplemented usefully[77,cf.,78] by a phenylthio-substituted oxazine (15) that shows propensity towards substitution either with inversion (by Bu^nCu) or with retention (Bu^nZnI). This behaviour has been seen in several similar cases before, and continues to defy rationalization. Williams' oxazinone (Scheme 7), converted into the boron enolate and alkylated with acetaldehyde, yields L-allothreonine on work-up,[79] thus showing the opposite stereoselectivity from that of the corresponding reaction undergone by Seebach's imidazolinones. Enantiomeric excesses between 82 and 94% are reported for C-arylglycines prepared by either Friedel-Crafts or cuprate couplings with the bromo-oxazinone (16).[80]

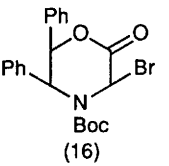
A new chiral imine approach uses the hydrazone (17 in Scheme 8); and 100% diastereoselectivity is claimed for a representative L-alanine synthesis employing it.[81]

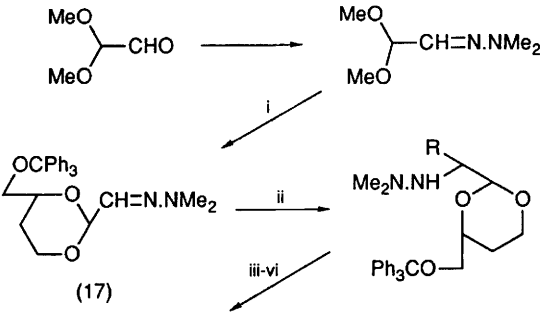
Small-ring chiral synthons complete this crop of related routes. Ammonolysis of chiral oxiranes (resulting from Sharpless oxidation of crotyl and allyl alcohols) gives L- and D-allothreonines and (S)- and (R)-isoserines, respectively,[82] and a similar methodology is involved in the synthesis of (2S,3S)- and (2R,3R)-3-hydroxyleucine (Scheme 9).[83] Turning things on their heads, an aziridine-2-carboxylate prepared from D-threonine serves as starting material for alkylation by an N-alkylindole (catalyzed by BF_3–OEt_2) to give (αR,βR)-1,β-dimethyltryptophan (Scheme 10).[84] The same approach using the C2-symmetric diethyl N-toluene-p-sulphonyl aziridine-2,3-dicarboxylates prepared from (+)- and (-)-tartaric acids yields products formally derived from the β-cation of L- and D-aspartic acid respectively, through nucleophilic ring-opening (with $LiCuMe_2$ to give homochiral β-methyl aspartates, for example).[85] Natural (2S,3R)-tartaric acid serves as starting material in a straightforward synthesis of (2S,3R)-N-Boc-3-benzyloxyaspartate.[86] Other 'carbohydrate-based' asymmetric syntheses are more interesting; N-Boc D-glucosamine through successive $NaBH_4$ reduction and $NaIO_4$ oxidation gives L-serinal (in its stable polymeric form in aqueous solution) from which D-dehydroglutamic acid was prepared through aldehyde processing (-CHO → -CH=CHCOR).[87] Di-acetoneglucose yields (2S,3R,4R)-3,4-dihydroxyproline (and the route can be adapted to give the corresponding pipecolic acid) through azidolysis of the protected methyl acetal, to give (18), and hydrogenation of the separate α- and β-anomers.[88] A similar exploitation



Reagents: i, NEt_3, $Bu_2BOCOCF_3$; ii, MeCHO; iii, $PdCl_2/H_2$

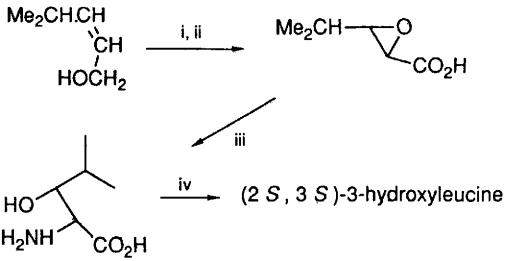
Scheme 7





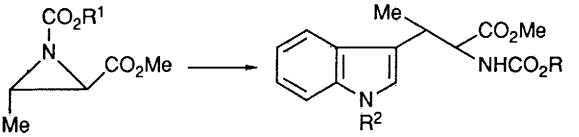
Reagents: i, (*S*)-HO.CH(CH_2OH)CH_2OH, Ph_3CCl; ii, RLi; iii, H_2/Ni; iv, phthaloylation; v, HOCl; vi, NH_2NH_2

Scheme 8

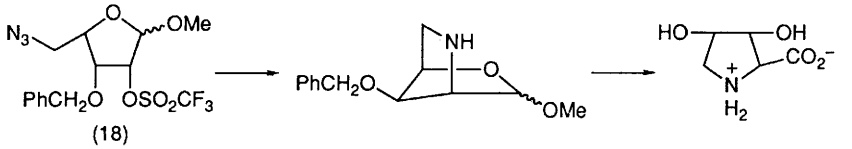


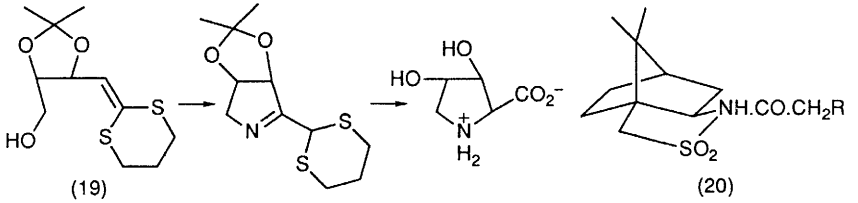
Reagents: i, Sharpless oxidation; ii, RuO_4; iii, $PhCH_2NH_2$; iv, H_2/Ni

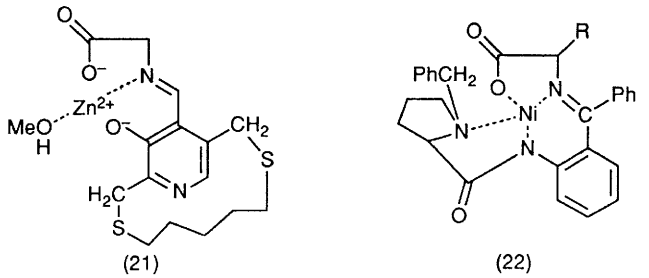
Scheme 9



Scheme 10





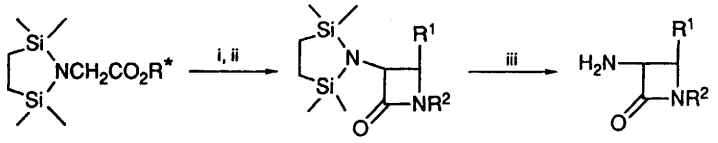


of azide chemistry, leading to (2S,3S,4R)-3,4-dihydroxyproline, introduces a very promising use for chiral ketene dithioacetals (19; cf. Scheme 1).[89] In the latter example, the routine conversion OH → N_3 is followed by intramolecular cycloaddition.

Asymmetric alkylation of a glycine derivative, implicit in some of the preceding examples, continues to offer an attractive route to homochiral α-amino acids. A striking example leading to α-amino-β-lactams (Scheme 11) that has a famous ancestor in a total synthesis of penicillins and cephalosporins, is also a hidden illustration of a chiral synthesis of β-amino acids.[90] In this case, a chiral ester moiety R* induces the enantioselectivity, and needs to be chosen through trial and error so as to give maximum enantiomeric excess in any particular case.

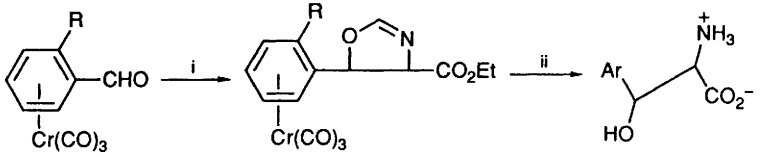
Oppolzer's bornanesultamylglycine (20; cf. Vol.22, pp.12,13) has found a new compatible reagent, 1-chloro-1-nitrosocyclohexane, to carry out amination of its enolate, so as to offer N-hydroxyamino acids as well as its original purpose, asymmetric synthesis of the amino acids themselves, obtained through Zn^{2+}/aqueous acid reduction of the hydroxylamines.[91] Chirality in the Schiff base moiety of glycine imines is a more favoured, and probably a better, choice within this approach. Examples range from the simplest glycine Schiff bases (see previous Volumes of this Report) to conformationally rigid (and therefore more complex) cases. The latter category is illustrated by the chiral pyridoxal-like pyridinophane Zn complex (21), used for aldol condensations leading to α-amino-β-hydroxy acids giving 27-77% enantiomeric excess[92] and benzylation giving substituted phenylalanines.[93] A similar application for Ni^{2+} complexes of Schiff bases (22) formed between glycine (22; R = H) or alanine (22, R = Me) and (S)-N-(N-benzylprolyl)aminobenzophenone has been developed over several years, this year for o-, m- and p-fluorophenylalanines and their α-methyl analogues[94] and for allo-isomers of β-substituted (S)-2-aminobutanoic acids.[95] Nucleophilic substitution of bromoglycine complexes (22; R = Br) by diethyl malonate or n-C_4H_9ZnCl gives L-aspartic acid (80% e.e.) and L-norleucine (68% e.e.) respectively.[96] Chiral arylaldehyde-$Cr(CO)_3$ complexes add to the glycine equivalent, ethyl isocyanoacetate,[97] accomplishing an asymmetric aldol reaction (Scheme 12) that is effectively the same route as that leading to α,γ-diamino-β-hydroxycarboxylic acids using (S)-dibenzylaminoalkanals with ethyl isocyanoacetate,[98] via the same oxazoline intermediate.

A near relative of glycine alkylation, providing a new principle in enantioselective amino acid synthesis, is based on nucleophilic ring-opening of 1-nitrocyclopropanecarboxylic acid salts. With an L-amino acid methyl ester the route gives 4-(α-methoxycarbonylalkylamino)-2-nitroalkanoic acids,[99] which on reduction with Zn/AcOH in the presence



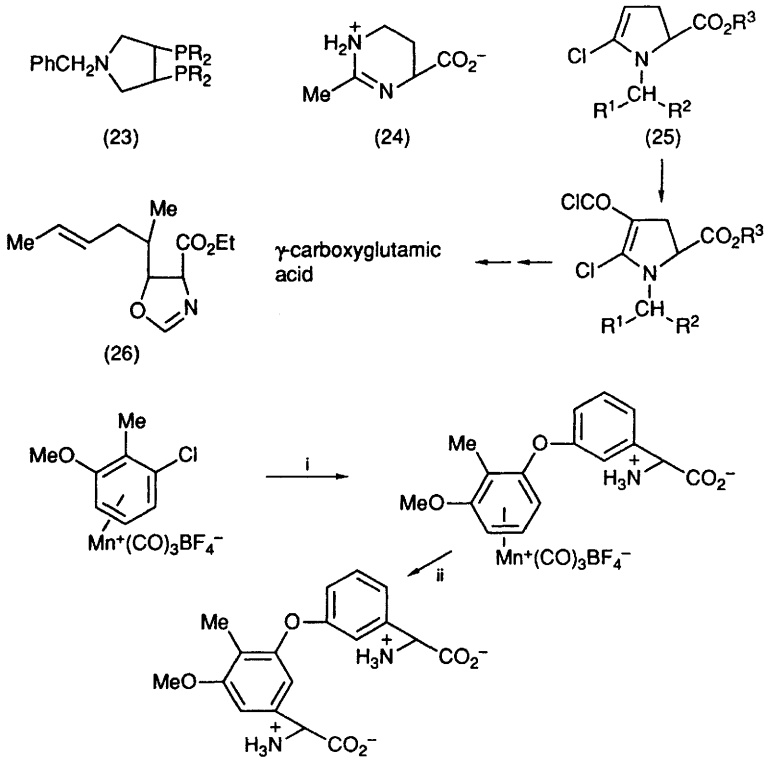
Reagents: i, LDA; ii, $R^1CH{=}NR^2$; iii, H_2O

Scheme 11



Reagents: i, $CN.CH_2.CO_2Et$; ii, ArCHO

Scheme 12



Reagents: i, *N*-Boc-(*S*)-(3-hydroxyphenyl)glycine; ii, Schöllkopf alkylation (*cf.* Scheme 4)

Scheme 13

of acetic anhydride gives the corresponding L-amino acid in moderate enantiomeric excess.

"Asymmetric hydrogenation" of α-acylamido-cinnamic acids using rhodium - chiral phosphine catalysts, is a long-ongoing interest of several research groups. This can be very effective in terms of high enantioselectivity, with up to 87% enantiomeric excess being achieved with mineral-supported catalysts,[100] and better in other cases[101] (e.g. 95% e.e. in synthesis of dihydroxyphenylalanines).[102] The role of the approach pathway of hydrogen is important in determining stereoselectivity, and relatively rigid chiral phosphines, e.g. (23), seem to have a particularly effective role. The contribution of molecular graphics in determining structural features in the catalyst, that allow only that pathway that must lead to the desired enantiomer, has been reviewed.[103] This essentially expands the report by the same author cited last year (Vol.22, p.10).

4.3 Synthesis of Protein Amino Acids and Other Naturally Occurring α-Amino Acids.- Examples of amino acids synthesis under this heading can also be found elsewhere in this Chapter, particularly in the preceding two sections. However, enzymatic methods and laboratory synthesis of the more unusual natural amino acids are reserved for this Section.

Reviews have appeared of microbial and enzymatic production of L-tryptophan,[104] of L-lysine and L-glutamic acid (use of L-lysine oxidase from Trichoderma viride, and L-glutamic acid oxidase from Streptomyces sp., respectively),[105] and of D-amino acids.[106] Representative papers cover bioreactors with NH_3 or urea as nitrogen source, for the production of branched side-chain L-amino acids,[107] production of L-lysine with methionine and threonine double auxotrophic mutants from Bacillus megaterium,[108] and use of the same means for L-alanine and L-valine production.[109]

A Volume entitled "Biochemical Engineering 6" includes several papers dealing with fermentative production of amino acids, covering L-aspartic acid,[110] L-phenylalanine (use of Escherichia coli),[111] and fermentative production of D-amino acids from DL-hydantoins.[112] The use of hydantoins in this context for the synthesis of L-amino acids continues to develop, with Arthrobacter showing the appetite for the task of L-tryptophan production.[113,114] Enzymatic conversion of DL-hydantoins into L-amino acids has been reviewed,[115] and a thoughtful exposition on the production of either D- or L-amino acids from hydantoins in this way concentrates on the three enzymes involved.[116] D-Glyceric acid provides a substrate for the synthesis of L-serine by successive operation of glyoxylate reductase and alanine dehydrogenase.[117] Reductive amination of phenylpyruvic acid by

phenylalanine dehydrogenase from Bacillus sphaericus, and the more general involvement of this system in L-amino acids synthesis has been explored.[118]

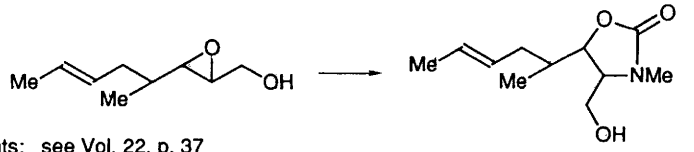
Non-protein amino acids D-p-hydroxyphenylglycine (from the action of Agribacterium sp. on the appropriate DL-hydantoin),[119] L-DOPA (tyrosinase from A. terreus 104),[120] S-adenosyl-DL-homocysteine, (Saccharomyces cerevisiae cells transformed with a plasmid containing an ethionine resistance gene),[121] and S-adenosyl-L-methionine[122] are also accessible by enzymatic methods. A Tolypocladium inflatum mutant has been reported to accumulate MeBmT, i.e. (4R)-4-((E)-2-butenyl)-4,N-dimethyl-L-threonine.[123]

No attempt is made in this Chapter to cover the literature of the more academic aspects of the biosynthesis of amino acids, though a note on the origins of ectoine (24) from phosphorylated L-aspartic acid in Ectothiorhodospira halochloris and Halomonas elongata catches one's interest.[124]

An improved β-carboxyaspartic acid synthesis based on alkylation by sodium dibenzyl malonate,[125] and another efficient γ-carboxylation of protected (S)-pyroglutamate via the γ-enolate have been reported.[126] N-Benzhydryl-L-pyroglutamic esters give α-chloro-enamines (25) with phosgene, from which γ-carboxyglutamic acid can be obtained.[127] A concise route to L-phenylalanine from (R)-epichlorhydrin is available.[128] Laboratory synthesis of thyroxine and tri-iodothyronine has been reviewed.[129] More sophistication is needed, based on organomanganese chemistry, for the synthesis of a deoxy-ristomycinic acid derivative (Scheme 13),[130] in which the α-amino acid formation step uses the standard Schöllkopf asymmetric synthesis methodology (cf. Scheme 4).

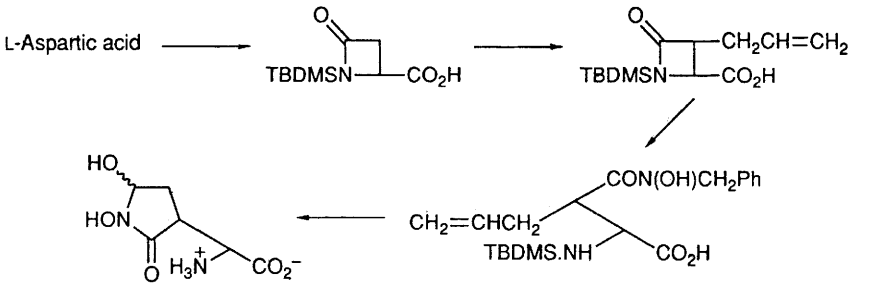
The remaining examples in this section will be recognized by faithful readers as even more challenging synthetic targets that have already featured in these reviews. Other similarly-daunting natural amino acids that will come into readers' minds will be found in a later section where they fall within the β-amino-and-higher acid category.

Among α-amino acids, syntheses of the cyclosporin component MeBmT [(4R)-4-((E)-2-butenyl)-4,N-dimethyl-L-threonine] are being achieved in fewer steps than the marathon accomplishments of previous years (see Vol.22, p.37). Gold(I)-chiral ferrocenylphosphine catalysis of the aldol reaction between (2R,4E)-MeCH=CHCH$_2$CH(Me)CHO and ethyl isocyanoacetate gives the oxazoline (26) carrying the appropriate stereochemistry. Two further steps to reach the target, constitutes the shortest synthesis (so far) of this α-amino acid.[131] A "chiral epoxide" methodology (Scheme 14) involving base-catalyzed rearrangement of β-hydroxyurethanes, has been used to synthesise a 3-hydroxy-MeBmT



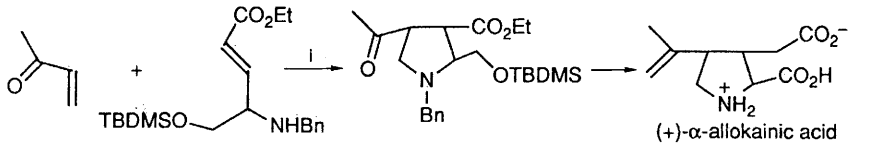
Reagents: see Vol. 22, p. 37

Scheme 14



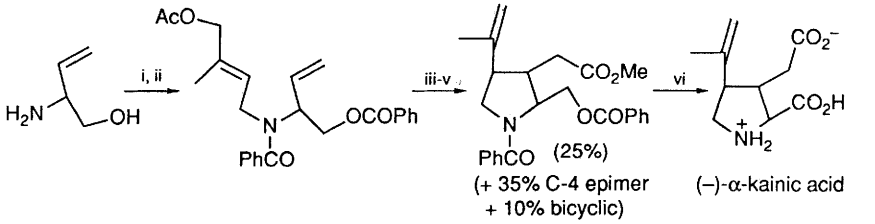
Reagents: as noted in text

Scheme 15



Reagents: i, reactants in EtOH, 15 days; or, with catalytic tetramethylguanidine or, in two separate stages (a) $FeCl_3$, (b) tetramethylguanidine (90% yield)

Scheme 16



Reagents: i, $AcO.CH_2.CMe{=}CH.CH_2Cl$; ii, PhCOCl; iii, $Pd(DBA)_2$, PPh_3, CO, AcOH, 80°C;
iv, hydrolysis, then CH_2N_2;
v, repeat of conditions (iii), but with higher pressure (3 atm.) of CO;
vi, routine elaboration

Scheme 17

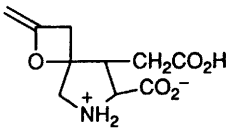
stereoisomer,[132] and analogously for the synthesis of the δ-oxygenated analogue from (4S,2Z)-$PhCH_2OCH_2$.CHMe.CH=CH.CO_2Me.[133]

Several examples of the use of protein amino acids with side-chain functional groups, for the synthesis of more complex non-protein amino acids, emphasise the growing importance of this approach. Indeed, some examples have been included in a preceding section (4.1 General Methods of Synthesis of Amino Acids) since they could be judged to have entered into this category. A synthesis, from L-aspartic acid, of de-alanylalahopcin, the non-protein moiety of the dipeptide, has been described (Scheme 15).[134] Notable features are the diastereoselective alkylation of the azetidinone and thiolate-catalyzed ring opening with benzylhydroxylamine via the readily aminolyzed thiolester.

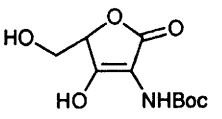
Protected L-pyroglutamic acid is efficiently alkylated in terms both of yield and trans-stereoselectivity, after Y-anion formation using $LiNPr^i_2$ or $LiNBu^t_2$ in THF.[135] A not-too-distant relative is Bulgecin C, for which the first total synthesis is reported, starting from (2S,4R)-hydroxyproline.[136] This synthesis exploits the electrochemical methoxylation of the protected hydroxyproline acetate and ensuing routine elaboration. Syntheses of kainic acids start from a protected serine, employing Co(II)-mediated radical cyclization of a derived halide already well-established by Baldwin's group, in a route leading to (-)-α-kainic acid,[137] and tandem Michael reaction methodology [leading to (+)-α-allokainic acid; Scheme 16] in another study.[138] Pd(0)-mediated alkene-insertion and carbonylation (Scheme 17)[139] has been described. The Michael route is notable in being a one-pot process that generates three chiral centres in one stage.

Members of the kainoid family (kainic acid, acromelic acids, domoic acid) share the ability to inflict marked depolarization effects on L-glutamate receptors, and syntheses of analogues are very much of interest in exploring structure-activity relationships. Synthesis of analogues has been reported,[140] simple analogues of acromelic acid (o-hydroxyphenyl in place of 3-pyridonyl; see Vol.22, p.15) being more highly active agonists of kainic acids than any natural kainoid. Scheme 18 outlines this synthesis, starting from L-vinylglycinol. In another account, synthesis of a novel oxetane-containing analogue of kainic acid (27) is described, synthesized starting from kainic acid, with allylic hydroxylation proceeding with complete retention at C-4.[141] Loss of affinity for glutamate receptors accompanies this structural change.

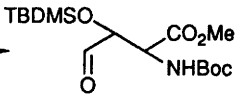
A synthesis of the Y-azetidinyl-β-hydroxy-α-amino acid moiety of mugineic acid starts with (R)-glyceric acid,[142] converted into (28) through an earlier-established sequence (Vol.21, p.20) and then elaborated stereospecifically into the serinal derivative (29). The extraordinary antibiotic duocarmycin A (30; from Streptomyces sp.) and related pyrindamycins A and B have been synthesized through



(27)



(28)



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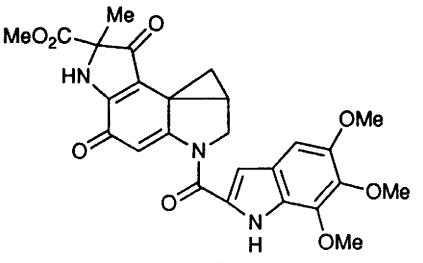

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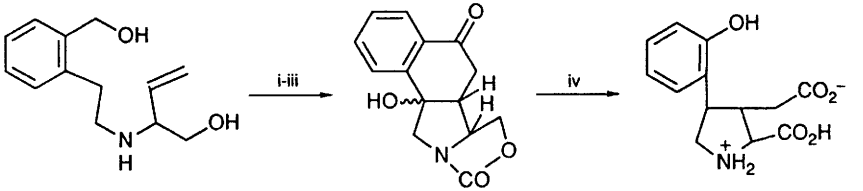
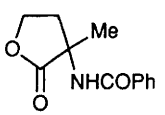


Reagents: i, Z.Cl, K_2CO_3, then KOH, MeOH; ii, MnO_2 (-CH_2OH→ -CHO); iii, *h* ν, then MnO_2; iv, routine elaboration

Scheme 18



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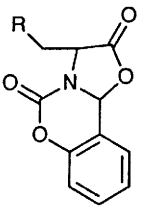


(32)

Reagents: i, α-glucosyl bromide + $ZnBr_2$; ii, H_2O

Scheme 19

constructing the amino acid moiety on to an NH_2 group carrying the rest of the structure using $MeCHBr.CO_2Me$.[143]

4.4 α-Alkyl and Aryl Analogues of Protein Amino Acids.- These are important as potential disruptors of metabolic processes, whether in their own right or as components of peptides. They can be prepared through certain standard routes - e.g. by Strecker synthesis from ketones - or through α-alkylation of a protein amino acid derivative. Recent examples in the latter category are the conversion of N-benzoyl-DL-alanine methyl ester into α-methyl homoserine lactone (31) through di-anion formation and reaction with ethylene oxide,[144] (nucleophilic ring-opening by $PhSe^-$ is followed by β-elimination to give the α-vinyl alanine derivative), and catalytic phase transfer alkylation of an alanine Schiff base ester by an imidazolylmethyl acetate (or the alternative methylation of the histidine Schiff base) to give α-methylhistidine.[145]

An N-protected tryptophan methyl ester survives the conditions of alkylation if the indole nitrogen atom is also Boc-blocked, demonstrated by α-anion formation from the isocyano analogue, using LDA, and reaction with an alkyl bromide.[146] N^{in}-Alkylation competes with α-alkylation in Michael additions to N-benzylidene tryptophan methyl ester.[147]

Stereoselective double alkylation of some of the chiral synthons covered in the earlier Section, 4.2 "Asymmetric Synthesis", is already well-researched, but a new example is an interesting resurrection of the chiral oxazolidinone (32) formed between an amino acid, salicyaldehyde and phosgene emphasises the fact that these saturated heterocycles are not newly-discovered synthons.[148] Stereoselective alkylation after anion formation [lithium bis(trimethylsilylamide] is efficient in the modest number of cases tried.

Further work has been reported from Burger's group, extending their methodology for preparation of trifluoromethyl amino acids (see Vol.22, p.25). α-Alkynyl "trifluoro-alanines" are available through Grignard alkynylation of $CF_3.C(=NZ).CO_2R$ (or corresponding use of $NaC{\equiv}CR$),[149] and ω-carboxyalkyl analogues have been prepared through routine elaboration of corresponding ω-alkenyl analogues.[150] New 2-phenyl-4-(α-arylalkyl)-4-trifluoromethyl oxazolones,[151] and the 4-methyl-4-(α-hydroxybenzyl) analogue (threo:erythro = 3:1)[152] have been described, as have analogous oxazinones[153] from which the respective α-trifluoromethyl or α-methyl α-amino acids could be secured through aqueous acid hydrolysis.

An interesting series of α-substituted α-amino acids becomes available through the establishment of an α-C-glucosylation route using ketene acetals and α-glucosyl bromide/$ZnBr_2$,[154] (Scheme 19). There is no reference to asymmetric induction in this study.

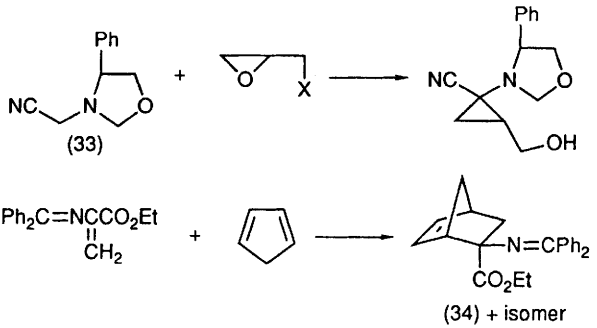
Selective monophenylation of active methylene compounds in the synthesis of α-phenyl-α-amino acids (the work of M.J.O'Donnell's group) has been reviewed.[155] α-Arylamino acids are formed in good yield by treating Schiff bases with (arene)halotricarbonylchromium(II) complexes.[156]

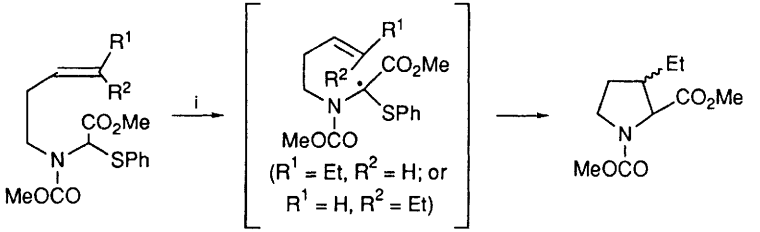
4.5 α-Amino Acids Carrying Alkyl Side-Chains, and Cyclic Analogues.- This section is intended to be the repository for papers covering the synthesis of aliphatic non-protein α-amino acids lacking side-chain functional groups, and has become more and more concerned with alicyclic representatives (amino function outside the ring). Saturated heterocyclic examples including proline analogues (amino function within the ring) are also covered here.

(1R,2S)- and (1S,2R)-1-Amino-2-hydroxymethylcyclopropanecarboxylic acids have been prepared through cycloalkylation of dimethyl malonate with epichlorhydrin (the nucleophile attacks the epoxide in preference to displacing the halogen), followed by a classical Hofmann rearrangement to deliver the α-amino group and separation of diastereoisomers.[157] Epichlorhydrin, or alternatively, glycidyl triflate, has also been used for alkylation of the chiral aminonitrile synthon (33) to give two of the four possible diastereoisomers of 2,3-methanohomoserine, separated by conventional crystallization.[158] 1-Amino-2-oxo-3-oxabicyclo[3.1.0]hexane, the lactonized isomer of the amino acid just mentioned, has been synthesized through cyclization of the carbenoid derived thermolytically from an alkyl allyl diazomalonate $RO_2C,CN_2,CO_2,CH_2,CH{=}CH_2$, followed by selective elaboration of the alkyl ester function (RO_2C- → HO_2C- → H_2N- with diphenylphosphoryl azide).[159] The other approach to preparing conformationally-constrained analogues of protein amino acids is to place the cyclopropyl ring one carbon further away from the "glycine moiety", as in (2R, 3S, 4R)-α-carboxycyclopropylglycine, alias "D-cyclopropylglutamic acid".[160] This synthesis was achieved through dirhodium(II) tetra-acetate catalyzed thermal decomposition of ethyl diazoacetate in the presence of Z-D-vinylglycine methyl ester. A similar approach using photolysis of diazomethane, has been used for the preparation of 2,3-methanoproline.[161]

Geometrical isomers of 4-guanidinocyclohexylglycine (proposed as arginine analogues), have been prepared through standard methods.[162] Pyroglutamic acid processing (→ N-Boc (S)-pyroglutaminol, and alkylation) gives (2S,4S)- and (2S,4R)-$HO_2C.CH(NH_2).CH_2.CH(CO_2H)(CH_2)_nPh$, (n = 1,3,5), as 4-substituted glutamic acid analogues for neuroexcitatory activity studies.[163]

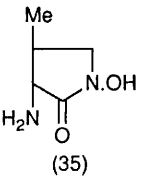
The four stereoisomers of 3-phenyl-1H-aziridinecarboxylic acid are the outcome of a route starting with racemic ethyl (E)-2-





Reagents: i, Bu_3SnH/azo-bis-isobutyronitrile, toluene, 80°C/N_2

Scheme 20



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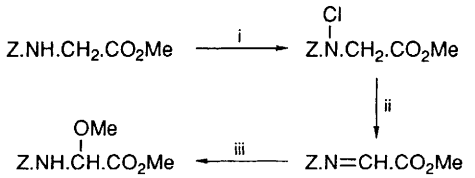
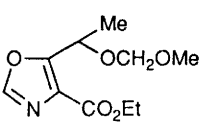


Reagents: i, Bu^tOCl; ii, base; iii, NaOMe, MeOH

Scheme 21



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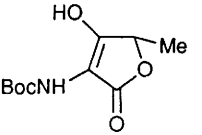


(37)

phenyloxiranecarboxylate, prepared by Darzens reaction between benzaldehyde and ethyl chloroacetate,[164] azidolysis after resolution, and cyclization with Ph_3P.

Like the corresponding cyclopropanes, azetidine-2,4-dicarboxylic acid[165] and its 4-alkyl analogues[166] are of considerable interest as potential agonists of N-methyl-D-aspartate receptors. There are no particular targets justifying the synthesis of "norbornane amino acids" (34) other than a useful extension of Diels-Alder methodology.[167]

Prolines and pipecolic acids have been prepared through cyclization of the C2-radical of 1-methoxycarbonyl-2-aza-5-hexenyl phenyl sulphides (Scheme 20).[168] Photocyclization of αω-di-amino acids giving prolines and pipecolic acids involves what has been called an aqueous semi-conductor suspension (water/TiO_2 or CdS/PtO_2).[169] Less spectacular syntheses of pipecolic acids are based on processing of substituted 2-cyanopyridines formed from pyridines by N-oxidation followed by Me_3SiCN.[170]

A near relative to these classes is the pyrrolidinone (35), a potent glycine and N-methyl-D-aspartic acid receptor antagonist that has been synthesized through a well-planned stereoselective route.[171]

4.6 Prebiotic Synthesis of Amino Acids.- The simple-chemical-mixture/sophisticated-energy-source combination that has been the main feature of this section over the years is repeated in a variety of ways. Sixteen amino acids are present in the sputtered material when graphite is bombarded by high energy molecular beams in which the elements hydrogen, nitrogen and oxygen are represented.[172] A similar experiment involves 3 MeV proton irradiation (van de Graaff generator) of an atmosphere of carbon monoxide and nitrogen over water, which produces various amino acids (and imidazole) during 2 - 5 h.[173] From this result, it is reasoned that cosmic radiation and/or solar flares should be considered to have a place in theories of the origins of life.

Higher up the pathway leading to amino acids - or so the originators of these experiments presumably speculate - are carboxylic acids, which through high-pressure explosive amination using ammonium carbonate or ammonium hydrogen carbonate (no further information in the abstract of this paper) form glycine, phenylalanine and aspartic acid.[174] A similar treatment of ^{14}C-methylamine through catalyzed carboxylation with CO_2 gives glycine, glutamic acid, and β-alanine,[175] the radiolabel allowing conclusions to be drawn to the effect that CO_2 only contributes the carboxyl carbon; and that glycine was the precursor of the other two amino acids. KrF Excimer laser irradiation of ethylamine in aqueous HCl results in stepwise oxidation to give ethanolamine and glycine, through cleavage of water into H and OH radicals.[176] The maximum *yield*

of glycine is poor at 10%, but is of a level that suggests that the results of serendipitous experiments of this type may feature in future production processes that create a cocktail of more or less useful organic chemicals (though probably not for the production of amino acids!).

Erythrose and formamidine, both known to be formed in prebiotic conditions, have been shown to react to give imidazole-4-acetaldehyde.[177] Since HCN and NH_3 (required for Strecker amino acid synthesis) were also abundant at prebiotic times, the formation of histidine in 3.5% yield through presenting these compounds to the erythrose - formamidine reaction mixture is a convincing proposal for the genesis of this amino acid.

4.7 α-Alkoxy α-Amino Acids.- α-Hetero-atom substituted glycine derivatives continue to play a useful role in amino acid synthesis. Examples have been mentioned earlier in this Chapter, and protected α-alkoxy α-amino acids achieved the status of carving out their own Section in this Chapter some years ago as a result of their simple electrochemical synthesis. A new synthesis of α-methoxyglycine from the N-chloro derivative of Z.Gly.OMe has been described (Scheme 21).[178]

4.8 Halogeno-alkyl α-Amino Acids.- All the examples in this Section this year concern fluorine-substituted protein amino acids - which is not to be interpreted as saying that no other halogeno-alkyl amino acids have been prepared in ways that are chemically-interesting, but that (unlike the fluorinated compounds) these others are intermediates en route to amino acids that are mentioned elsewhere in this Chapter.

Syntheses of fluorinated amino acids[179] and more specifically, α-(β-fluoroalkyl) α-amino acids[180] have been reviewed. (-)-D-erythro- and (+)-L-threo-4-fluoroglutamic acids have been prepared from trans- and cis-4-hydroxyprolines, respectively, through substitution of OH by F after N-acetylation and esterification, followed by RuO_4 oxidation to the pyroglutamate.[181] 4,4-Difluoroglutamic acid has been prepared through Michael addition of a 2,2-difluoroketene silyl acetal [$F_2C{=}C(OMe)OSiR_3$ from $F_2CI.CO_2Me$] to a homochiral N-propenoyl 5-benzyloxazolidin-2-one (cf. Scheme 5),[182] and a simpler version of the same methodology was used to prepare Ph.CONH.CH_2.CF_2.CH_2.CHO for use in a Strecker synthesis of 5,5-difluoro-lysine. 5-Fluoro-L-lysine is accessible from L-homoserine and ethyl bromofluoroacetate through a Horner-Emmons reaction.[183]

More direct fluorination approaches in which fierce reagents are presented to protected amino acids usually cause multiple and untargeted substitution, as with the reaction of XeF_2 with N-trifluoroacetyl S-benzyl cysteine.[184] Monofluorination of the benzyl

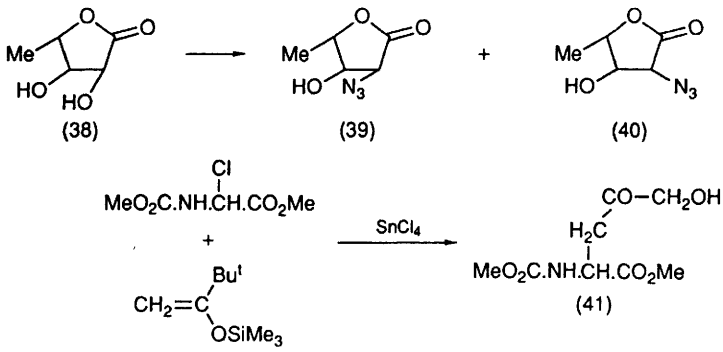
methylene group, and substitution of the benzylthio-group, accompany the formation of a useful protected β-fluorocysteine, which over 24 h spontaneously eliminates HF to give the mixed Z/E-dehydrocysteine derivative.

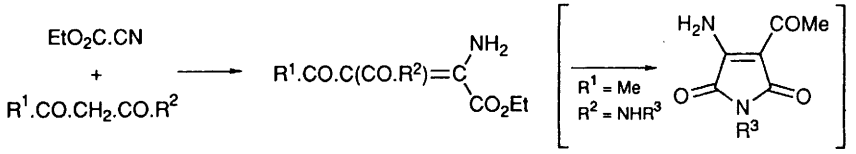
4.9 α-(ω-Hydroxyalkyl) α-Amino Acids.- There are many examples of syntheses of α-(β-hydroxyalkyl) α-amino acids, not least because there are several important natural compounds of this family (and for this reason, this year's crop of examples will be found in other sections of this Chapter). An interesting use of enzymes is seen in preliminary results for the synthesis of these compounds through the aldol reaction of an aldehyde with glycine catalyzed by aldolase enzymes extracted rabbit liver and corn seedlings.[166] The alternative stereoselective synthesis methodology for this process is represented in the Zn(II) or Cu(II)-catalyzed aldolization of a homochiral glycine imine [derived from (1R)-3-hydroxymethylbornan-2-one for this study], reaction with benzaldehyde giving β-phenylserine diastereoisomers.

A preparation of the oxazoline (36) from ethyl isocyanoacetate and (S)-$MeOCH_2$.OCHMe.CO_2Me and its use as a chiral β-hydroxy-α-amino acid synthon has been described.[166] For example, reaction with diphenyl phosphorazidate and NaH and routine steps, leads to lactone (37) that yields a mixture of γ-hydroxynorvaline diastereoisomers on hydrogenation. A more stereoselective route to the same target employs (38), derived from D-ribolactone, as starting material,[167] proceeding through azides (39) and (40). More routine methods underlie the syntheses of γ-hydroxyvalines (modified Erlenmeyer synthesis) and δ-hydroxyleucine and δ-hydroxyisoleucine (Michael addition).[168]

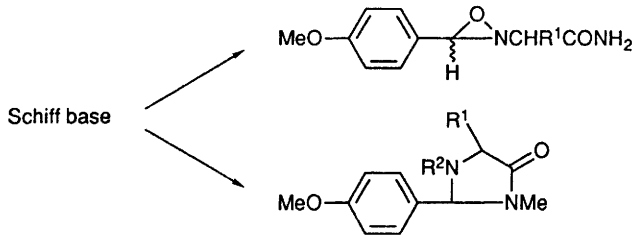
Lewis acid-catalyzed coupling of N-methoxycarbonyl chloroglycine methyl ester with a silyl enol ether has been used for the synthesis of the antitubercular/antifungal 5-hydroxy-4-oxo-norvaline (41).[169]

4.10 α-Amino Acids Carrying Unsaturated Side Chains.- 2-Aminoalken-2-oic acids ("αβ-dehydro-amino acids") call for simple methods in the simplest cases [condensation of secondary amines + pyruvate esters catalyzed by $AsCl_3$;[130] dehydration with di-isopropylcarbodi-imide/Cu(I)Cl of β-hydroxy-N-diphenylmethyleneamino acids;[131] and condensation of EtO_2CCN with a 1,3-dicarbonyl compound (Scheme 22)[132]] but more sophisticated procedures are needed for polyfunctional examples. 4-Bromo-N-tosylindoles add to N-protected α-amino acrylates under $PdCl_2$ catalysis to give dehydro-tryptophans, though a side-reaction due to benzo-substitution is troublesome.[133] A similar approach gives β-vinyl and β-aryl dehydro-amino acids, using vinyl and aryl triflates as reagents and methyl α-acetamidoacrylate as substrate under Pd(II) catalysis.[134]

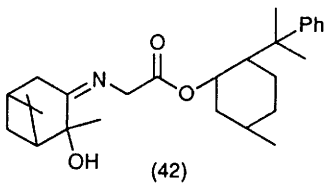


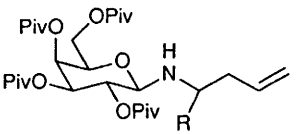


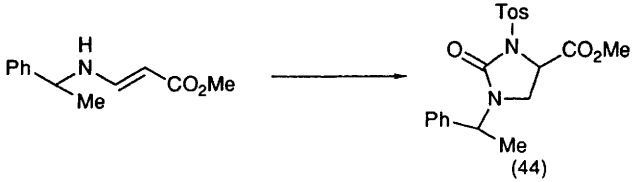
Scheme 22



Scheme 23







An interesting observation, that oxidative dehydrogenation of α-aminoacidato complexes of cobalt(II), viz. $[Co(en)_2(aa)]^{2+}$, is brought about by $SOCl_2$, could be exploitable for a practical synthesis of αβ-dehydroamino acids through rearrangement of the resulting imines $HN{=}CRCO_2H$.[195]

An effective synthesis of N-benzyloxycarbonyl L-vinylglycine methyl ester from the L-methionine derivative is based on facile elimination following conversion to the sulphoxide.[196] Other 3,4-dehydroamino acids synthesized recently include (Z)-3,4-dehydronorvaline and the (E,Z)-3,4-dehydro-ornithine and 2,5-di-aminopimelic acid analogues, through addition of Grignard reagents to diethyl acetyliminomalonate.[197]

γδ-Unsaturated amino acids have been prepared through the substitution of a protected α-chloro- or α-methoxyglycine with an allylsilane.[198]

The first synthesis of an ethynylglycine derivative has been achieved through substitution of N-acetyl α-chloroglycine diphenylmethyl ester and $Me_3SiC{\equiv}CSnBu_3$ followed by deblocking.[199]

4.11 Aromatic and Heteroaromatic α-Amino Acids.- As is the case for other nearby sections in this Chapter, covering particular side-chains, relevant information will also be found in the later section 'Specific Reactions', where reactions at the side-chain of one of the well-known aromatic or heteroaromatic amino acids are described that can produce a new addition to the same family. General methods for amino acid synthesis have also been applied, for example to the preparation of α-amino phenylacetonitrile $H_2N.CHPh.CN$, from which phenylglycinamide may be prepared through HCl - mercaptoethanol treatment in THF.[200]

Carbalkoxyalkylation - replacement of the OH group of a hydroxyphenylalanine - has been demonstrated through reaction of a protected tyrosine triflate with an acrylate ester catalyzed with $(Ph_3P)_2PdCl_2$, followed by hydrogenation.[201]

A 1975 preparation of L-homohistidine has been improved through the use of formamidine acetate and NH_3 in the final imidazole-forming stage.[202] The next higher homologue, but with the imidazole moiety linked through nitrogen, i.e. δ-(1-imidazolyl)norvaline, has been synthesized as an arginine analogue.[203] A photo-activatable heteroaromatic amino acid analogue, 2'-diazo-histidine, has been synthesized as its N^{α}-Boc methyl ester using routine imidazole chemistry through the 2'-amino histidine.[204]

4.12 N-Substituted α-Amino Acids.- This section serves here for unusually-modified amino or imino groups; protection or transient modification as part of a reaction pathway is either covered in the

later Section 'General Reactions' or excluded from the Chapter if its details are routine.

N^{α}-Hydroxy-L-amino acid amides are conveniently prepared from oxaziridines produced by oxidation of the Schiff base (Scheme 23), or (as second best) from N^{α}-oxidation of the imidazolidinone formed from the Schiff base.[205]

An alternative synthesis of N^{α}-benzyloxycarbonyl-N^{δ}-hydroxy-L-ornithine methyl ester has been announced, in which the N^{δ}-acetyl derivative is reacted with benzoyl peroxide.[206]

4.13 α-Amino Acids Containing Sulphur, Selenium, or Tellurium.- There is one citation for each element, as it happens, for this year's review. 2'-Arylthio-L-histidines have been prepared[207] for use in peptide synthesis. A routine selenomethionine synthesis uses MeSeH and an α-protected-amino Y-butyrolactone or α-protected-amino methyl cyclopropanecarboxylate,[208] while telluromethionine is available in the same way (α-amino-Y-butyrolactone + LiTeMe).[209]

4.14 Phosphorus-Containing α-Amino Acids.- As is the tradition of this Chapter, α-amino acids in which the carboxy group is replaced by a phosphorus oxy-acid group, are not covered (nor are amino-sulphonic, amino-boronic etc, acids). Where a phosphorus side-chain function is involved, as in the obviously-important competitive glutamate antagonist (at the N-methyl-D-aspartic acid complex), (R)-4-oxo-5-phosphono-norvaline,[210] there is every reason to put such information side-by-side with that on other amino-carboxylic acids. The synthesis of this compound from D-aspartic acid in six relatively straightforward steps, via the ketone $RCH_2.CO.CH_2.PO_3H_2$, is described in this paper.

The racemic homologue, E-2-amino-5-phosphonopenten-3-oic acid, E-$HO_2C.CH(NH_2).CH{=}CH.CH_2.PO_3H_2$, has been synthesized from the unsaturated β-acetoxynorvaline $EtO_2C.CH(NHBoc)CH(OAc)CH.CH{=}CH_2$ through Pd(II)-catalyzed [3,3]-sigmatropic rearrangement followed by elaboration to the phosphonic acid.[211]

Enantiomerically-pure D- and L-2-amino-3-phosphonopropanoic acid has been prepared from the homochiral Boc-serine α-lactones and $(MeO)_3P$.[212] Phosphinothricin and analogues have been prepared by Michaelis-Becker alkylation of $R^1R^2P(O)H$ by acetylaminolactams.[213]

4.15 Labelled Amino Acids.- This is the repository for papers that demonstrate the use of reliable standard methods of amino acid synthesis in the context of isotopically-labelled compounds. Given the high cost of intermediates, whether in terms of cash or in investment of effort, in many of the examples in this section, the reader seeking

an optimized preparative procedure would do well to consult these papers to see how the last available milligram might be extracted from an amino acid synthesis. As usual for this Section, labelled amino acids are grouped in order of increasing atomic number (and subdivided in order of increasing relative atomic mass) of the labelled atom(s).

Simple α-^{2}H-labelling of protein L-amino acids has been claimed using tryptophanase-containing whole cells of E.coli B/1t7-A in $^{2}H_2O$.[214] Variously ^{2}H- and ^{13}C-labelled indoles have been included in fermentative production of L-tryptophans leading to six different isotopomers.[215] An alternative approach to the same objective is pyridoxal-catalyzed α-^{1}H - ^{2}H exchange with inversion of configuration, demonstrated for valine.[216] ^{2}H - I Exchange brought about for N-acetyl 3,5-di-iodotyrosinamide depends on parameters such as the nature of the catalyst used, and the protocol followed.[217] ^{2}H-Exchange both sides of the sulphur atom in D-methionine has been accomplished using $NaO^{2}H$ with the sulphonium salt, followed by mercaptoacetic acid reduction.[218] Use of the recently-established methionine elimination permitted the extension of this route to the preparation of D-[4-$^{2}H_2$]vinylglycine.

Routes to [3,4-$^{3}H_2$]-1-aminocyclopropane-1-carboxylic acid by tritium addition (Pd/C catalyzed) to the corresponding cyclopropene,[219] and to [4,5-$^{3}H_2$]-DL-leucine and -isoleucine (using the acetamidomalonate synthesis) and to [2,3,4,5-$^{3}H_4$]-DL-proline (pyrrole/$(NH_4)_2CO_3$ followed by $^{3}H_2$-Pd/C)[220] use standard methods.

^{11}C-Labelling continues to be a strong feature of this section, with its own fascination associated with the need for deft chemical operations as a result of the short half-life of this isotope. It has extra interest also, generated by a controversy[221] over the value of direct recoil ^{11}C-labelling of L-valine and 2-aminobutanoic acid, with retention of chirality, by brehmstrahlung from a 65 MeV linear electron accelerator.[222] This results in ^{11}C-atom insertion, and in reply, it was acknowledged[223] that useful radioactivity levels may not be achieved in this way. Other papers follow the conventional pathway in applying procedures occupying less than one hour, from generation of $^{11}CO_2$ or H^{11}CN to the finished product, such as the double chiral induction process using the glycine Schiff base (42) for a synthesis of [β-^{11}C]-L-alanine employing $^{11}CH_3Li$,[224] and the alkylation of Belikov's nickel-complexed chiral Schiff base (22) with $^{11}CO_2$ as starting point for the synthesis of alkylating agents for preparation of β-^{11}C-labelled amino acids.[225] Enzyme-catalyzed routes seem to be entering Langstroem's thinking, with [β-^{11}C]-L-serine as target, starting with $^{11}CO_2$, en route to $^{11}CH_3OH$ and H^{11}CHO via N^5,N^{10}-[^{11}C]methylenetetrahydrofolate (1 - 2% yield within 50 - 65 min after preparation of $^{11}CO_2$).[226] Enzymatic conversion of DL-[3-^{11}C]alanine (formed from $^{11}CO_2$ → $^{11}CH_3I$ + $Ph_2C{=}N.CH_2.CO_2Bu^t$), into L-[β-^{11}C]tryptophan and its 5-hydroxy-

derivative, has been described,[227] so also have [β-^{11}C]-L-tyrosine and -DOPA.[228] A multi-enzyme synthesis of ^{11}C-carboxy group-labelled tyrosine, DOPA, tryptophan, and 5-hydroxytryptophan from H^{11}CN,[229] and of 1-[^{11}C]-DL-homocysteine thiolactone using $^{11}CO_2$ and α-lithiated S-tetrahydropyranyl-thiopropyl isonitrile,[230] has been described. L-[5-^{11}C]Ornithine has been prepared through processing the K^{11}CN - γ-bromohomoserine lactone reaction product.[231]

[ε-^{13}C]-L-α-Amino-adipic acid and five of its isotopomers, variously labelled with ^{13}C, ^{15}N, and ^{2}H in δ and ε positions, have been synthesized through the Schöllkopf bis-lactim ether procedure (Scheme 4) with the use of K^{13}CN and routine elaboration, as far as the ^{13}C isotope is concerned.[232] [1,2-$^{13}C_2$]Lysine has been prepared by Co-catalyzed hydroformylation of 3-cyanopropene using ^{13}CO and CH_3CONH_2, via 5-cyano-2-acetamidopentanoic acid (some 4-cyano-2-acetamido-3-methylbutanoic acid is also formed).[233] Another of the protein amino acids is represented among the ^{13}C-labelled group of papers this year, in the form of [2-^{13}C]-DL-glutamic acid (DABCO-catalyzed addition of diethyl [2-^{13}C]acetamidomalonate to methylacrylate),[234] and both enantiomers of the non-protein [1-^{13}C]-2-amino-2-methylmalonic acid by straightforward means.[235] The last-mentioned preparation was the means by which stereospecific decarboxylation of this malonic acid derivative was demonstrated to involve the 2-pro-R-carboxy group in the biogenesis of D-alanine.

A lengthy synthesis involving a resolution with (-)-N-methylephedrine at its final stage to give [2-^{14}C]-L-glutamic acid, has been detailed.[236] It starts from sodium [2-^{14}C]acetate, which is converted into ethyl [2-^{14}C]-2-bromoacetate for reaction with the morpholine enamine of ethyl pyruvate, to give diethyl [4-^{14}C]-2-oxoglutarate. $LiAlH_4$ Reduction of the oxime, and resolution, completes the synthesis. 5-Amino-[4-^{14}C]laevulinic acid has been prepared,[237] a key step being the Pd(0)-catalyzed coupling of 2-phthaloylamino-[1-^{14}C]acetyl chloride (from K^{14}CN) to $EtO_2C.CH_2.CH_2.ZnI$. [2,3-^{14}C]-1-Aminocyclopropanecarboxylic acid is produced in low yield from $Br^{14}CH_2.{}^{14}CH_2Br$ and $NC.CH_2.CO_2Et$,[238]

Enzymatic methods enter again, and particularly logically, into the labelled amino acid field for syntheses of [^{15}N]-L-phenylalanine and [^{15}N]-L-tyrosine, employing [^{15}N]-ammonia and glutamic or pyruvic acids.[239] A synthesis of the neurotoxin $Me^{15}NH.CH_2.CH(NH_2)CO_2H$ from N-acetyldehydroalanine and [^{15}N]-methylamine uses the enzyme acylase I in the traditional end-of-synthesis manner for resolution.[240]

The synthesis of [^{18}F]-substitution products of m-tyrosine[241] and of 6-trifluoroacetoxymercuriDOPA[242] leads to mixtures of 2-, 4- and 6-mono-[^{18}F]-fluoro-m-tyrosines, and [^{18}F]-6-fluoroDOPA, respectively, when [^{18}F]-acetyl hypofluorite is the reagent. The latter product has been prepared through an alternative route,[243] based on displacement by $^{18}F^-$ +

crown ether of the nitro group in 3,4-dimethoxybenzaldehyde or 6-nitropiperonal, followed by a standard azlactone synthesis.

DL-[^{35}S]Cysteine has been obtained through addition of [^{35}S]-thioacetic acid to α-acetamido-acrylic acid followed by routine deprotection and purification.[244] L-[^{35}S]homocysteine thiolactone is also accessible through standard methods.[245]

L-6-[^{123}I]Iodo-m-tyrosine is formed through the reaction of Chloramine-T - $^{123}I_2$ with L-m-tyrosine,[246] while $^{123}I^-$ - Br exchange involving 6-bromoDOPA is particularly simple (35 min at 97°, pH 4).[247] In this latter study, a process was worked out for iodo-demercuration of a mercuriDOPA derivative based on I_2 composed of the normal iodine isotope. Similar approaches form the bases of syntheses of 3-[^{123}I]iodo-D-tyrosine[248] and of 3-(4'-[^{125}I]-iodophenyl)-4-aminobutyric acid, a radioactive analogue of Baclofen.[249]

4,16 β- and Higher Amino Acids.- This Section continues to expand, illustrating the growing importance of amino acids in which a larger separation of amino and carboxy functions is involved. Much of the expansion is associated with their importance as constituents of biologically-active natural products, and the interest in synthesis of peptide analogues.

Standard methods to β-amino acids, undergoing development, include Michael addition of primary or secondary amines to silyl acrylates $R^1R^2NH + CH_2{=}CHCO_2SiMe_3$.[250] and the equivalent process, addition of imines to ketene silylacetals catalyzed by FeI_2 or trityl hexachloro-antimonate.[251] Addition of the N-dialkylamino group of 1-(N-dialkylamino)benzotriazoles to ketene silylacetals leading to the same outcome, has been reported.[252] Use of N-(α-alkoxycarbonylalkyl)benzotriazoles in a general β-amino acid ester synthesis, based on Reformatzky reagents, has been described.[253] The overall sequence α-amino acid → β-amino acid is represented in the regio- and stereoselective conversion of chiral N-toluene-p-sulphonylaziridines (prepared from L-α-amino acids) using cyanotrimethylsilane.[254,255] also [256]

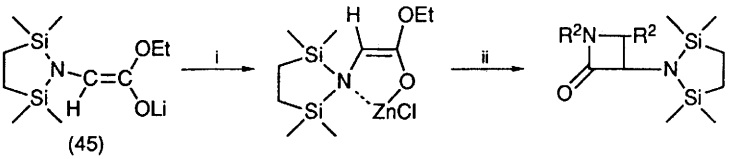
L-Asparagine serves in a general synthesis of enantiomerically pure β-amino acids, via β-cyano-alanine, thence to the methanesulphonate $(PhCH_2)_2NCH(CH_2.CN)CH_2.OMes$ which is subjected to substitution by a lithium dialkylcuprate.[255] Alternative ways in which an enantiospecific route can be organized include condensation of 3-methyl-1-nitrobutane with (-)-8-phenylmenthyl glyoxalate (KF in THF) to give a mixture of diastereoisomers including 77% of that needed for processing so as to give (2S,3R)-3-amino-2-hydroxy-5-methylhexanoic acid,[256] and (a rare example of the citation of a patent in this Chapter) a synthesis of β-amino-α-hydroxyalkanoic acids from a malic acid enantiomer.[257] Chiral

homoallylamines, e.g. (43) formed by diastereoselective addition of the familiar (see Vol.22, pp.15, 34) chiral tetra-O-pivaloylaminopyranose to allyl trimethylsilane, are cleaved by aqueous acid, to give (S)-β-phenyl-β-alanine in this particular example after $KMnO_4$ oxidation at the alkene function.[258] Transfer of chirality observed[259] to accompany DBU-catalyzed rearrangement of imines $CF_3CPh{=}NCHMePh \rightarrow CF_3CHPhN{=}CMePh$ is to be investigated for the synthesis of α-substituted β-amino acid analogues, initial experiments indicating that a much higher temperature (225°) is required for the β-amino acid synthesis than in the satisfactorily-demonstrated case (120°). Another chirality transfer is seen in the addition of toluene-p-sulphonyl isocyanide to (S)-PhCHMe.NH.CH=CH.CO_2Me, which provides $Me_3N^+.CH_2.CH(NHTos).CH_2.CO_2^-$ via the intramolecular Michael adduct (44).[260]

Improvements in syntheses of azetidin-2-ones (alias β-lactams) amount to improved β-amino acid syntheses, and provide in some cases useful exploitation of glycine and other α-amino acid synthons. The glycine ester-derived enolate (45) undergoes $ZnCl_2$-catalyzed addition to imines in the conventional way (Scheme 24).[261] (2R,3S)- and (2S,3R)-3-Amino-2-hydroxyalkanoic acids have been prepared from methyl (R)- and -(S)-mandelate, respectively, through [2 + 2]-cycloaddition of the derived chiral imines $PhCH(OR')CH{=}NR_2$ to benzyloxyketene (from $PhCH_2O.CH_2.COCl$ + NEt_3).[262] 3-Trimethylsilyloxyazetidin-2-ones and α-alkylidene-β-lactams, prepared from α-bromo-esters and azetidin-2,3-diones[263] have been used in stereoselective syntheses of α-hydroxy-β-amino acid constituents of the peptide antibiotics taxol and bestatin.[264]

For γ-amino acids, an equivalent process to those seen in the preceding paragraphs [$MeNO_2$ + $CH_2{=}C(CF_3)CO_2Bu \rightarrow NO_2.CH_2.CH_2.CH(CF_3)CO_2Bu$] leads to 2-trifluoromethyl-4-aminobutanoic acid[265] or to 3-alkyl analogues.[266] (R)- and (S)-4-Amino-3-methylbutanoic acids have been prepared through a route starting with enantioselective hydrolysis (pig liver esterase) of dimethyl 3-methylglutarate to give methyl (R)-3-methylglutarate, followed by the conversion of the ester group into NH_2 with one portion, and conversion $CO_2H \rightarrow NH_2$ for the other.[267] α-Methoxy-γ-lactams (46) undergo substitution with 1,3-dicarbonyl compounds and other active methylene compounds to give γ-aminoalkanoic acids.[268] Stereoselective $NaBH_4$ reduction of the Boc-L-valine-derived allyl ketone $BocNH.CHPr^i.CO.CH_2.CH{=}CH_2$ is the crucial step in a synthesis of (3S,4S)-BocNMe.CPr^i.CH(OMe)CH_2CO_2H.[269]

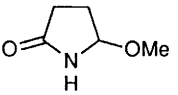
The statine synthesis industry is in ever-expanding mood, with new papers describing methods that run over well-used tracks. L-Malic acid has been used as a starting point in two independent routes, both through the chiral pyrrolidin-1,5-dione (47 in Scheme 25) and depending on regioselective carbonyl addition.[270,271] Similar strategy for a stereospecific synthesis of (-)-(3S,4S)-statine based on tetramic acid



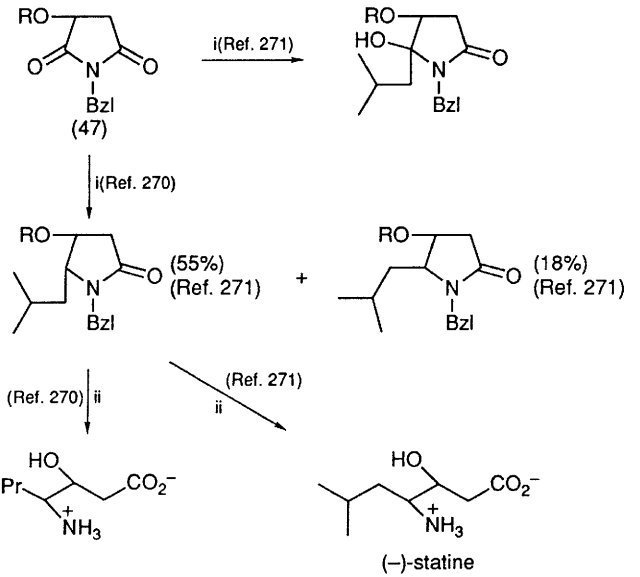
(45)

Reaction: [(45) is formed using LDA in THF at –78°C]
i, $ZnCl_2$; ii, $R^1N{=}CHR^2$

Scheme 24



(46)



(–)-statine

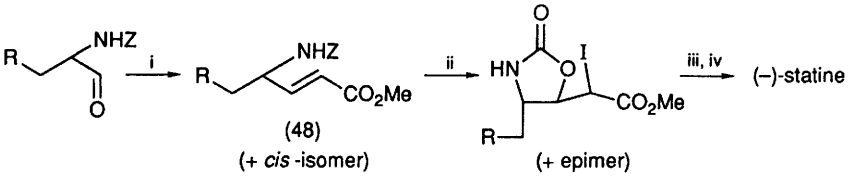
Reagents: (Ref. 271) i, $CH_2{=}CMeCH_2MgBr$; ii, dehydration, then H_2/Pd-C in CH_2Cl_2;
(Ref. 270) i, (R = H) → (R = Ac), $NaBH_4$, allyltrimethylsilane,
H_2/Pd (→ Pr^n in place of Pr^i)

Scheme 25

chemistry through the same intermediate has been demonstrated.[272] Diastereoselective $TiCl_4$-catalyzed allyl-metal addition to (S)-α-Boc-aminoalkanals[273] follows earlier precedent (Vol. 22, p.34) and involves erythro/threo ratios favouring the natural amino acid. (-)-Statine is also accessible from an (S)-α-Boc-aminoalkanal through Horner-Emmons reaction to give the cis-trans alkene (48 in Scheme 26) which is then subjected to "iodocyclocarbamation" and simple further processing.[274] The cyclic carbamate technology that is being increasingly used (Vol. 22, pp.19, 53) has been fully written up, and illustrated with a statine synthesis (Scheme 27).[275]

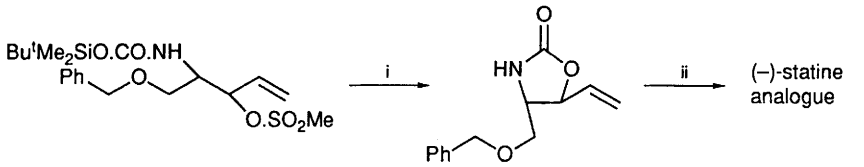
Alternative ways of inducing the correct stereochemistry at the C-3 chiral centre are available, one somewhat cumbersome method using (S)-phenylethylamine for reductive amination of isobutyl 2,5-dimethoxyphenyl ketone and depending on Birch reduction of the aryl moiety followed by ozonolysis to give $Pr^iCH(NHBoc)CO.CH_2.CO.Me$ calling for further routine processing.[276] The all-S diastereoisomers of statine and cyclohexylstatine are formed in a highly diastereoselective (94:6) aldol route involving an (S)-α-isopropoxycarbonylaminoalkanal with O-methyl-O-trimethylsilylketene acetal.[277] This is described by its originators as "the most practical synthetic route" to these compounds, a phrase that will be used more often to justify future statine papers, now that so many effective routes are available. A paper from the same group could even be seen as challenging the claim, involving $CeCl_3$-catalyzed stereoselective Grignard addition to the imine derived from (2S,3S)-tartaric acid in a synthesis of the corresponding (2R,3S)-3-amino-2-hydroxyalkanoic acid, alias cyclohexylnorstatine (Scheme 28),[278] the same product as obtained starting from L-phenylalanine in a route established by these workers to prepare the aldehyde of its cyclohexyl analogue in the form of its N-isopropoxycarbonyl derivative; the route includes a highly diastereoselective acetoxycyanohydrin formation step.[279] A correspondingly simple route to the same target starts with N-Boc-L-phenylalaninal.[280] A chiral thioacetamide PhCHMe.NH.CS.Me has been used to start a statine synthesis through Michael addition of its carbanion (Bu^nLi) to acrolein, followed by diastereoisomer separation and stereoselective iodolactamization (Scheme 29).[281]

"Isostatine", in which another chiral centre is created as an isopropyl methyl group is moved to C-5, is nevertheless an easier synthetic challenge since Fmoc-D-alloisoleucine offers a convenient starting point, either in the form of the methyl ester[282] or as the acid chloride.[283] Full details in the former paper include a synthesis of D-alloisoleucine from L-isoleucine (5 steps) as well as the 6 further steps needed for reaching (3S,4R,5S)-isostatine; the other paper covers the simpler acylation of $LiCH_2.CO_2Bu^t$, KBH_4 reduction and flash



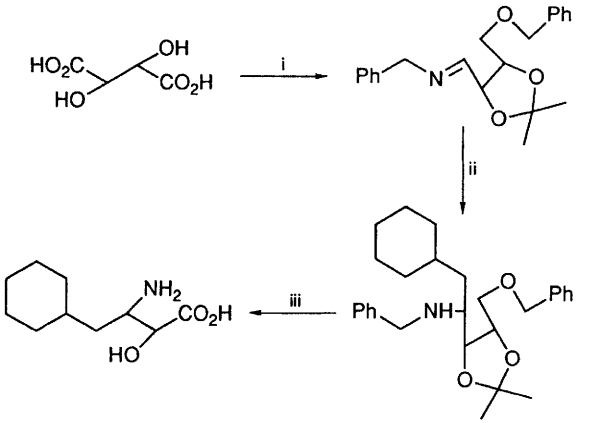
Reagents: i, $(MeO)_2POCH_2CO_2Me/NaH$; ii, $I_2/MeCN$; iii, $Bu^n{}_3SnH$; iv, alkaline hydrolysis

Scheme 26



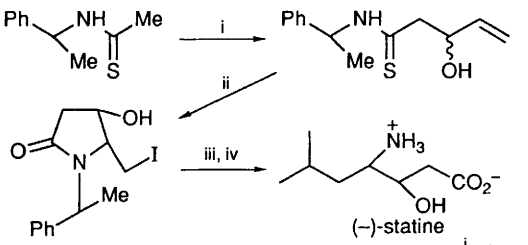
Reagents: i, F^-; ii, routine elaboration

Scheme 27



Reagents: i, known sequence; ii, $C_6H_{11}CH_2MgX$, $CeCl_3$; iii, deprotection, *etc.*

Scheme 28



Reagents: i, Bu^nLi, acrolein; ii, resolve, then MeI, then I_2; iii, $I \rightarrow Pr^i$; H_2/Pd; iv, hydrolysis

Scheme 29

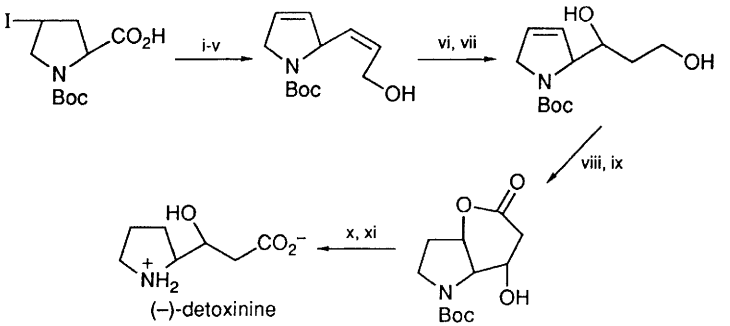
chromatographic separation stages, and also explores the corresponding use of Fmoc-L-leucine in a statine synthesis.

The earlier-mentioned use of cyanotrimethylsilane[284] for cyanohydrin formation from an N-protected α-amino-aldehyde is also used in a one-pot anti-diastereoselective route to β-amino-α-hydroxyesters for bestatin and amastatin synthesis,[284] also in a route to corresponding formyl anion synthons (thiazole moiety in place of the ester function) when 2-trimethylsilylthiazole is used in place of the cyanide.[285]

More complex natural β-amino acids are covered in a (−)-detoxinine synthesis (already the subject of three total syntheses), starting from N-Boc-(2S,4S)-4-iodoproline methyl ester, easily prepared from 4-hydroxyproline, and proceeding through highly diastereoselective stages (Scheme 30),[286] and a synthesis of "ADDA" [(2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid] starting from the (2S,3R)-epoxide of 4-benzyloxy-cis-2-buten-1-ol in which all chiral centres are generated with the correct configurations (Scheme 31).[287]

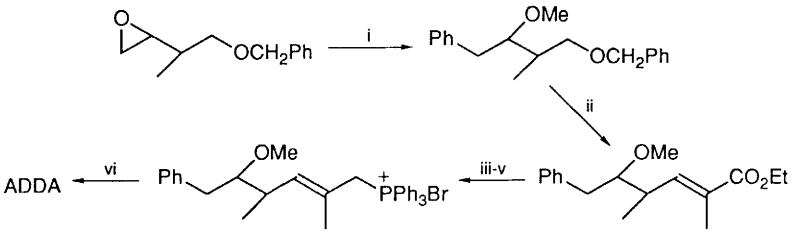
γ-Amino acids result from carboxylation of lithiated di-allylamines (Scheme 32).[288] Both enantiomers of carnitine $Me_3N^+.CH_2.CH(OH).CH_2.CO_2^-$ (the L-isomer is often referred to as Vitamin B_T) have been synthesized from malic acid, through relatively straightforward functional group transformations.[289] Other γ-amino acids also requiring more than a little skill for their synthesis include "(R)-GABOB" - alias (R)-γ-amino β-hydroxybutyric acid - for which several efficient syntheses have been reported. Claisen condensation (lithium diethylamide) of N-benzyloxycarbonylglycinal with $(R)\text{-}MeCO_2CHPhC(OH)Ph_2$ occurs with induction of the correct stereochemistry (Scheme 33).[290] A route from $CH_2{=}CHCH_2CONHCH_2Ph$ to the γ-lactam [a substituted (R)-GABOB] exploits a chiral phenylethylamine for the induction of the correct stereochemistry, and of course, the route equally conveniently provides (S)-GABOB.[291] (+)-Tartaric acid is the starting point in another (R)-GABOB synthesis.[292]

δ-Amino acids are of increasing interest since they provide dipeptide isosteres for routes to peptide analogues. γ-Keto-δ-amino acids ("ketomethylene pseudopeptides" in the jargon of peptide analogues), have been synthesised through an efficient route.(Scheme 34).[293] Bamberger cleavage of ethyl 3-(4-imidazolyl)butanoate (see Vol.22, p.37) using (−)-menthyl chloroformate gives the 3,4-di-aminobutanoates as their carbamates though not particularly high enantioselectivity, a process applicable also to L-histidine methyl ester.[294] Naturally-processed dipeptides incorporating thiazole moieties are, from one point of view, peptide analogues, and a synthesis of the thiazole (49)[295] is properly located in this section since it is effectively a dipeptide derivative and at the same time, a δ-amino acid derivative.



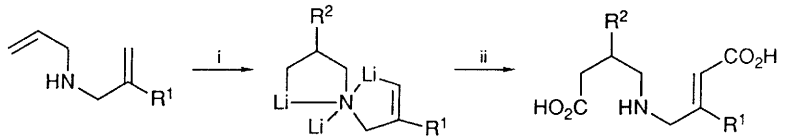
Reagent: i, $NaBH_4$-LiCl; ii, $COCl_2$/DMSO; iii, $(CF_3CH_2O)_2P(O)CH_2CO_2Me$; iv, DIBALH; v, PhSeNa; vi, MCPBA, then diastereoisomer mixture is separated; vii, *syn* -isomer reduced with Red-AL ®; viii, Pt-O_2; ix, Br_2-EtOH; x, Bu_3SnH; xi, TFA and work up

Scheme 30



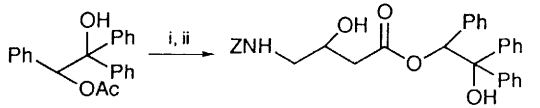
Reagents: i, PhMgBr/CuI, then NaH/MeI; ii, Pd/C; H_2, then $COCl_2$/DMSO, then $Ph_3P{=}CMe.CO_2Et$; iii, DIBALH; iv, CBr_4/PPh_3; v, PPh_3/MeCN; vi, Bu^nLi, then condensation with modified C-1 to C-4 segment of ADDA (in the form of the C-4 aldehyde), followed by routine elaboration

Scheme 31



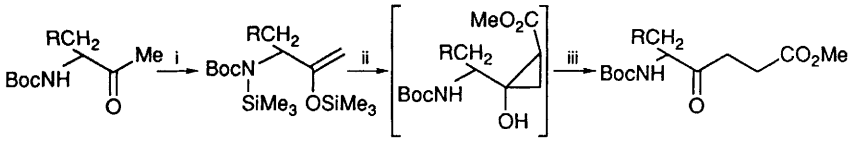
Reagents: i, RLi; ii, CO_2

Scheme 32



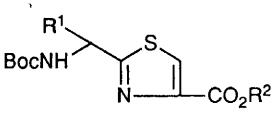
Reagents: i, LDA; ii, Z.NH.CH_2.CHO

Scheme 33

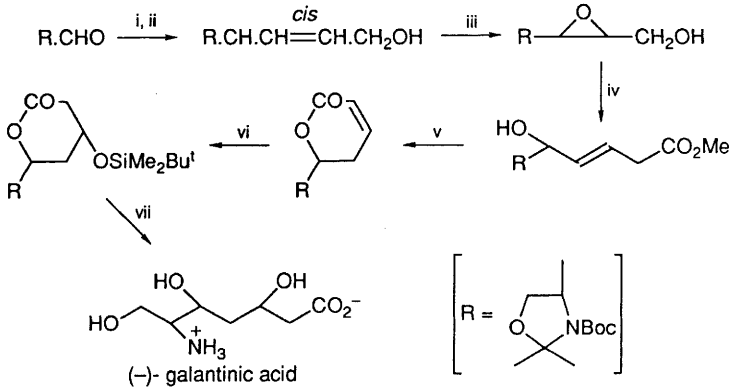


Reagent: i, LDA, Me_3SiCl; ii, N_2CHCO_2Me, $Cu(acac)_2$; iii, $Bu_4N^+F^-$

Scheme 34



(49)



Reagents: i, $(CF_3CH_2O)_2P(O)CH_2CO_2Me$, NaH; ii, Bu^i_2AlH; iii, MCPBA;
iv, $COCl_2$/DMSO, then Ph_3PCHCO_2Me; v, DBU; benzene, reflux;
vi, Bu^tOOH (→ oxirane) then PhSeH, TFAA/DMSO, NH_3-BH_3, $TfOSiMe_2Bu^t$;
vii, TFA-CH_2Cl_2

Scheme 35

A synthesis supporting a revised structure for (-)-galantinic acid has been described (Scheme 35).[296] This does not invalidate another synthesis[297] so far as its strategy is concerned, which is based on the chiral oxazolidin-2-one methodology mentioned earlier. A point of interest in this strategy[297] is the substitution of a 4-phenylthio group on the oxazolidinone by photochemical radical allylation.

Qualifying for last mention in this section, organized as it is in order of increasing separation of amino and carboxy functions, is the synthesis of cis-12-amino-9-octadecenoic acid methyl ester and derivatives, using standard functional group transformations.[298]

4.17 Resolution of DL-Amino Acids.- The main subsections of this topic remain under active investigation, and are described here as in preceding Volumes. Although resolution through chromatographic and other physical principles is included here, it is also covered in analytical terms in the later sections covering t.l.c., g.l.c., and h.p.l.c.

Classical non-enzymatic methods of resolution of DL-amino acids involve diastereoisomer salt formation (mentioned at appropriate points elsewhere in this Chapter - refs. 48, 188, are representative), or conversion into diastereoisomeric derivatives, an unusual example this year being the esterification of N-phthaloyl-β-phenyl-γ-aminobutyric acid with (R)-(-)-pantolactone.[299] A review has appeared covering the resolution of multigram quantities of enantiomer mixtures.[300]

Crystallization of the reaction mixture from DL-phenylalanine + $[Cr(L\text{-}Phe)_2(NCS)(OH_2)]$ [i.e. aqua(isothiocyanato)bis(L-phenylalaninato)chromium] from ethanol gives successive crops of $[Cr(L\text{-}Phe)_2(NH_2CS\text{-}D\text{-}Phe)(OH_2)]$ + $(\underline{fac})\text{-}(-)_{546}\text{-}[Cr(L\text{-}Phe)_3]$,[301] thus achieving resolution of the DL-amino acid. Other crystallization processes based on physical phenomena, are continuing to be studied, and a Symposium Volume has been dedicated to this topic.[302] Two papers from this source deal with batch crystallization purification of L-isoleucine[303] and with growth rate and impurity occlusion in crystals of S-carboxymethyl-D-cysteine from solutions of the seeded supersaturated racemate.[304] An extension the latter study describes the promotion of crystallization of S-carboxymethyl-L-cysteine from aqueous solutions through addition of NaCl or KCl.[305] Four papers from Shiraiwa's group fall within the latter area, one dealing with the "replacing crystallization" principle and illustrated for DL-threonine solutions containing L-proline as optically-active co-solute.[306] D-Threonine of 91% optical purity crystallizes out to the extent of 78% of that available, and L-threonine crystallizes from the mother-liquors. A merging of two classical resolution methods is represented in asymmetric transformation, in which transient, racemizable

intermediates are formed as one diastereoisomeric salt crystallizes out; illustrated for (RS)-N-methyl-2-phenylglycine/aldehyde/(S)-camphor-10-sulphonic acid[307] and for the corresponding system based on (R)-α-methylbenzylamine/N-acetyl-(RS)-2-phenylglycine[308] and the 4-hydroxyphenyl analogue.[309]

An example of more interest in synthesis concerns the epimerization of β-methyl L-aspartate by heating in solution in MeCN with salicylaldehyde and (-)-PhCHMe.SO_3H, relying on the fact that the salt of the D-isomer is practically insoluble.[310]

Aminolysis of oxazolones with L-phenylalanine methyl ester continues to be studied for what is essentially an asymmetric transformation process, current results establishing that triethylamine usefully augments diastereoselectivity by increasing both racemization and reaction rates.[311] *Reductive aminolysis of 4-alkylidene-oxazolones* ("azlactones") in this way gives only 9 - 27% diastereoisomeric excesses of the D,L-dipeptide ester,[312] and little better using (R)-phenylglycine methyl ester.[313] Further results (Vol.22, p.53) concerning the asymmetric induction that accompanies the aminolysis of 2-phenyloxazolones with an L-amino acid ester[314] confirm the predominance of the D,L-dipeptide derivative in the product, even in a polar solvent, with an influence of temperature inconsistent with that reported by Benoiton ten years previously.[315]

A continuing high level of interest in uses for enzymes for "resolution" of DL-amino acids is partly explained by the growing awareness of methods by which their selectivity can be "broadened" considerably. A review of resolution by enzymes emphasizes the mechanistic organic chemistry of the process.[316] A novel demonstration of the classical use of enzymes for the present purpose is the conversion DL-histidine → D-histidine + histamine,[317] while other papers cover applications in which moderately successful processes are achieved for compounds somewhat different from the enzymes' natural substrates; such as methyl, ethyl, or butyl esters of amino acids (modest stereoselectivity using Sulfolobus solfataricus whole cells trapped in calcium alginate),[318] and Pseudomonas whole cells used for the liberation of L-cysteine from DL-thiazolidine-4-carboxylic acid.[319] A notable feature of the last-mentioned study is the inclusion of hydroxylamine to prevent further enzyme-catalyzed changes, so making this a viable process. The first illustration of the formation of D-amino acid N-alkylamides in this way from a DL-amino acid ester and immobilized D-amino acid peptidase has been reported.[320] Although no microbial methods yet exist for the isolation of L-methionine from its racemate, the process can be achieved in better than 95% yield and better than 99% enantiomeric excess by a roundabout method (via α-oxo-γ-methyl thiobutyrate) in which a cocktail containing *D-amino acid*

oxidase, catalase, leucine dehydrogenase, and formate dehydrogenase are employed.[321] Of academic interest, perhaps, is the fact that this useful method is successful also with alanine and leucine, but more important is the implication that it is applicable to any leucine dehydrogenase substrate.

The lipase-catalyzed n-butanolysis in di-isopropyl ether, of 2-phenyl-4-methyloxazol-5(4H)-one with in situ racemization of the oxazolone so as to give N-benzoyl-L-alanine n-butyl ester[322] has successfully passed referees' attention to enter the literature, though the principle was well-established many years ago for thiazolones, using trypsin.[323]

Methyl N-acetyl phenylserinate and threoninate are "resolved" by α-chymotrypsin, subtilisin, or bromelain.[324] The last-mentioned enzyme shares the preference of α-chymotrypsin for the (R)-enantiomer of the esters of phenylserine.[324] Various kinetic and structural parameters relating to the resolution of N-acetylphenylalanine ethyl ester by α-chymotrypsin have been considered.[325] A production method for L-phenylalanine[61] employs chymotrypsin resolution.

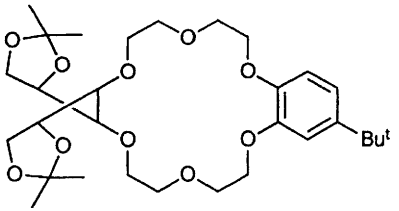
Preparative chromatographic resolution of DL-amino acids follows established methods, some of recent origin, such as use of "chirally imprinted" polymers, and others of almost antique character but of immense value, such as resolution over cellulose. The imprint generated by copolymerizing L-tyrosinyl acrylate with a large excess of vinylbenzene, followed by hydrolysis in hot aqueous NaOH to remove the optically-active ester group, binds D-4-aminophenylalanine ethyl ester in preference to its L-enantiomer, maximum selectivity from a range of experiments being 1.35:1.[326] Methacrylate analogues[327] similarly imprinted using L-amino acid anilides are found to be efficient in resolution of DL-amino acids, not restricted to the imprinting amino acid. Other chiral polymers prepared similarly, but leaving the amino acid residue in place and attached, have been used as chiral stationary phases; specifically, (R)-N-(3,5-dinitrobenzoyl)phenylglycine-derivatized polymers,[328] (S)-N-(3,5-dinitrobenzoyl)tyrosine analogues,[329] similarly-formed mixed DL-N-(3,5-dinitrobenzoyl)valine methyl ester/(S)-2-(phenylcarbamoyloxy)propionic acid n-butylamide-derivatized polymers,[330] and silica gel treated with $ClSiMe_2CH_2CH_2NHCHPhCONHPr$, formed from Me_2SiCl_2 and N-acryloyl (R)-phenylglycine n-propylamide.[331] Much debate can be noted, on the mode of action of these polymers in discriminating between enantiomers, and one of these papers describes direct spectroscopic evidence for a chiral recognition mechanism that had been proposed earlier.[332] A crosslinked polystyrene + chiral di-amine or L-proline + a Cu(II) salt combination has been used for chromatographic resolution of DL-amino acids.[333] β-Cyclodextrin incorporated into silica gel acts as chiral discriminator in displacement chromatography of

dansyl-DL-amino acids,[334] as it does when incorporated into gels for isoelectric focussing on immobilized pH gradients.[335] 2-Amino-ω-phosphono-alkanoic acid enantiomers are rather inefficiently resolved using simple crown ether-based chiral stationary phases,[336] though the more formidable crown ether (50) incorporated into C-18 silica[337] is more successful for the resolution of DL-α-amino acids. (R,R)-(-)-NN'-trans-1,2-dicyclohexyl-hexanediamine is a suitable chiral selector for the resolution of DL-amino acids and their dansyl derivatives.[338] Proteins have been advocated as chiral selectors for large-scale resolution of DL-amino acids by centrifugal partition chromatography.[339]

The ligand exchange principle, in which discrimination is exerted through competitive interactions involving an achiral stationary phase and a mobile phase containing a copper(II) - derivatized-L-amino acid complex, works well for preparative resolution of DL-amino acids.[340] Dansyl-DL-amino acids have been resolved in this way, using copper(II) - mixed o-, m-, and p-xylenyl-L-prolinates,[341] and the related "continuous counter-current fractional extraction" technique, using a two-phase system prepared from aqueous butan-1-ol and copper(II) - N-(n-dodecanoyl)-L-hydroxyproline results in a concentration of the D-isomer in the upper (organic-enriched) layer when applied to DL-valine.[342]

Returning to the long-standing method, cellulose chromatography, referred to in the opening paragraphs of this Section, the effects of salts and of added methanol, on the resolution of 5-methyl-DL-tryptophan in aqueous media, has been investigated.[343] [^{14}C]-Labelled phenylalanine and methionine have been resolved efficiently (greater than 99% optical purity) through cellulose column chromatography,[344] and N-protected DL-amino acid esters have been resolved over 6-cellulose tris(phenylcarbamate)s and 5-amylose tris(phenylcarbamate)s, the L-enantiomer emerging first.[345]

Somewhat obscure calculations are purported to demonstrate that amorphous cellulose shows 1% discrimination between the alanine enantiomers as far as the energetics of attractive forces are concerned,[346] and adsorption of L-alanine on kaolinite has been shown through SCF calculations to be favoured, relative to adsorption of the D-isomer, by 0.14 and 0.04 kJ mol^{-1} for the positive ion and for the zwitterion, respectively.[347] Interestingly, these microscopic energy differences are many orders of magnitude greater than the energy difference between amino acid enantiomers that arises from the "electroweak" parity-violating energy difference. There is some connection between the purpose of these calculations, and theories of prebiotic "resolution" of DL-amino acids, for which reviews[348] and further experimental studies have been published. In this latter category, "resolution" through the differential destruction of



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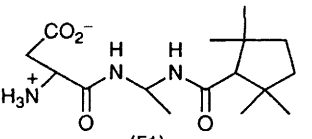

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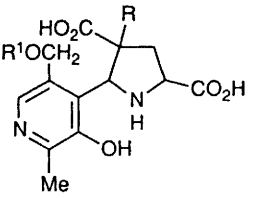

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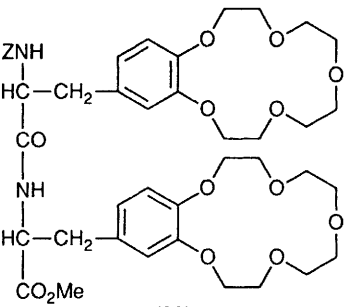

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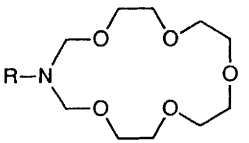
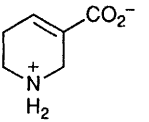


[(54) R = Z.Gly, Z.Phg-Gly, or MeO.CO.CHPh-Gly]



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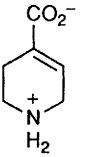

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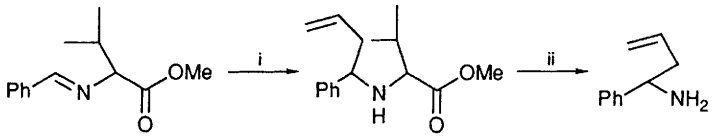
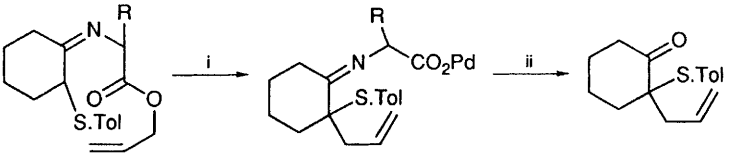


(+ 5% diastereoisomer)

Reagents: i, $CH_2{=}CHCH_2Br/TiCl_4$ (catalytic amount); ii, OH^-, then e^-

Scheme 36



Reagents: i, $Pd(0)Ac_2$; ii, hydrolysis

Scheme 37

enantiomers of an amino acid has long been speculated to accompany high-energy β-irradiation and positron annihilation. The analysis by pulse-height spectroscopy, of Cerenkov radiation emitted as β-particles pass through enantiomerically-pure samples, verifies that chiral electrons actually do distinguish between molecules of opposite chirality.[349]

5 Physico-Chemical Studies of Amino Acids

5.1 Crystal Structures of Amino Acids and Their Derivatives.- With one or two exceptions where some commentary is added, studies are merely listed as having been reported.

Protein amino acids subjected to X-ray crystal analysis are L-serine, L-cysteine, and L-cystine,[350] potassium hydrogen L-glutamate monohydrate,[351] strontium L-aspartate trihydrate and barium L-aspartate trihydrate,[352] L-asparagine monohydrate,[353] DL-lysine mono- and di-hydrochlorides,[354] DL-arginine acetate hydrate and DL-lysine acetate,[355] DL-arginine hemisuccinate dihydrate and the corresponding L-arginine salt,[356] and DL-arginine DL-glutamate hydrate and the corresponding DL-aspartate salt.[357] Compared with the L-arginine and L-lysine acetates, the DL-salts show quite different crystal structures as far as their hydrogen bonding patterns are concerned, a fact that the authors speculate might be of relevance to the prebiotic ascendancy of the L-amino acids.

N-Methyl-D-aspartic acid hydrate is an important natural protein amino acid derivative that has been included in this year's published X-ray work,[358] as has L-lanthionine.[359] X-Ray studies on derivatives that are more familiar in laboratory synthetic operations or molecular orbital studies include N-trityl-L-4-hydroxyproline methyl ester,[360] N-phenylacetyl-L-aspartic acid,[361] various N-acylureas of N-benzyloxycarbonyl-L-valine,[362] N-acetyl-DL-methionine and its calcium salt,[363] α-(N-acetylamino)-α-n-butylnorleucine,[364] and Nᵃ-acetyl-N-methyl-L-tryptophanamide.[365]

The crystal structure of the intensely sweet L-aspartamide (51), an inverso-dipeptide derivative from one structural point of view, has been reported.[366]

5.2 Nuclear Magnetic Resonance Spectrometry.- A spectacular application, 2D-COSY ^{1}H-n.m.r. assignments to cerebral metabolites L-alanine, N-acetyl-L-aspartic acid, L-aspartic acid, γ-aminobutyric acid, and L-glutamic acid, has been achieved for a living animal using

a surface coil probe.[367] Other n.m.r. papers published this year can hardly live up to that, but are worthy in their own right.

^{1}H-N.m.r. studies continue to provide practical analytical support for amino acid studies, as in assignments of absolute configuration to α-methyl α-amino acids through detection of the precise chemical shift for the methyl proton resonances in aqueous solutions containing the chiral lanthanoid shift reagent 1,2-propanediamine tetra-acetato europium(III).[368] The resonance for the (S)-enantiomer is upfield relative to that of its (R)-isomer. The enantiomeric purity of N-Boc N-methyl α-amino acid methyl esters can be assessed through Eu(hfc)$_3$-induced shift separation of the Boc and N-methyl signals.[369]

Phenacyl esters of Boc-proline, prepared from the imino acid in a one-pot procedure, exist in solutions in a 1:1-cis:trans mixture.[370] Magnetic asymmetry is revealed for the phenacyl group in this study. Similar cis:trans-mixtures occur for 3-benzamido-2-piperidonecarboxylic acid, which adopts a distorted chair conformation in dimethyl sulphoxide-$^2H_6$.[371] Higher up the sophistication scale, conformational assignments have been made to N-benzoyl-L-phenylalanine through combined rotation and multiple pulse ^{1}H-n.m.r. (CRAMPS).[372] N.m.r. spectra of N-acetyl N'-methylamides of aliphatic amino acids, a well-studied category of compound for molecular orbital calculations, have been analyzed after specific ^{13}C-labelling of the carbonyl carbon atom, in terms of the dependence of $^3J_{HNC\alpha H}/^3J_{C'NC\alpha H}$ values on the dihedral angle Ø.[373] The dihedral angle increases regularly with increasing side-chain bulk.

^{1}H-N.m.r. data show that caffeine stacks in a parallel ("pack of cards") fashion with L-tryptophan in a 1:1-ratio in aqueous solutions (as it does with several other more-or-less two-dimensional heteroaromatic species).[374] Establishment of the existence of intermolecular structuring of this sort, both between different molecules and between two or more identical molecules, is a continuing feature of n.m.r. studies of amino acids that has been extended to mono- and di-thiocarbonyl analogues of methylamides of N-acylamino acids and dipeptides.[375] The intramolecular hydrogen bonding patterns in N$^\alpha$-thioacetylproline methylamide have been located through a combination of i.r., ^{1}H- and ^{13}C-n.m.r. in this study.

^{13}C-N.m.r. spectra of strategically-^{13}C-labelled L-lysine[232] have established the correct assignments for β- (31.2 ppm) and δ- and ε- (27.6 ppm) side-chain resonances hitherto thought to be the other way round. Synchronous ^{13}C-/^{15}N-n.m.r. monitoring has been used to follow the metabolism of [1-^{13}C,^{15}N]glycine on whole liver cells, through the development of serine resonances.[376] In CP-MAS ^{13}C-n.m.r. of polycrystalline L-leucine, the splitting of the β-carbon resonance is due to site differences in the $P2_1$ unit cell, not to long range residual

dipolar ^{14}N - ^{13}C coupling.[377] ^{17}O-N.m.r. spectrometry has also been applied to polycrystalline L-leucine with a view to establishing hydrogen bonding patterns.[378] A more familiar ^{17}O-n.m.r. application in the amino acids field is the measurement of spin-lattice relaxation times T_1 for $H_2{}^{17}O$, as a function of structure for apolar amino acid solutes and various physical parameters of the solutes.[379]

^{19}F-N.m.r. data for 1:1-inclusion complexes of N-trifluoroacetyl-D- and -L-4-fluorophenylalanines and -phenylalanines with cyclomaltahexaose (alias α-cyclodextrin) have been reported, contributing to understanding of the penetration and relative geometry of the aryl moiety into the cavity of the host.[380]

5.3 Optical Rotatory Dispersion and Circular Dichroism.- A careful study of the c.d. of L-phenylalanine, compared with that of (R)-3-amino-4-phenylbutanoic acid, has been reported.[381] The two compounds, though of the same configurational family, show oppositely-signed ^1L_a Cotton effects associated with the phenyl chromophore, and caution is advocated for empirical configurational assignments based on the sign of a 200 - 400 nm Cotton effect developed in a phenyl chromophore perturbed by a β-chiral centre.

5.4 Mass Spectrometry.- Excluding routine results, and leaving analytical studies such as g.l.c. - m.s. of derivatized amino acids to a later section, results cited here relate to pioneering m.s. studies of the amino acids and their significant reactions.

Negative ion m.s. of deprotonated amino acids have been interpreted in terms of specific H^+ transfers to carboxylate anions followed by simple fragmentation processes through ion complexes.[382] Positive ion m.s. studies for 2-amino-alken-2-oic acids ("dehydro-amino acids") have been reported.[383]

DL-γ-Carboxyglutamic acid reacts with pyridoxal phosphate in water to give (52; R = H or CO_2H), identified by FAB-m.s. more conveniently than other derivatives and therefore proposed to have potential analytical value.[384]

5.5 Other Physico-chemical Studies.- Spectroscopic studies, using techniques in addition to those (n.m.r., o.r.d./c.d., and m.s.) specifically located in sections preceding this one, continue to be applied in amino acids science, but are either too routine to deserve citation here, or arise in isolated, pioneering, papers; and are therefore discussed here. Photo-electron He(I)- and He(II)-spectroscopic studies of N-acetyl dehydroalanine methylamide[385] with objectives in conformational assignments, and i.r. and p.e. spectroscopy of glycine, L-alanine and β-alanine on a copper surface[386]

have been reported. U.v. Resonance Raman saturation spectroscopy, a new technique concentrating on relaxation measurements with associated vibrational band resolution, has contributed a new aspect to the very substantial body of Raman data on tryptophan and its derivatives,[387] together with data on u.v. resonance Raman excitation profiles of this amino acid.[388] Sub-picosecond fluorescence anisotropy of tryptophan in water,[389] and the underlying cause of oscillating absorption and fluorescence of tyrosine in water,[390] have been studied.

As with some of the papers mentioned in the preceding paragraph, many of the papers located in this section are aimed at providing information of use in understanding the reaction behaviour and particularly, aspects of the physiological properties of amino acids. This is particularly clear with adsorption and other more obvious transport properties, such as calorimetric studies yielding heats of dilution, from which chiral interactions involving protonated amino acids in aqueous hydrochloric acid may be deduced.[391] Dilution enthalpies thus obtained are identical for such solutions containing only one enantiomer or containing both enantiomers of an amino acid. Therefore, the recently uncovered evidence that there is a greater attraction between an L- and a D-enantiomer of an amino acid in aqueous solution than between two enantiomers of the same configuration is deduced to involve the zwitterionic forms of the amino acids. Enthalpies of dilution of solutions of N-acetyl N'-methylamides of D- and L-amino acids with alkyl side-chains[392] and for L-serine, L-threonine, and hydroxy-L-proline and their enantiomers.[393] The same data have been collected for glycine, alanine, valine, leucine, proline, sarcosine, and N-methyl-alanine in aqueous media,[394] (see also ref. 395) confirming that interaction between an L-amino acid derivative with its D-enantiomer is significantly less exothermic than that between two identical molecules. L-Phenylalanine – α-cyclodextrin inclusion complexation[396] has been studied, and thermodynamic data relating to 298.15K, for stable 1:1-amino acid : "Cryptand 222" complexes[397] formed in methanol have been reported. Within this same topic area, but with quite a different objective, is the thermoenergetic identification of enantiomers, through study by n.m.r. and differential scanning calorimetry, of D- or L-amino acid:sodium chloride:water eutectic mixtures.[398] The abstract source of this information leaves the scientific basis of this study unfathomable. Hydrophobic interactions have been shown to be the basis of complexation of amino acids by the water-soluble porphyrin, 5,10,15,20-tetrakis(4-sulphonatophenyl)-21H,23H-porphine.[399]

New clarity is provided for mechanisms of amino acid transport by demonstrations of the effectiveness of the chiral 15-crown-5-ether (53)[400] and of lariat-type ligands (54)[401] in carrying protected DL-amino

acids through CH_2Cl_2 and $CHCl_3$ membranes, respectively. Potassium salts of dipeptides were also suitable passengers in the latter study, in which the L-enantiomers of Z-amino acids were favoured, though minimal enantioselection was observed in the former study. Studies of this type can lead to useful practical advances, through establishing means by which amino acids can be taken into organic solutions. At the extreme limit of this process is the solubilization of tryptophan and proline in ethane and propane through the use of reverse micelles in the supercritical fluids,[402] while the possibility of enriched concentrations of amino acids at phase interfaces through adsorptive bubble separation in aqueous media by foam flotation has been theoretically modelled.[403] Concentration of amino acids at interfaces, detected by oriented crystallization,[404] may have important implications. Liquid emulsion membranes have been devised for the separation and concentration of amino acids using di-(2-ethylhexyl)phosphoric acid as cation carrier.[405] At another level of thinking, helical bilayer membranes can be formed from L-glutamic acid derivatized with bis(dodecylamide) groups.[406]

Adsorption of amino acids and their derivatives from solutions has been studied for hydroxylapatite (aspartic acid, lysine, alanine; see also Vol.22, p.2),[407] for silica gel (glutamine, methionine, phenylalanine, and tryptophan),[408] and for an aminocarboxy-cellulose-based ampholyte.[409] The object in these studies has a practical preparative side to it, and the preferential adsorption of one enantiomer of an amino acid has been a topic of long-standing study in relation to prebiotic chemistry (see also Section 4.17 Resolution of DL-Amino Acids). Adsorption isotherms of N-benzoyl D- and L-alanine at different temperatures allow enthalpy of adsorption data to be established. For a protein, these data give support to a bimodal retention mechanism for the enantioselection.[410]

More academic studies concern relationships between structure and pK values, for α-trifluoromethyl-α-amino acids (lowering of pK values for CO_2H and NH_3^+ groups),[411] L-Alaninehydroxamic acid shows a higher pK value for its NH_3^+ group than for its other acidic group, but the order is reversed for the corresponding *β*-alanine derivative.[412] As well as the usual potentiometric methods, ^{13}C-n.m.r. data were also employed in this study. Routine evaluation of acidity constants of amino acids[413] and dissociation constants of DL-amino acids in aqueous dioxan (cf. Vol.22, p.45, ref.298) has been reported.[414] Activity coefficient data confirming the destabilization of an amino acid through transfer from water to aqueous alcohols.[415] A purely theoretical study has been completed, modelling the effects of temperature and of pH on the solubility of an amino acid in water, with reference to activity coefficient data.[416]

5.6 Molecular Orbital Calculations.- Theoretical studies dealing with amino acids fall into categories of conformational analysis on the one hand, and calculations of physical parameters on the other. The conformational theme continues to dominate this group of papers, with protein amino acids being represented as zwitterions in the gas phase (glycine, alanine, and serine),[417] and in less specific situations (L-cysteine,[418] and L-arginine and its des-amino analogues[419]). Atom-centred partial charges have been calculated for amino acids.[420]

Conformations of amino acid residues in peptides and proteins are modelled by N-acetylamino acid methylamides, and new calculations of conformational energies have been reported.[421] N-Formyl-L-serinamide has been treated in a similar way.[422] Calculations of entropy and solvent effects on conformational energies have been reported for some conformations of N-acetylalanylglycinamide,[423] and for hydration energies of the twelve lowest energy conformations of N-acetylalanine methylamide.[424]

These same methods and objectives have been applied to non-protein amino acids too, dealing with the identification of the most stable conformation for L-2,4-di-aminobutanoic acid[425] and β-alanine.[426] Conformational calculations for γ-aminobutyric acid have been matched with those for two compounds that inhibit its neurotransmission properties [guvacine (55) and isoguvacine (56)].[427]

A summary has appeared of molecular dynamics simulation of the conformational behaviour of dityrosine in an attempt to account for its non-exponential fluorescence decay.[428] Calculated energy barriers relating to the diphenyl moiety of thyroxine are in qualitative agreement with those measured from n.m.r. data.[429]

6 Chemical Studies

6.1 Racemization.- Microwave irradiation of solutions of isoleucine and phenylalanine in acetic acid leads to quantitative racemization.[430] Slow acetylation of amino acids in acetic acid was established long ago, and it remains unclear how these results should be interpreted. The racemization of L-proline as a component in milk subjected to microwave treatment is a cause of anxiety because D-proline is known to be toxic.[431] Rate studies have been reported showing the effect of neighbouring functional groups on the racemization of βγ-unsaturated amino acids in acetic acid.[432] (E)-2,4-Di-aminobuten-3-oic acid racemizes somewhat faster than its N^4-benzyloxycarbonyl derivative, but both racemize at rates several orders of magnitude faster than 3,4-dehydrovaline. All these racemize more readily than ornithine and

norvaline. The pH - rate profile for the racemization of L-5-benzylhydantoin demonstrates catalysis by hydroxide ion.[433]

L-Phenylalanine, -leucine, -isoleucine, and -tyrosine subjected to high pressure (several GPa) at ambient temperatures, undergo substantial racemization.[434] Positive catalysis by minerals used as supports for the amino acids is observed, with silica gel and alumina inducing the greatest rate enhancements. Results such as these fuel the controversy over applications of racemization levels of amino acids in fossils as a date index, but those proponents of the method can point to careful calibration of their results that tends to promote their credibility. An interesting example is the finding that ancient eggshell samples for the African ostrich (Struthio camelus) retain their indigenous organic matrix, and their L-isoleucine:D-alloisoleucine ratio can be used to date Pleistocene archaeological sites.[435] Fossil bones from two sealed catacombs in Rome dating to the 4th Century BC provide ideal samples for calibrating the L-aspartic acid racemization scale, since constant temperature and humidity conditions have prevailed, and the sites are presumed to be free from human contamination. Relatively high D-aspartic acid content was found, showing that bone collagen decomposes and racemizes faster in conditions of high humidity.[436] A new study (see Vol.22, p.47) of the dating of human remains through the D-aspartic acid content of dental collagen (alias dentin) based on teeth from 18th and 19th Century burials gives good agreement with known interment dates.[437] A feature of this study, from the point of view of analytical chemistry, is an improved derivatization procedure using D-leucine N-carboxyanhydride, to provide the diastereoisomer mixture from which D:L-aspartic acid ratios are obtained. The amino acids in tooth enamel of a 230,000 y fossil (from the Hexian-Man site, Anhui Province, China) show a quite different profile from that of a modern mammal tooth,[438] implying that caution is required in interpreting amino acid data in any context for very old fossils.

6.2 General Reactions of Amino Acids.- Reactions at the amino and carboxy groups (or at both) are covered in this section; the following section is devoted to papers that deal exclusively with side-chain processing.

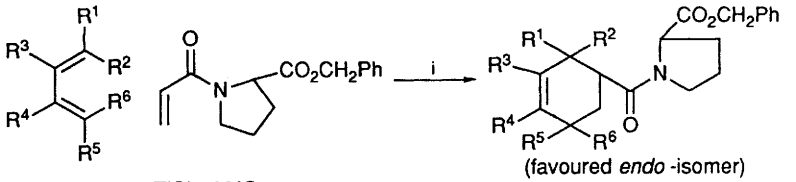
Thermal decomposition of amino acids has featured in this section in earlier years, the interest being in the nature of the pyrolysis products. A current example is the formation of pyrroline-2,5-dione and its 3-methyl and 3,4-dimethyl analogues when aspartic acid is maintained at 220° at 10 mm Hg pressure under nitrogen.[439] Asparagine behaves similarly but seems to undergo degradation at a slower rate. A study by thermogravimetry and differential scanning calorimetry of the

thermal stability of representative amino acids has been completed, with no product information.[440] At a more energetic level, irradiation of alanine with 200 KeV helium and argon ions leads to breakdown into H_2, NH_3, CO_2 and hydrocarbons.[441]

Solubilization of amino acids in organic solvents has been claimed for tetrabutylammonium salts formed by evaporating aqueous solutions of amino acids neutralized with tetrabutylammonium hydroxide.[442] Perhaps this is a good example of a short communication providing too few details, since attempts by some of us to reproduce the results are not successful.

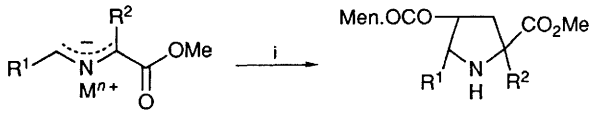
Water-soluble acylating agents p-R.CO.O.C_6H_4.SMe_2^+ $MeSO_4^-$ continue to be advocated (see Vol.21, p.46) for clean N-acylation of amino acids (that is, avoiding the involvement of the carboxy group in mixed anhydride formation, and its consequences).[443] A simple preparation of t-butyl fluoroformate starts from a 1-chloroethyl carbonate - an unusual example of a conversion of an ester into an acid halide.[444] This reagent makes the economics of large-scale preparation of Boc-amino acids more attractive, particularly because of the higher stability of the reagent (it does not react with DMF or DMSO).[445] More familiar acylating agents are used for preparing N$^{\alpha}$-urethanes from L-histidine methyl ester, and this is described in a useful practical account that includes an ion exchange purification procedure for the products.[446]

Other reactions at the amino group include allylation (O-protected tyrosine gives the N,N-diallyl derivative with allyl bromide),[447] and t-butoxycarbonylation [to give N,N-bis(t-butoxycarbonyl)amino acid esters through exhaustive acylation].[448] These bis(Boc)amino acids, converted into active esters, are slow to couple in peptide synthesis and show an enhanced tendency towards hydantoin formation. The NN-bis(diformyl) homologue accompanies N-formylglycine t-butyl ester, when prepared through standard reactions.[449] Stereospecific decarboxylative allylation of N-benzylidene-L-valine methyl ester using allyl bromide catalyzed by $TiCl_4$ with electrolytic cleavage of the valyl chiral residue leads to (S)-2-phenylallylamine (Scheme 36).[450] Stereoselective allylation of aldehydes and ketones can be accomplished through converting the carbonyl compound into its imine with an L- or D-amino acid allyl ester, followed by Pd-catalyzed rearrangement (Scheme 37).[451] There are numerous examples in this year's literature, as in earlier years, of the use of homochiral amino acids in stereoselective synthesis, another example of the type being Lewis acid-catalyzed Diels-Alder reactions (Scheme 38).[452] Reactions under the general heading of N-alkylation include reductive condensation ($NaBH_3CN$) with a ketone, illustrated for "N-menthylation" using menthone,[453] and reaction with malonaldehyde (studied more from the point of view of determining enthalpies of interaction).[454]



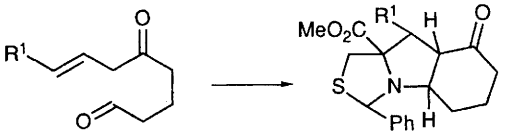
Reagents: i, 1 eq. $TiCl_4$, 20°C

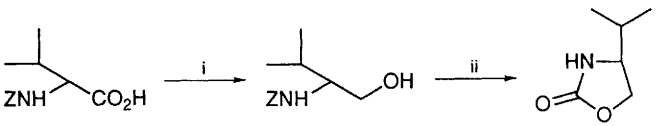
Scheme 38



Reagents: i, menthyl acrylate

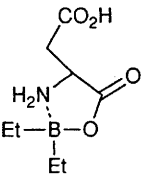
Scheme 39



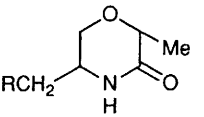


Reagents: i, BH_3-THF; ii, trace Bu^tOK, Δ

Scheme 40



(58)



(59)

A curious pathway is described[455] for the otherwise routine reaction of glycine with 2,4-dinitrochlorobenzene in the presence of $KHCO_3$, followed by nitration to give N-trinitrophenylglycine, purportedly via the N-nitro compound.

Further studies of 1,3-dipolar cycloaddition reactions of amino acid imines continue to reward the research groups responsible for current knowledge of their wide range of synthetic applications. The course of new proline syntheses involving metal ion-catalyzed asymmetric 1,3-dipolar cycloadditions to imines formed with menthyl acrylate, is determined by the metal chosen; Ag(I), Li, and Tl(I) salts direct the reaction to one regioisomer, while Ti(IV) salts give the other (Scheme 39).[456] Similar results and the same conclusion have been described for the cycloaddition of N-titanated azomethine ylides from t-butyl benzylideneamino-acetate to αβ-unsaturated esters, compared with lithium analogues.[457] Very high diastereofacial selectivity is seen in all these processes, with four contiguous chiral centres being generated when αβ-unsaturated esters of optically-active amino acids are used, and after the chiral auxiliary has been removed.[458] Intramolecular cycloaddition of azomethine ylides from 5-oxo-6-heptenals or 4-oxo-5-hexenals and methyl 2-phenylthiazolidine-4-carboxylate illustrates this point, with the formation of (57).[459]

3,4-Dehydroprolines are formed through cycloaddition of arylidene-imines of amino acid esters to alk-2-ynoic esters.[460] Imines formed between 1,8-di-azafluorenone and amino acids proceed along the newly-established ninhydrin pathway via azomethine ylides (Vol.22, p.49) to give a red fluorescent dye, of forensic use for detecting latent fingerprints since it is substantially more sensitive than ninhydrin for this purpose (and for the purposes of mainstream amino acid analysis).[461] Fluorogenic labelling of amino acids at their NH_2 groups can be efficiently accomplished using N-chloroformyl carbazole.[462] An unusual reaction at nitrogen is "borylation"; $R'B{=}NBu^t + NH_2.CHR^2.CO_2R^3 \rightarrow Bu^tNH.BR'.NH.CHR^2.CO_2R^3$.[463]

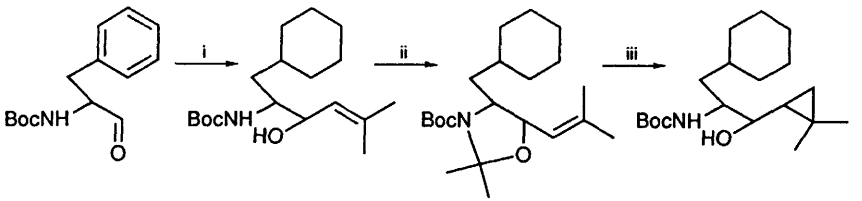
The flexible use of standard N-protecting groups has been extended with new reagents; $ZnBr_2$ in CH_2Cl_2 offers a useful mild means for selective Boc removal from secondary amines,[464] though it is difficult to imagine that familiar methods in use for 60 years for benzyloxycarbonyl group cleavage will be abandoned in favour of a 10 - 36 hour procedure using a 10-fold excess of $BF_3.OEt_2/EtSH$![465]

Oxidation studies of α-amino acids and their derivatives are as voluminous as ever, and while some routine work deserves to be mentioned, what is here is representative of a much larger body of effort. A study of electrogenerated manganese(III) sulphate for oxidation of L-histidine in aqueous H_2SO_4[466] and of alkaline $K_3[Fe(CN)_6]$ oxidation kinetics for lysine, arginine, and histidine have been

described.[467] There are common features in studies of oxidative decarboxylation of amino acids by N-chlorosuccinimide in aqueous alkali,[468] by N-chlorobenzamide in aqueous perchloric acid, catalyzed by Cl^-,[469] and the kinetics of the decomposition of N-chloroamino acids in aqueous solutions (pH 6 - 13).[470] There is considerable preparative value to be had from oxidative decarboxylation of amino acids, shown in a preparation of α-amino phosphonic acids through $Pb(OAc)_4$ treatment followed by reaction with $(MeO)_3P/TiCl_4$; N-acylamino acids give roughly 50:50-mixtures of 1-acylamino-1-acetoxyalkanes and their hydroxy analogues when treated with $Pb(OAc)_4$ in DMF.[471] This process is related to the increasingly-useful anodic oxidation of L-N-acylamino acids in methanol to give 1-methoxy analogues of the lead tetra-acetate reaction products, de-methoxylated by Et_3SiH/Lewis acid to give the corresponding optically-active amine.[472]

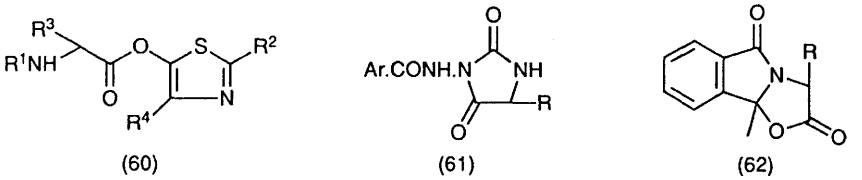
Carboxy-group processing in more explicit forms is seen in racemization-free reduction of esters to alkanols with sodium acetoxyborohydride in dioxan at elevated temperatures,[473] and with di-isobutylaluminium hydride, of a Z-amino acid methyl ester[474] or of an N-benzylidene-amino acid ester,[475] followed by a Grignard reagent to give threo-2-aminoalkanols. This last-mentioned example proceeds via the aldehyde, a class of compound with increasing value in synthesis for which an Organic Syntheses procedure using $LiAlH_4$ for the conversion Boc-L-Leu-NMe.OMe → Boc-L-leucinal *will be found useful.*[476] An *improved* $LiAlH_4$ reduction of phenylalanine to phenylalaninol has been reported.[529] The Evans chiral auxiliary (S)-4-isopropyloxazolid-2-one (cf. Scheme 40) is easily prepared from Z-L-valine through BH_3-THF reduction to the valinol, followed by thermal cyclization using a trace of Bu^tOK.[477] Selective reduction of the α-carboxy group of L-aspartic acid involves first, formation of the boroxazolidinone (58) with BEt_3 in refluxing THF followed by BH_3-THF reduction at 0°, cyclisation to L-homoserine lactone occurring with HCl.[478] This is a convenient route that is also adaptable for ^2H incorporation. B_2H_6 Reduction of N-Boc L-glutamic acid diethyl ester to the glutaminol provides a synthon for chiral lignan lactones [e.g. (-)-ninokinin].[479] Selective protection of the α-carboxy group of aspartic acid via oxazolidinone formation with formaldehyde allows elaboration of the side-chain carboxy group leading to (S)-2,3-di-aminopropanoic acid via the N-Boc-oxazolidinone.[480]

Employment of amino-alkanals in the synthesis of statines and pseudopeptides, among others, has been mentioned earlier in this Chapter, and further illustrations are the use of Boc-L-prolinal in a synthesis of muscarinic agents (starting with -CHO → -CH=CBr_2),[481] a use of Boc-L-phenylalaninal leading to *conformationally-restricted* transition state analogues (Scheme 41),[482] and stereoselective formation of cyanohydrins, to be converted into homochiral 3-amino-2-



Reagents: i, $Me_2C{=}CHMgBr$; ii, $MeCH{=}CHOMe$; iii, $N_2CH.CO_2Bu^t$

Scheme 41



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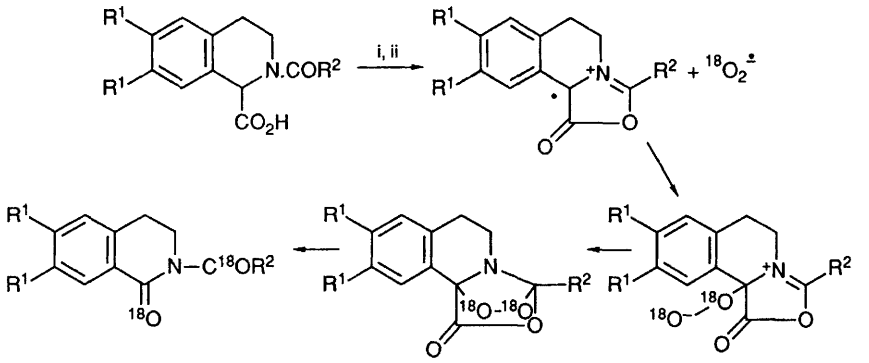
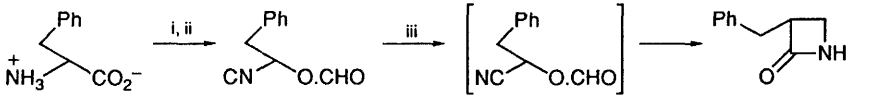


Reagents: i, DCCI; ii, $^{18}O_2$

Scheme 42



Reagents: i, $LiAlH_4$; ii, $POCl_3$, Pr^i_2NH; iii, 585°C, 10^{-4} Torr

Scheme 43

hydroxyesters via the derived imidate hydrochloride.[483] Reduction with $NaBH_4$ in THF or MeOH, of mixed anhydrides formed from N-protected amino acids, is a convenient route to 2-amino alcohols.[484] Conversion of optically-pure morpholinones (59) formed from N-acylated aminoalkanols, into imino-ethers leads on to ring-opening possibilities (giving depsipeptides) and the process emphasizes that mild cleavage of lactams is practicable.[485] Ethyl N-alkenylpyroglutamates have been subjected to a comparative study showing that reduction with $LiBH_4$ gives poor results, with $LiAlH_4$ – silica gel coming out best.[486]

Renewed interest in uses for N-protected α-aminoacyl halides, particularly in peptide synthesis, has led to further exploration in their preparation. Fmoc-Amino acid fluorides are easily prepared using cyanuric fluoride, a procedure that is compatible with the presence of many side-chain functional groups protected, for example, as their Boc or t-butyl derivatives.[487] Pd-Catalyzed coupling of an N-protected L-prolyl chloride with vinylstannanes provides the corresponding N-protected α-amino αβ-unsaturated ketones.[488] Other types of acylating agents reported in the year under review include triazolides formed between an Fmoc-amino acid and 2,4,6-mesitylenesulphonyl-3-nitro-1,2,4-triazolide (used for coupling the first residue on to a polymer hydroxymethyl group in solid-phase peptide synthesis),[489] imidazolides,[490] and N-acylthiazolidine-2-thiones.[491] Unstable mixed anhydrides formed from N-protected amino acids and isopropenyl chlorocarbonate are effective esterification agents towards alcohols if 4-dimethylaminopyridine is employed as catalyst;[492] and because of this, racemization must be accepted as a side-reaction.

Esters of N-protected amino acids fall into two categories for the purpose of this review; either as acylating agents ("active esters"), or as substrates for mechanistic studies concerning ester hydrolysis or transesterification. In the former category are Fmoc amino acid pentafluorophenyl esters, conveniently prepared using pentafluorophenyl trifluoroacetate,[493] and N-[α-(N'-benzyloxycarbonylaminoacyl)]-N-arylhydroxylamines, for which an N- → O-acyl transfer has been studied as a model for the transformation in vivo of arylamines into "ultimate carcinogens".[494] β-Cyanoethyl esters are little-used as active esters but possibilities are offered for transformations of aspartate derivatives by the sequence Boc.Asp.OCH_2CH_2CN → Boc.Asp(OR).OCH_2CH_2CN → Boc.Asp(OR).OH using piperidine in MeCN for the ester cleavage.[495] Thiolacids are, in their way, activated forms of carboxylic acids, and Z.Ala.SH is a substrate for papain for peptide synthesis (though poor yields are secured with isoleucine and with β-t-butyl aspartate derivatives).[496]

A new class of active esters has been studied as models for the putative oxazolone self-acylation product (60; O in place of ring S, R^1

= R^2, R^3 = R^4), that constitutes a novel racemization mechanism applicable to the methodology of peptide synthesis.[497] Hydrazinolysis of these thiazol-5-yl esters (60) displaces the prochiral leaving group in optically-active form, the first evidence for synchronous proton-capture from the incoming amine by the leaving group in aminolysis of active esters (several authorities[8,9,499] have written the aminolysis mechanism for certain active esters as an electrocyclic process, without evidence). New vinyl esters $ZNH.CHR^1.CO_2.C(=CH_2)CR^2=CH_2$ are obtained by $RuCl_2(PMe_3)$(p-cymene)-catalyzed addition of a Z-amino acid to the corresponding alkyne.[499] Photo-cleavable 2-nitro-4,5-dimethoxybenzyl esters prepared from the corresponding bromide and a Boc-protected neurotransmitter amino acid are of potential value for release at receptor sites.[500]

Acyl migration giving β-(5-hydroxy-4-pivaloyloxyphenyl)-L-alanine accompanies the hydrolysis of the catechol mono-ester of N-pivaloyl-L-DOPA.[501] The rearrangement product exists as an equilibrium mixture with its 3-pivaloyloxy isomer in solution.

Esters of L-DOPA are formed through α-chymotrypsin-catalyzed transesterification in organic solvents, of other amino acid esters; yields no greater than 50% are obtained using various alcohols as acyl acceptors.[502] Accelerated esterification of amino acids has been reported using lipoglycosylated α-chymotrypsin in polar solvents,[503] and esterases of various sorts catalyze the transesterification of N-benzyloxycarbonyl-L-tyrosine p-nitrophenyl ester with methanol.[504] L-Amino acid - ZnO catalysts bias the methanolysis of DL-amino acid active esters in favour of the D-enantiomer.[505]

Continuing a general theme of growing interest in recent years, and implicit from the preceding paragraph, the rate of chiral micelle-catalyzed hydrolysis of N-dodecanoyl-L-phenylalanine p-nitrophenyl ester is more than 19 times faster than for its enantiomer, when co-aggregates of phosphatidylcholine, Triton X-100, and Z-L-Phe-L-His-L-Leu-OH are present.[506] The topic is full of apparent uncertainties: there are remarkable substituent effects when the isomeric nitrophenyl groups are substituted for the generally-used p-isomer,[507] and rates are dependent upon the ionic strength of the medium.[508] The hydrolysis is inhibited by flavanoids present in the micelles.[509] An identical study, though using the β-cyclodextrin - Z-L-His-OH inclusion complex, demonstrated diminished rates though the hydrolysis was enantioselective.[510]

Cyclization reactions via derivatized amino acids, requiring the involvement of both amino and carboxy groups, are represented in the formation of imidazolin-2,4-diones (61) from L-amino acids and 2-phenyl-1,3,4-oxadiazolin-5-ones in m-cresol at 150°C,[511] and in the formation of novel benzo-fused tricyclic oxazolidinones (62) through

condensation of L-amino acids with o-acetylbenzoic acid.[512] The latter study corrects an earlier mis-assignment of an oxazolone structure to these products.[513] 4-Acylation of oxazolones formed between an N-benzoylamino acid and a fluoralkanoic anhydride (the Dakin-West reaction) has been illustrated further as a means of synthesis of N-α-acylaminoalkyl fluoroalkyl ketones through decarboxylation in oxalic acid.[514] Imino acids yield mesoionic oxazolones that are prone to autoxidation; ^{18}O-labelling studies (Scheme 42) have clarified the course of this reaction.[515]

Thiohydantoins are available from N-acylamino acid vinyl esters (as formed from the acid by reaction with Woodward's Reagent K) by condensation with trimethylsilyl thiocyanate in MeCN.[516] N-Alkoxycarbonyl oxazolidin-2,4-diones (alias N-carboxyanhydrides, NCAs), hitherto considered to be somewhat fragile, are accessible through careful operation of a previously-established procedure.[517] An illustrative procedure showing the usefulness of Fmoc-L-leucine-NCA in solid-phase peptide synthesis amounts to a trouble-free derivatization of the Rink resin.

Maillard reactions (condensation of an amino acid with a carbohydrate) involve a more complex pathway than any other amino acid reaction - or more correctly, more complex families of pathways, since the reactions lead to a variety of products. Part of the problem of studying this system lies in the lability of the initial products, and the glycine - glucose reaction buffered at pH 7 has been studied using a trapping technique. Initially-formed aldehydes or ketones give benzimidazoles with o-phenylenediamine, and a lactic acid ester and two furanolactones were identified through their derivatives.[518] The presence of sulphite is said to inhibit the Maillard reaction, but this is loose talk for stating that the system is diverted along another pathway; thus, glycine and glucose give 3,4-dideoxyhexosulose-4-sulphinic acid instead of the normal 3-deoxyhexosulose.[519] S-Alkyl-L-cysteines react with D-glucose to give alkylpyrazines - a common class of Maillard product - and 2,4-bis(propylthio)butanal and an unprecedented 2,4-bis(propylthio)but-2-enal.[520] Of course, numerous other compounds accompany these, and (given the fact that many of the research groups working on this reaction are based in food research) some of these are described as "useful flavour compounds". The tryptophan - glucose system would be expected to involve further complexities, and breakdown of the Amadori rearrangement product that appears early in the pathway (leading to hydroxymethylfurfural, maltol, tryptophan, indole, norharman, and harman) has been subjected to kinetics study at 110° and at 140°.[521] H.p.l.c. study of this Amadori rearrangement product itself shows that various tautomeric carbohydrate moieties are involved (α- and β-furanoses and -pyranoses, as well as

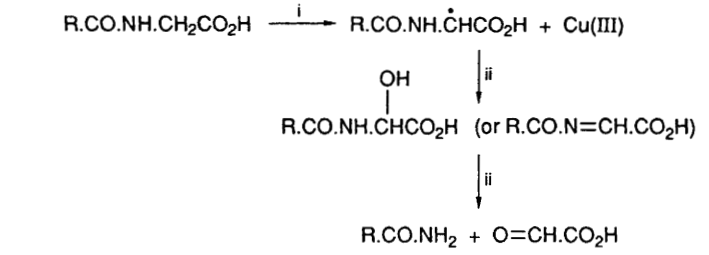
open-chain isomers including ketoses).[522] H.p.l.c. has been brought to bear on the preparative-scale isolation of the major browning compound from the lysine - glucose reaction,[523] and at the opposite end of the scale, capillary zone electrophoresis profiles have been reported for the Maillard reaction products of ribose with glycine, alanine and leucine.[524]

β-Lactams provide the target for much of the research involving β-amino acids, and conversely, their availability through cycloaddition processes provides a useful means for the synthesis of this class of amino acid. The latter aspect has been covered in the earlier section 4.16, and methods for the cyclization of β-amino acids continue to be developed, with ever more unusual reagents ethyl dichlorophosphate and phenylphosphonic dichloride,[525] 1-(methanesulphonyloxy)-6-trifluoromethylbenzotriazole,[526] and diethyl 2-(3-oxo-2,3-dihydro-1,2-benzoisosulphonazoyl) phosphonate.[527]

N-3-(Haloacyl)-α-and β-amino acids $ClCH_2.CRMe.CO-X-OH$ (X = Gly, Val, Trp, or β-alanine) can be cyclized in aqueous NaOH in an unusually facile reaction leading to 3-methyl 3-substituted β-lactams.[528] α-Amino acids provide a chiral source for enantiomerically-pure β-lactams through application of the isonitrile - nitrile rearrangement (Scheme 43); if the intermediates can survive the drastic conditions required (585°C, 10^{-4} Torr), the flash pyrolysis can be performed on 20g batches![529]

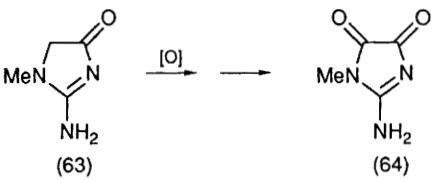
6.3 Specific Reactions of Amino Acids.- The perennial problem (faced particularly in this Section), of grouping material in one part of this Chapter, that could be equally well placed in some other part (or parts), is not solved easily if repetition is to be avoided. Thus, reactions that modify amino acid side-chains amount to the synthesis of one amino acid from another, and could have been described in an earlier "Synthesis" section. However, such work is covered here if it is of a self-contained nature, but reactions that have developed into general synthesis methods are mentioned in the earlier Section 4.1.

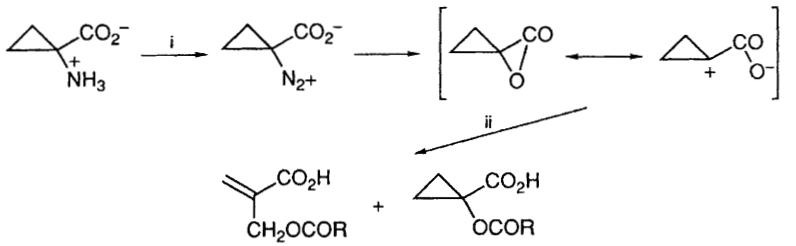
Interesting developments in mild oxidation of acylamino acids as models for the processing that occurs at the C-terminus of a peptide so as to give the amide, have been described for copper(II)-mediated oxidation of N-acylglycines (Scheme 44).[530] The work supports a non-enzymatic oxidative mechanism for peptide amidation that was advocated some time ago.[531] Oxidative processing of the glycine derivative, creatinine (63), to give the ring-opened product $MeNH.C(=NH)NH.CO.CO_2H$ has been re-investigated to assign the correct structure (64) to the re-cyclized intermediate, rather than the isomeric imidazolidinedione structure previously allocated.[532]



Reagents: i, Cu(II) $\xrightarrow{e^-}$ $\xrightarrow{O_2}$ $\xrightarrow{e^-}$ Cu(II)OOH/(IV)=O; ii, H_2O

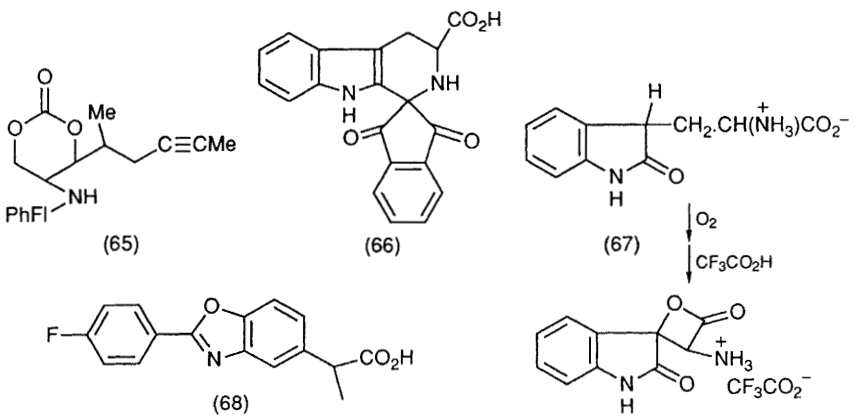
Scheme 44





Reagents: i, $NaNO_2$, RCO_2H, H_2O; ii, RCO_2H

Scheme 45



N-Bromosuccinimide treatment of methyl esters of N-phthaloyl amino acids (leucine, valine, and phenylalanine) followed by $AgNO_3$ in aqueous acetone gives the corresponding β-hydroxy-α-amino acid derivatives with complete diastereoselectivity.[533] Clearly, water is permitted to attack only the less-hindered face of the intermediate carbocation in this process. The same reagent, with protected αβ-dehydroamino acids, gives β-bromo-α-imino acids [$R^1R^2CBr.C(=NCO_2R^3)CO_2R^4$] that are useful in further reactions (if R^1 or R^2 = H), that place a β-heterocyclic structure on the side-chain.[534] There may be a common mechanistic theme underlying this study, and the ability of N-acyldehydroalanines to scavenge superoxide and hydroxyl radicals that leads to their promotion as X-irradation protection agents.[535]

Ring-opening of 1-aminocyclopropanecarboxylic acid that follows diazotization, leads to products of attack by the carboxylic acid used with $NaNO_2$ to provide nitrous acid (Scheme 45).[536] Although the expected product, an α-alkanoyloxymethylacrylic acid, is formed, the retention of configuration in the substitution of $-NH_2$ permits reasonable speculation to be languished on the nature of the intermediate carbocation (a "chimeric zwitterion"?) and gives the first evidence for the existence of the cyclopropyl α-lactone. The ring-closure that occurs through spontaneous hydrolysis of (α-halogenomethyl)-diaminopimelic acid leads to 2-(4-amino-4-carboxybutyl)aziridine, which like other aziridines is a potent irreversible enzyme inhibitor.[537]

Hydroxyalkyl side-chains are represented in cyclization reactions, of L-serine benzyl ester to benzyl (S)-2-aziridinecarboxylate[538] and in the intramolecular Mitsunobu reaction (Ph_3P – diethyl azodicarboxylate) undergone by N-trityl trans-4-hydroxy-L-proline to give the corresponding bicyclic lactone.[539] This is already established as a useful route to the β-lactone from serine, and is used in this study to initiate the route to the cis-hydroxyproline isomer through further routine steps. Boc-D-or -L-serine lactone undergoes ammonolysis to give the corresponding 2,3-diaminopropanoic acids.[540] The α-aminoketone derived from N-phenylfluorenyl-L-serine has been elaborated into the cyclic anhydride (64).[541]

Co-enzyme PQQ, already known to bring about oxidative decarboxylation of acylamino acids to form oxazoles,[542] catalyzes the oxidative fission (de-aldolization) of β-hydroxy-α-amino acids under very mild conditions.[543]

A route from methionine to homoserine is described[544] that conventionally follows sulphonium salt formation with bromoacetic acid and hydrolysis in refluxing aqueous acetic acid. The product is most easily isolated as its lactone, formed using 4M HCl-dioxan. Base-induced ring closure of methylsulphonium salts of N-trityl L-methionine hydroxamide through Me_2S displacement could involve either N or O in the

hydroxyamide moiety as nucleophile. Rather the previously-claimed formation of (S)-4-(N-tritylamino)-1,2-oxazin-3-one (in 3% yield), the product, whose yield can be increased to 34%, is found to be (S)-2-hydroxyimino-3-(N-tritylamino)tetrahydrofuran resulting from nucleophilic attack by carbonyl oxygen.[545]

S-Trimethylacetamido-L-cysteine is easily prepared using N-hydroxymethylpivalamide and trifluoroacetic acid as reagent.[546] Surprisingly, the S-protection is stable to HF but removable by $Hg(OAc)_2$ in TFA or by I_2 in aqueous acetic acid. The high nucleophilicity of the cysteine side-chain function is involved in this reaction, also in a very real analytical problem that explains "losses" of cysteine on polyacrylamide gels through addition to traces of un-polymerized acrylamide.[547] A similar source of loss is through the actions of traces of persulphate (the polymerization initiator) that can both oxidize acrylylamines (used to create a pH gradient) to N-oxides, and cysteine to the sulphonic acid.[548] Cysteine thionitrites continue to be studied (Vol.22, p.59),[549] providing new knowledge of this unusual functional group that may have important physiological functions.

Ammonium persulphate oxidation of L-tyrosine gives only 20% yield of L-DOPA 3-O-sulphate, but this must nevertheless be considered a convenient practical process, considering the difficulties of other standard routes.[550] The fact that photo-oxidation of phenylalanine to o-, m-, and p-tyrosines and DOPA is prevented by radical scavengers and exclusion of oxygen is taken as evidence for the involvement of the hydroxyl radical.[551] Mushroom tyrosinase catalyzed oxidation of α-methylDOPA methyl ester results in iminochrome formation similar to the well-known DOPA - dopachrome conversion. The product is stable at pH 5 but in neutral or slightly alkaline media, it is tautomerized to a quinone methide. These findings strongly support a similar sequence of events as a stage in melanogenesis.[552] Relative iodination rates for tyrosine and di-iodothyronine are roughly 5:1.[553] Diaryl ether analogues of tyrosine have been prepared through aromatic substitution of N-Boc- or N-acetyl-L-methoxytyrosine sodium salt, without racemization, using bis(2-methoxy-5-formylphenyl)iodonium bromide.[554] Similar processing of phenylalanine has been reported, using $ClCH_2OMe/ZnCl_2$.[555]

Peroxomonophosphoric acid brings about the oxidative cleavage of L-tryptophan at pH 0 - 2.5 to give indole-3-acetaldehyde.[556] The ninhydrin reaction, normally an oxidative decarboxylation, gives the condensation product (66) with L-tryptophan, and a kinetics study of this reaction has been reported,[557] also for the corresponding reaction with the DL-amino acid.[558] The cation radical and the neutral radical formed from tryptophan by pulse radiolysis undergo reversible one-electron transfer processes.[559] Indoxylalanine (67) epimerizes at C-3 within 2 - 3 h, and

undergoes C-3 hydroxylation in aqueous NaOH with O_2; easy trifluoroacetic acid cyclization to the oxetanone is notable.[560]

Protection of the arginine side-chain through bis(t-butoxycarbonyl)tetrachlorobenzoylation is reversed through a two step procedure (trifluoroacetic acid, then very dilute acid hydrolysis),[561] a process that should represent a viable competitor for current awkward or expensive protection protocols for the guanidine grouping. Features of the arginine biosynthesis pathway (the urea cycle) have been simulated starting from a protected ornithine, requiring amidation (with nitro-urea) and cyano-ornithine and arginosuccinate synthesis.[562]

Whereas lysine is more nucleophilically reactive in an aqueous buffer relative to cysteine, the order is reversed in a water/oil microemulsion (i.e. a medium of lower polarity).[563]

Rosenmund reduction of acid chlorides of side-chain carboxy groups of Z- or Boc-protected aspartic and glutamic acids after first forming the oxazolidinone from the N-hydroxymethyl compounds is an economical route to the β- and γ-semi-aldehydes.[564] An interesting alternative method for the preparation of glutamic semi-aldehyde employs ozonolysis of a suitably protected 4-vinyl-4-aminobutanoic acid;[565] of no hindrance to the growing use of these aldehydes in synthesis is the fact, shown by n.m.r. data gathered in this study, that hydration of the aldehyde group occurs in solution, and that concentration-dependent dimerization of the hydrate is also prominent.

γ- and δ-Keto-α-amino acids are formed from aspartic and glutamic acids respectively, through the Masamune protocol [$-CO_2H \rightarrow -CO.CH_2.CO_2CH_2CH{=}CH_2 \rightarrow -CO.CR^1R^2.CO_2CH_2CH{=}CH_2 \rightarrow -CO.CHR^1R^2$ with $Pd(PPh_3)_4$].[566] More routine results concerning side-chain fluorenylmethyl esters,[567,568] t-butyl esters (from the amino acids and isobutene, with α-esters as easily-separated side-products,[569] and α-ethyl N-trifluoroacetyl-L-aspartate (formed by hydrogenolysis of the β-benzyl derivative),[570] have been reported. Anodic oxidation of β-enamino-esters derived from pyroglutamic acid (ring $C{=}O \rightarrow C{=}CR^1CO_2R^2$) in methanol gives vinylogous N-acyl-N,O-acetals as a result of replacement of the α-carboxy function by OMe.[571] Melting an alkali metal salt of L-glutamic acid gives L-pyroglutamic acid as an amorphous glass, from which the previously unknown crystalline trihydrate has been obtained through recrystallization from water.[572]

The use of enzymes for selective processing of aspartic acid derivatives has been illustrated in papain-catalyzed hydrolysis, of di-allyl N-benzyloxycarbonyl-L-aspartate to give the β-allyl compound[573] and of an N^{β}-glycosylated Boc-L-asparagine methyl ester to open up the involvement of these sensitive compounds as intermediates for peptide synthesis.[574] Hofmann degradation of asparagine and glutamine with

$PhI(OTFA)_2$ is the basis of efficient syntheses of (S)-Nα-Boc-2,3-di-aminopropanoic acid and -2,4-di-aminopropanoic acid derivatives.[575]

7 Analytical Studies of Amino Acids

7.1 Gas-Liquid Chromatography.- Much of the work is routine application of standard methodology, reported from laboratories that have set up effective systems, especially in conjunction with a mass spectrometry facility.

This is well illustrated by a g.l.c. - m.s. study of $^{12}C:^{13}C$-isotope ratios of amino acids, derivatized as their N-trifluoroacetyl methyl esters.[576] Considerable care is needed to avoid losses in the derivatization of samples, and this paper is excellent reading from this point of view. The same derivatization protocol is applied in analysis of 4-hydroxyproline in collagen samples,[577] and in a thorough study of the homochiral purity of commercial amino acid samples using a Chirasil-Val column.[578] Another common amino acid derivative for g.l.c. analysis is the N-trifluoroacetyl n-butyl ester.[579] An uncommon procedure, used for the analysis of phenylalanine in brain tissue, involves benzoylation and formation of the pentafluorobenzyl ester using dicyclohexylcarbodi-imide and the alcohol.[580]

It goes without saying that this technique is chosen for studies in which sub-nanogram levels of analyte are routinely encountered, and where rapid analysis can also be pointed to as an advantage; both aspects are illustrated in g.l.c. - m.s. of N-heptafluorobutyroylamino acid isobutyl esters.[581] Analysis of phenylalanine, tyrosine and DOPA in a single ventral thoracic nerve cord from the locust (Schistocerca gregaria) established the presence of 194, 347, and 11 ng respectively per sample, through successive conversion of the amino acids into their hexafluoroisopropyl esters and pentafluoropropionylation after azeotroping away with MeCN, the hydrochloric acid used in sample extraction.[582]

G.l.c. is commonly resorted to for the analysis of naturally-derivatized amino acids, such as N-acetylaspartic acid (as its n-butyl ester),[583] and N,N-dimethylglycine (as its ethyl ester).[584]

In view of the crucial importance of clean, quantitative derivatization, it is surprising that one-step processes are little used. However, another look (see Vol.17, p.35) has been taken at 1,3-dichlorotetrafluoroacetone as a derivatization reagent in a g.l.c. - m.s. study of the oxazolidinones formed in this way with glycine, phenylalanine and tyrosine.[585]

7.2 Ion-Exchange Chromatography and Related Techniques.- The classical amino acid analysis protocol is becoming more fully automated (for a review see ref.586) and an auto-hydrolysis - amino acid analysis system has been described.[587] Movement away from the empirical basis of the method is offered in a survey of the theory of strong-acid cation-exchanger equilibria involving amino acids.[588] Free amino acids separated by reversed-phase ion-pair chromatography, have been subjected to post-column derivatization with o-phthaldialdehyde, and estimated fluorimetrically.[589,590]

7.3 Thin-Layer Chromatography.- T.l.c. separation of phosphotyrosine from corresponding serine and threonine phosphates has been described.[591] This contributes useful information on these sensitive derivatives for which mild methods for their release from biologically-important peptides are being sought. It also sets the tone for this section, restricted to less routine studies.

T.l.c. of derivatives of amino acids is covered in reviews of dansyl and dinitrophenylamino acids,[592a] and of the adsorption and partition behaviour of amino acids between a solution and solid in a static relationship compared with the mobile + stationary situation that is the basis of t.l.c. separation.[592b] High-performance t.l.c. quantitative analysis of phenylalanine phenylthiohydantoin has been established with a sensitivity of 0.5 mg L^{-1}.[593]

Chiral t.l.c. has been reviewed (in conjunction with a review of chiral h.p.l.c.).[594] and illustrated for phenylalanine and tyrosine derivatives.[595] Chiral t.l.c., dependent upon chiral solutes in the mobile phase rather than a chiral stationary phase, is particularly effective using the ligand exchange principle, employing copper(II) complexes of diastereoisomeric N-(2-hydroxydodecyl)proline derivatives formed between hydroxy-L-proline and (R,S)-1,2-epoxydodecane are used.[596] N-Benzyloxycarbonyl-L-amino acids are suitable mobile phase components for this approach to t.l.c. resolution of enantiomers.[597]

7.4 High Performance Liquid Chromatography.- A discussion of the relative advantages of pre-column and post-column derivatization[598] is overwhelmingly answered by the sheer volume of work in the former category. If counting papers is a reasonable guide, the o-phthaldialdehyde - thiol protocol for pre-column derivatization has returned to front place, a position it had appeared to lose in the face of competition from N-phenylthiocarbamoyl derivatization.

The typical application of the o-phthaldialdehyde + thiol reagent for amino acid analysis is recorded in papers dealing with tyrosine-O-sulphate,[599] amino acids extracted from dried blood spots by sonication into phosphate-buffered saline,[600] β-amino-isobutyric acid in urine,[601]

N-Bromosuccinimide treatment of methyl esters of N-phthaloyl amino acids (leucine, valine, and phenylalanine) followed by $AgNO_3$ in aqueous acetone gives the corresponding β-hydroxy-α-amino acid derivatives with complete diastereoselectivity.[533] Clearly, water is permitted to attack only the less-hindered face of the intermediate carbocation in this process. The same reagent, with protected αβ-dehydroamino acids, gives β-bromo-α-imino acids [$R^1R^2CBr.C(=NCO_2R^3)CO_2R^4$] that are useful in further reactions (if R^1 or R^2 = H), that place a β-heterocyclic structure on the side-chain.[534] There may be a common mechanistic theme underlying this study, and the ability of N-acyldehydroalanines to scavenge superoxide and hydroxyl radicals that leads to their promotion as X-irradation protection agents.[535]

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including a prototype automated system, and a review of dabsylamino acids advocating them favourably in relation to other methods.[621]

Specific derivatization is called for in some circumstances, such as for N-benzoylarginine ethyl ester converted into its side-chain N^{ω}-(2-pyrimidinyl) derivative,[622] and similar derivatization of DL-α-difluoromethylarginine with 9,10-phenanthrenequinone.[623] Acylcarnitines have been treated with 4'-bromophenacyl trifluoromethanesulphonate prior to h.p.l.c. analysis.[624] Automated assay of tryptophan and its metabolites has been developed.[625]

Developments in alternative detection methods include chemiluminescence generated by dansylamino acids with H_2O_2 and bis(2,4,6-trichlorophenyl)oxalate,[626] and post-column photochemical derivatization of aromatic amino acids and sulphur-containing amino acids followed by amperometric detection.[627,628] Electrochemical detection as an adjunct of h.p.l.c. analysis of amino acids has been reviewed.[629]

Enantiomeric analysis based on diastereoisomer-forming derivatization has been explored, with sarcosyl-L-phenylalanine methyl ester as reagent for N-benzyloxycarbonyl amino acids[630] and the acid chloride of (S)-flunoxaprofen (68) giving fluorescent derivatives.[631] A well-used system, o-phthaldialdehyde with an N-acyl-L-cysteine, was found to work best, as far as resolution was concerned, with N-isobutyroyl-L-cysteine for the estimation of D-isomers of alanine, aspartic acid, and glutamic acid in yoghourt.[632]

7.5 Fluorimetric Analysis.- This section runs naturally on from fluorescence-forming derivatization in h.p.l.c., but covering wider realms of analysis. Established h.p.l.c. derivatization reagents are used more widely in fluorimetry; o-phthaldialdehyde and naphthalene-2,3-dialdehyde have been reviewed for their potential for femtomole level analysis,[633] with o-phthaldialdehyde being involved in a procedure for the analysis of glycine (1-3 mM) in the presence of glutamic acid (25-100 mM),[634] and in a spectrophotometric total free amino acid assay,[635] and through time-resolved fluorescence of o-phthaldialdehyde - mercaptoethanol adducts prepared to estimate total amino acids in sea-water.[636] The same reagent system has been used for resolution of DL-glutamic acid on a cyclodextrin-bonded stationary phase.[637]

The other function of this Section is to feature the initial explorations in the amino acids field reported for new fluorogenic reagents that may enter the establishment, and 8-methoxy-5-quinolinesulphonyl chloride has been proposed, with modest credentials for this treatment since it shows similar characteristics with the dansyl family.[638]

7.6 Other Analytical Methods.- Pre-eminent now, in this category, is high-performance capillary electrophoresis, recently reviewed so as to cover also h.p.c.e. - m.s.[639] Protocols are being used in h.p.c.e. that are familiar from other areas of amino acid analysis, such as diastereoisomeric derivative formation (Marfey's reagent) for the determination of amino acid enantiomer ratios.[640] While amino acids derivatized with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate are not satisfactorily resolved by h.p.c.e., micellar electrokinetic chromatography in the presence of sodium dodecylsulphate gave excellent results.[641]

Preliminary results have been described for adsorptive stripping voltammetry as a technique for quantitative analysis of phenylthiohydantoins.[642]

The displacement chromatography principle is difficult to set up for individual cases but has useful characteristics as demonstrated for Fmoc-S-trityl-L-cysteine.[643]

7.7 Assays for Specific Amino Acids.- The analysis of L-lysine in amino acid mixtures using four different methods has been reported,[644] use of the amino acid analyzer, spectrophotometrically (ninhydrin or furfural), potentiometric/amperometric, with an enzyme electrode. The last-mentioned approach is of course the predominant feature of this section over the years, and continues to be so, with assays reported for N-acetyl-L-glutamic acid (as activator for carbamoyl phosphate synthetase),[645] L-glutamine (rose petal on ammonia gas sensor)[646] L-lysine (NADH formed with L-lysine dehydrogenase),[647] phenylalanine (NADH-dependent phenylalanine dehydrogenase),[648] and tyrosine and the three branched-chain protein amino acids by a fully-automated multienzyme method.[649] This broadened approach has also been applied in another laboratory to the branched chain amino acids.[650] A review has appeared of analytical approaches to carnitine and its esters.[651] The enzymatic approach using carnitine acetyltransferase has been evaluated using either radioassay or spectrophotometry for quantitation.[652]

The functional group in cysteine that is not shared with any other protein amino acid offers scope for its specific spectrophotometric[653] and amperometric assay.[654]

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2
Peptide Synthesis

BY D.T. ELMORE

1. Introduction

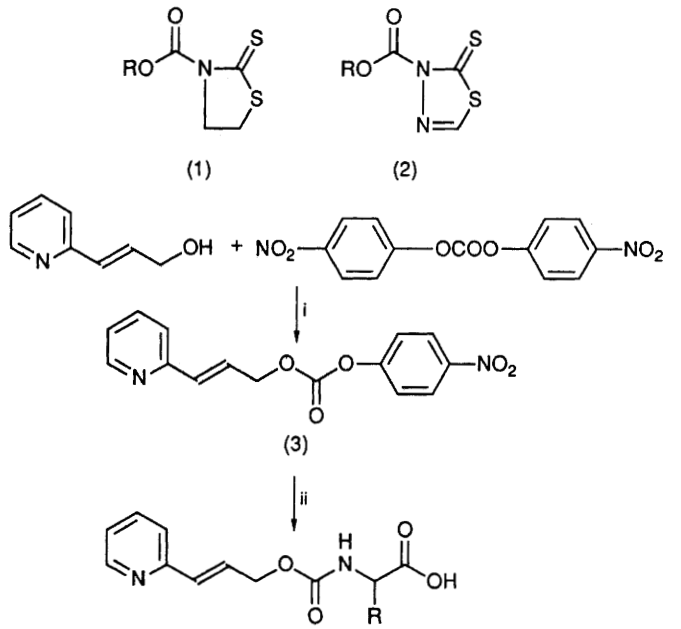
The format of this report is identical to that used last year.[1] Meetings abstracts and patents are not included. Two textbooks, one general in scope,[2] the other more specialized,[3] have been published. As usual, there are plenty of review articles,[4-30] some of which are not readily available to most readers[4-14]. Some reviews cover a wide spectrum while others are more specialized. For those readers who wish to acquire a large number of references to the original literature, attention is directed to reviews of methods of thiol group protection[15], the use of Fmoc-protection in solid phase peptide synthesis[16], enzymic synthesis of peptides[17-19], and the protection of the indole nucleus of Trp during peptide synthesis[20].

2. Methods

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

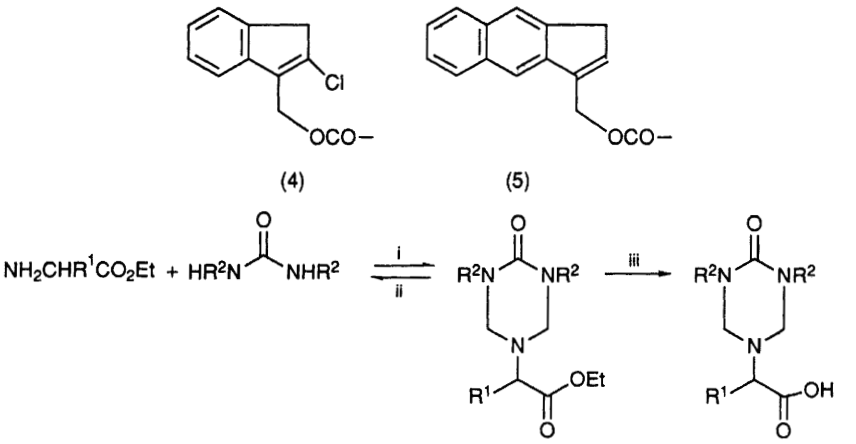
2.1.1 Amino-group protection

Amino-acid esters are frequently isolated as toluene-*p*-sulphonate salts. These can be conveniently converted into the free bases by treatment with tetramethylguanidine in $CHCl_3$; the toluene-*p*-sulphonate of the latter is precipitated by adding ether[31]. A one-pot method of *N*-protection of amino acids involves intermediate silylation[32]. *N*-Alkoxycarbonylthiazolidine-2-thiones (1) and the related compounds (2) have been synthesized and are stable, regioselective reagents for the



Reagents: i, Et_3N in CH_2Cl_2; ii, $NH_3^+CHRCO_2^-$ in dioxan–H_2O at pH 10 (pH–stat) or $NH_3^+CHRCO_2^-$, Me_3SiCl in $CHCl_3$–MeOH (1:1) under reflux, 2h then Pr^i_2EtN followed by (3) under reflux, 12h

Scheme 1



Reagents: i, aq. HCHO, 70 °C; ii, saturated aq. NH_4Cl, 70 °C; iii, LiOH in EtOH, room temperature

Scheme 2

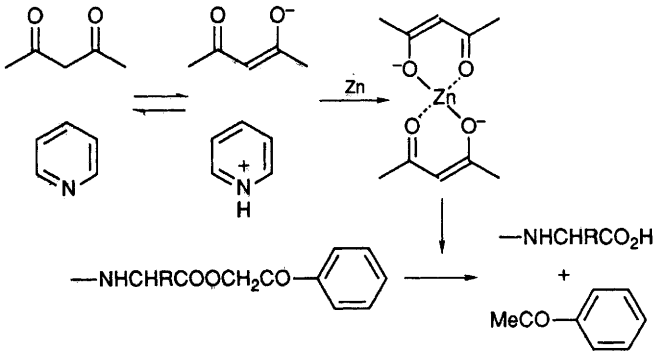
N-protection of amino acids and amino alcohols[33]. Z-Ser-OH (84%) was obtained showing that protection of the hydroxyl group is unnecessary despite the substitution observed with various polyols. A useful one-pot method for preparing Boc-Pro-OCH_2COPh and related compounds has been described[34]. The presence of *cis-trans* isomers can be detected and quantified by the presence of two AB quartets for the phenacyl $-CH_2-$ protons. The N^{α}-Boc derivatives of 2,3-diaminopropionic and 2,4-diaminobutyric acids have been prepared[35]. *NN*-$(Boc)_2$ amino acids are stable crystalline compounds[36] but their low reactivity in peptide couplings is likely to discourage their use. N^{α}-Boc amino acid esters can be converted directly into the corresponding Z-derivatives using benzyl trichloroacetimidate[37], but this does not appear to have much relevance to modern peptide synthesis. 3-(3'-Pyridyl)allyl(4"-nitrophenyl) carbonate (**3**), which is made by transesterification of bis-(4-nitrophenyl) carbonate with 3-(3-pyridyl)allyl alcohol, gives the corresponding *N*-protected (Paloc) amino acid derivative at pH 10 (Scheme 1)[38]. The Paloc group is stable to acids and to the treatment with rhodium(I) compounds that removes Aloc groups. It can be removed, however, by palladium(0)-catalysed transfer to weakly basic or neutral electrophiles. This could be a useful orthogonal protecting group and a detailed examination of the extent of racemization that occurs in coupling reactions of Paloc-amino acids and -peptides would be valuable. A systematic study of the ease of removal of Fmoc groups by hydrogenolysis has been carried out with different catalysts[39]. Two new related protecting groups, 2-chloro-3-indenylmethyloxycarbonyl and benz[*f*]inden-3-ylmethyloxycarbonyl (**4**,**5**), have been described[40]. The chloroformates and azidoformates are suitable reagents. Both groups are more sensitive than Fmoc to base. Diethyl phosphite has been used as a reagent to protect α-amino groups[41]; although deprotection is achieved under mildly acidic conditions, it is unlikely that the popularity of the Boc-group will be endangered. A much more innovative approach to the protection and deprotection of α-amino groups is the report[42] that monoclonal antibodies induced by

immunizing mice with the positively-charged tris(4-methoxyphenyl)phosphonium hapten possess catalytic activity for the removal of trityl groups at neutral pH. The pH-rate profile of the reaction suggests that the observed rate acceleration is not the result of general acid catalysis in the antibody binding site, but probably derives from electrostatic stabilization of a positively-charged transition state. The 3-nitro-2-pyridinesulphinyl protecting group has been used to block α- and ε-amino groups as well as thiol and hydroxyl groups in side-chains. Deprotection by thiolysis can be conveniently effected using 2-mercaptopyridine or 2-mercaptomethylimidazole for *O*- and *N*-Nps groups; $HSCH_2CO_2H$ and $HSCH_2CH_2OH$ can be used to remove *S*-Nps groups[43]. The suggestion that the Nps group should be used as a general protecting group is unlikely to be taken up because orthogonality is crucially important in peptide synthesis. The wider use of the Nps group for selective protection is a possibility, however, especially since deprotection can be monitored spectrophotometrically with the heterocyclic thiols. The use of 18-crown-6 as a noncovalent protecting ligand for the $-NH_3^+$ group of an amino acid or peptide has been investigated. With DCCI in $CHCl_3$ or MeCN, an amino acid gave the corresponding homopolymer[44]. Some simple couplings were satisfactorily accomplished in $CHONMe_2$ but the possibility of racemization during coupling was not studied. Nevertheless, the possibility of dispensing with an *N*-protecting group remains an attractive goal. A totally different strategy is involved in the protection of amino groups as triazinones[45]. Both protons on the primary amino group are replaced (Scheme 2). Unfortunately, the preparation of protected amino acids involves two steps. This new protecting group is stable to basic, nucleophilic, oxidative, reducing and alkylating conditions. Nevertheless, the reaction is readily reversed by hot NH_4Cl solution.

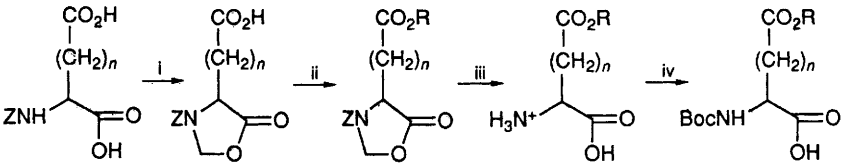
2.1.2 Carboxyl-group protection

Alkyl esters were the earliest type of protecting group for the *C*-terminal amino acid during peptide synthesis. Despite the

well known racemization risks during deprotection by alkali, new information continues to accumulate. The kinetics of alkaline hydrolysis of the methyl and ethyl esters of Z-dipeptides have been determined[46]. As expected, the sensitivity to hydrolysis depends heavily on the nature of the *C*-terminal residue. The *N*-terminal residue also influences the stability; for example, Phe exerts an accelerating effect. If a more hydrophobic ester group such as C_7H_{15} is used, the ester group can be removed by several lipases especially that from *Rhizopus niveus*[47]. Racemization is avoided and the n-heptyl esters can be prepared conveniently for this route by azeotropic esterification. 4-Chlorobutyl esters have also been proposed for peptide synthesis since their deprotection can be effected with sulphide anion in aqueous MeCN at room temperature with the formation of tetrahydrothiophene[48]. A one-pot synthesis of Fmoc-Asp(OBu^t)-OH and Fmoc-Glu(OBu^t)-OH involves reaction of the amino acid with isobutene and toluene-4-sulphonic acid followed by reaction with *N*-Fmoc-succinimide[49]. Phenacyl esters, which have occasionally been used in peptide synthesis and are usually deprotected by Zn/CH_3CO_2H, are efficiently cleaved by Zn reduction (Scheme 3) using a chelating agent such as pentan-2,4-dione in presence of pyridine[50]. Free carboxyl groups in amino acids or at the *C*-terminus of peptides to be coupled to an *N*-protected amino acid can be protected by phase-transfer reagents[51]. The substrate containing the free carboxyl group that requires protection is treated with one equivalent of a phase-transfer reagent such as tetrabutylammonium hydroxide in aqueous solution which is then freeze-dried. The *N*-protected amino acid is allowed to react with DCCI in presence of HOBt at room temperature. The product from the interaction of the *C*-terminal moiety and phase-transfer reagent is then added in $CHCl_3$. Good yields of *N*-protected di- and tri-peptides were reported. It is possible to use pentafluorophenyl esters as a temporary protecting group while attaching a carbohydrate moiety to the side chain of Asp or Ser and then to use the reactive ester to couple to a peptide to give *N*-linked Asn glycopeptides[52,53].

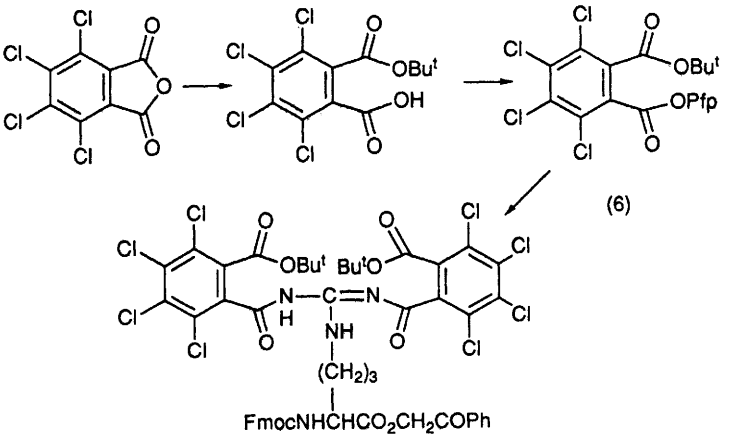


Scheme 3



Reagents: i, *s*-Trioxane, tosic acid; ii, 9-hydroxymethylfluorene, DCCl, 4-dimethylaminopyridine; iii, CF_3CO_2H, HBr; iv, $(Boc)_2O$, Et_3N

Scheme 4



Scheme 5

2.1.3 Side-chain protection

Convenient syntheses of fluorenylmethyl-based protected derivatives of Glu, Asp, Lys and Cys using conventional chemistry have been reported[54]. Alternative syntheses of the fluoromethyl esters of Boc-Asp-OH and Boc-Glu-OH have been described[55]; the α-CO_2H group was protected by forming the 5-oxo-4-oxazolidone derivatives (Scheme 4). Selective conversion of the side-chains of Asp and Glu into allyl esters has been achieved by reaction with CH_2:$CHCH_2OH$ in presence of Me_3SiCl[56]. ß-Esterification of Asp has been accomplished by first protecting the α-CO_2H as the 2-cyanoethyl ester then esterifying the ß-CO_2H group and finally removing the 2-cyanoethyl ester group with piperidine or 1,8-diazabicyclo-[5.4.0]-undec-7-ene[57]. Disymmetrical diesters of Boc-Glu can be prepared from Boc-pyroglutamate esters by alcoholysis in the presence of KCN as catalyst[58].

The synthesis of peptides containing -Tyr(OSO_3H)- residues as well as Ser and/or Thr residues requires the use of orthogonal protection since Ser and Thr hydroxy groups are converted into sulphate esters in preference to Tyr hydroxy groups[59]. Ser and Thr side chains are protected as 4-(methylsulphinyl)benzyl (Msib) derivatives because these derivatives are stable to CF_3CO_2H. The Tyr hydroxyl group is protected as the Bu^t ether. If a peptide is assembled by solid-phase methodology, the peptide can be removed from the matrix and all protecting groups except Msib can be removed by cleavage with acid. The sulphate ester can be introduced using the $CHONMe_2$-SO_3 complex in the presence of $HSCH_2CH_2SH$ to reduce the Msib groups to 4-methylthiobenzyl (Mtb) which is labile to CF_3CO_2H. This basic strategy was used to synthesize the *C*-terminal dodecapeptide of CCK. Hydroxyl groups can be protected by either the 4,4'-dimethoxytrityl or the 4-mono-methoxytrityl groups[60] using the corresponding fluoroborates in an aprotic solvent such MeCN in the presence of 2,6-di-t-butyl-4-methylpyridine. Deprotection is effected by mild acid treatment. Although this work was clearly aimed at the carbohydrate field and therefore should be applicable to the

synthesis of glycopeptides, it may also be relevant to the protection of the side chains of Ser, Thr and Tyr.

A problem has been reported when the N^{im}-benzyloxymethyl (Bom) group is used to protect the imidazole group of histidine and HF is used for deprotection[61]. The concomitant generation of HCHO produces methylated byproducts and these were not produced of course when the imidazole ring was protected by a DNP-group. The latter group is less effective than the Bom group at curtailing racemization because it is situated on the wrong nitrogen atom. Consequently, the Reporter does not find this observation a compelling reason for abandoning the use of the Bom group but rather indicating the desirability for using less vigorous conditions than exposure to HF for the general deprotection of synthetic peptide derivatives. The use of the Bom group followed by deprotection with HF can also lead to the cyclization of N-terminal cysteinyl residues to a thiazolidine derivative[61,62] as a result of the liberation of HCHO.

Readers of the previous Report[1] might have inferred that the development of the Pcm group for the protection of the guanidino function of Arg during peptide synthesis had said the last word on this subject. Already, a rival group incorporating a molecular safety catch has been described[63]. Tetrachlorophthalic anhydride was converted through its mono-t-butyl ester into the reactive ester (6)(Scheme 5). This afforded N^G,N^G-bis(2-t-butoxy-carbonyl-3,4,5,6-tetrachlorobenzoyl)(Btb) derivatives of Arg (7) which show early promise in peptide synthesis. The Btb-group is removed by a two-stage process. Once the t-butyl ester group is removed by CF_3CO_2H, anchimeric assistance by the liberated carboxyl group cleaves the amide bonds and frees the guanidino function of Arg. The synthesis of the octapeptide H-Lys-Asp-Tyr-Ala-Leu-Arg-Phe-Gly-OH in 25% overall yield is very promising and suggests that a more extensive survey is required.

Several papers have been concerned with the protection of thiol groups during peptide synthesis. Both the *S*-acetamidomethyl (Acm) and *S*-Fmoc groups have been satisfactorily used in

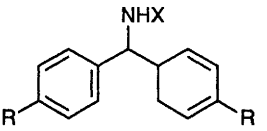
the synthesis of peptides of penicillamine[64]. The *S*-trimethylacetamidomethyl (Tacm) group is satisfactorily removed with $AgBF_4$ as convincingly demonstrated by the synthesis of porcine brain natriuretic peptide-32 and somatostatin[65,66]. H-Cys(Tacm)-OH is easily prepared from *N*-(hydroxymethyl)trimethylacetamide and cysteine in CF_3CO_2H[67]. The Tacm group can also be removed by treatment with either $Hg(II)(OAc)_2$ in CF_3CO_2H or iodine in acetic acid, but is stable to HF. For those who like to use a coloured label, reaction of ferrocenylmethanol with cysteine gives yellow *S*-(ferrocenylmethyl)cysteine. This protecting group can be removed after peptide synthesis by treatment with CF_3CO_2H and C_6H_5SH[68].

Protection of the thioether function of Met as the sulphoxide during peptide synthesis is now standard practice. Reduction back to Met after completion of peptide assembly can be effected by reduction with a mixture of the $CHONMe_2$-SO_3 complex and either a thiol (e.g.$HSCH_2CH_2SH$) or iodide anion[69,70].

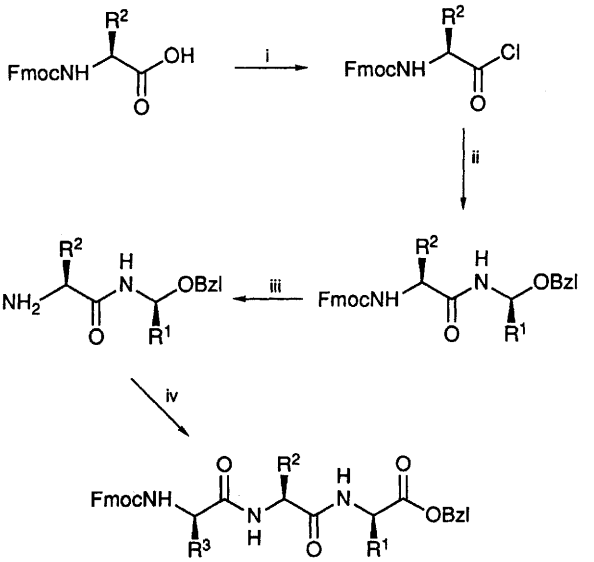
A side-reaction has been reported during the synthesis of secretin in which the amide function of a Gln residue was converted into the methyl ester[71]. Although protection of amide groups is not usual in peptide synthesis, there are clearly some occasions when this is desirable. Di-1-adamantyl di- and tricarbonates in the presence of 4-dimethylaminopyridine can be used to introduce Adoc groups on to amide nitrogen atoms[72]. A safety-catch type of protecting group (8) for synthesizing *C*-terminal peptide amides has been described, recalling a technique commonly used in solid-phase peptide synthesis[73]. Either Fmoc or Boc chemistry may be used and the amide protecting group is removed by one of several acidolytic reagents.

2.2 General deprotection

A further report has appeared on the use of tetrafluoroboric acid in CF_3CO_2H in the presence of thioanisole for removing several protecting groups such as Bu^t ethers and esters, N^{im}-Bom, *S*-MeBzl, Mbh, Mtr and of detaching peptides from the type of linkers used in Fmoc-based solid-phase peptide synthesis[74].

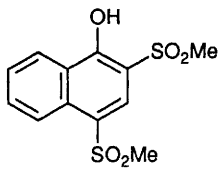


(8) R = MeS, MeSO



Reagents: i, $SOCl_2$, CH_2Cl_2; ii, $NH_2CHR^1COOBzl$, 5% $NaHCO_3$, $CHCl_3$;
iii, 4–(aminomethyl) pyridine, $CHCl_3$;
iv, $FmocNHCHR^3COCl$, 5% $NaHCO_3$, $CHCl_3$

Scheme 6



(9)

Human glucagon was synthesized on Wang resin using Fmoc chemistry in 33% yield based on the *C*-terminal residue and α-MSH was synthesized on MBHA resin in similar yield. An alternative mild acidolytic deprotection system for Fmoc-based solid-phase peptide synthesis comprises either 0.1 M-CH_3SO_3H or 0.1 M $CF_3SO_3SiMe_3$ in CF_3CO_2H and pentamethylbenzene[75]. The latter simultaneously accelerates cleavage and acts as an irreversible scavenger. Suitable conditions for the acidolytic deprotection of peptides containing Asp(OChx) residues have been worked out[76].

2.3 Peptide bond formation

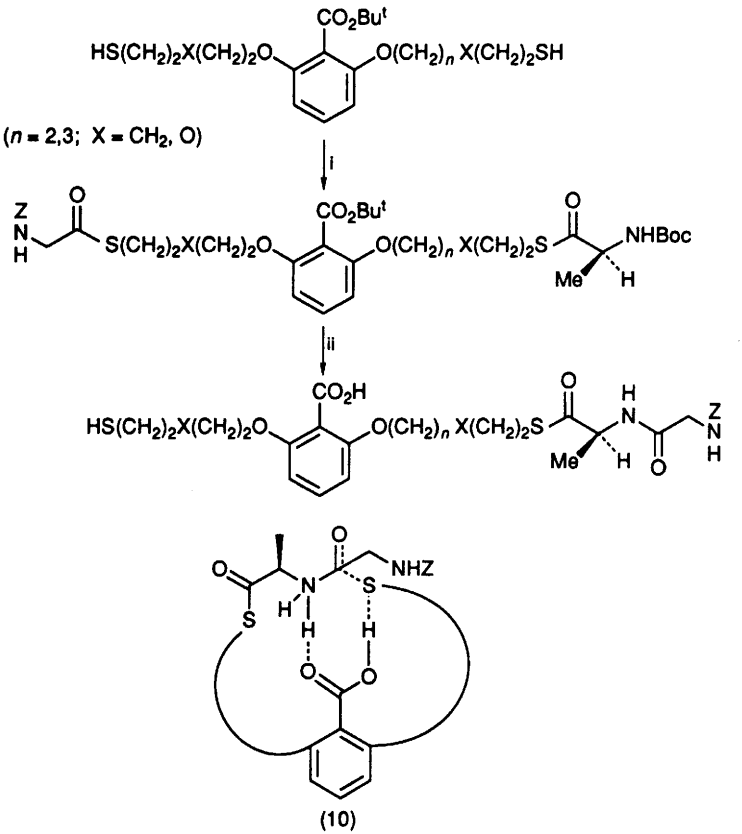
A new method for accomplishing repetitive couplings in solution uses the chlorides of Fmoc amino acids[77]. An excess of the latter can be employed and unused reagent is allowed to acylate 4-aminomethylpyridine. Sufficient of the latter is used to remove the Fmoc group ready for the next cycle (Scheme 6). Occasionally the workup is complicated by formation of emulsions or a voluminous precipitate. This kind of difficulty is avoided if 4-aminomethylpyridine is replaced by tris(2-aminoethyl)amine[78]. The chlorides of amino acid derivatives with a protecting group containing a Bu^t moiety in the side chain were too unstable to be used. Attempts to prepare Fmoc-Asp(OBu^t)-Cl, for example, gave only the cyclic anhydride presumably due to the elimination of Bu^tCl. If it is desired to use intermediates with side chains containing a Bu^t group, the pentafluorophenyl ester can be used instead of the acid chloride although the acylation step is slower. More recently, Carpino has shown that the acid fluorides of Fmoc amino acids are considerably more stable than the chlorides[79]. Reaction of Fmoc-Asp(OBu^t)-OH with excess cyanuric fluoride in presence of one molar equivalent of pyridine in CH_2Cl_2 under reflux affords the acyl fluoride (68%). Most yields were rather better than this.

Pentafluorophenyl esters of Fmoc amino acids have been obtained in high yield and purity by transesterification with pentafluorophenyl trifluoroacetate[80]. A useful collection of kinetic data has been made for the reaction of several types of reactive esters of Boc-Ala-OH, Boc-Phe-OH and Boc-Cys(Bzl)-OH

with H-Val-OMe in tetrahydrofuran at 23° C[81]. 2,4-Bis(methylsulphonyl)-1-naphthol (9) was synthesized by treating 1-naphthol with KSCN and Br_2 to give 2,4-bisthiocyano-1-naphthol; this was treated with Na_2S and MeI and then oxidized with H_2O_2 to give the desired compound. Esters of *N*-protected amino acids and (9) were used in peptide coupling reactions[82]. Two groups have examined the effect of high pressure on the reaction between an *N*-protected amino acid or peptide methyl or phenyl ester and an amino acid ester[83,84]. Under suitable conditions, quite satisfactory yields can be obtained but an unacceptable degree of racemization may occur depending on the solvent.

An interesting method of peptide bond formation has been designed to simulate an enzyme-controlled process[85,86]. The introduction of an intramolecularly catalysed aminolysis of a thiol ester certainly leads to a substantial increase in velocity. A typical process is summarized in Scheme 7. A possible transition state for intramolecular peptide bond formation is represented by structure (10). The authors report that bifunctional catalysis is inhibited by polar solvents presumably because intramolecular hydrogen bonding is disrupted. Et_3N is the most effective basic catalyst found and the amount used (1-2 molar equivalents) is important. The presence of ether links in the side chains marginally improves the rate constant. One would like to have more information about the optical purity of the peptide products particularly if it is envisaged that the method will be used to couple two peptides.

As part of a study to use crown compounds as noncovalent protecting groups in peptide synthesis[44,87], the reactivity of 18-crown-6 ether-dipeptide complexes with DCCI in dimethyl sulphoxide was explored. When the concentration of reactant was ≈ 0.02 M, DCCI did not activate the dipeptide and the *N*-acyl urea was slowly formed. When the reactant concentration was ≈ 0.2 M, the complexes were unstable and reacted with solvent. Although the formation of *N*-acylureas in peptide syntheses mediated by carbodiimides is an unwanted phenomenon, it is desirable to understand the mechanisms of all possible reactions. It has been



(10)

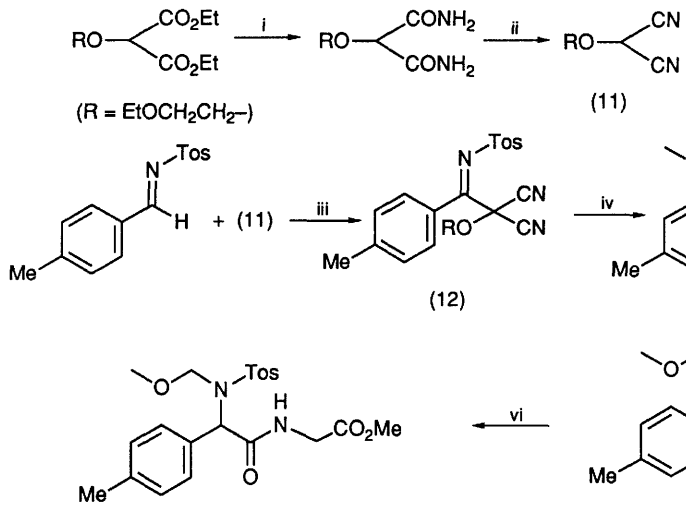
Reagents: i, Z–Gly–OH, DCCI, 4–pyrrolidinopyridine, CH_2Cl_2 then diphenylphosphoryl azide, Boc–Ala–OH, Et_3N, $CHONMe_2$; ii, CF_3CO_2H, CH_2Cl_2 at O °C then Et_3N in C_6H_6

Scheme 7

shown that if an unsymmetrical *NN'*-dialkylcarbodiimide is allowed to form an *N*-acylurea through the intermediate *O*-acyl-isourea, the acyl group becomes attached to the least hindered nitrogen atom[88]. Thus, EtN=C=NBu^t and Z-Val-OH give ZNHCHPr^{i}CONEtCONHBu^t. Any peptides that are formed in a reaction in which *N*-acylureas are a significant byproduct tend to be extensively racemized yet the *N*-acylurea is chirally pure[89]. When two amino acids and a carbodiimide are allowed to react in aqueous solution, the composition of the product is not stochastical[90]. For example, when glutamic acid and leucine were treated with *N*-cyclohexyl-*N'*-(3-dimethylaminopropyl)carbodiimide and unreacted amino groups and carboxyl groups were protected by benzoylation and esterification respectively, the major product (72%) was Bz-Glu(OMe)-Leu-OMe.

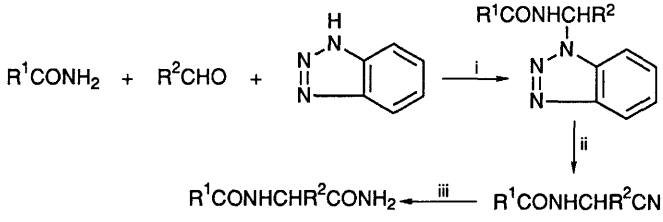
A new acyl anion equivalent, a protected hydroxymalononitrile (11), has been developed as a masked reactive ester[91] (Scheme 8). One of several elegant features of the scheme is the possibility of inserting the amino-acid side chain as part of the peptide synthesis (11 ⟶ 12). Removal of the *O*-ethoxyethyl protecting group unmasks the activated intermediate and coupling with an amino acid ester can proceed. In order to be generally accepted, R must be readily removable and if (12) is a pure stereoisomer, the final coupling step must take place with negligible racemization and in high yield.

The use of dialkyl phosphites as precursors of dialkylphosphorochloridate by reaction with CCl_4 and Et_3N and the generation of unsymmetrical anhydrides with *N*-protected amino acids for peptide synthesis[92] evokes memories of similar techniques that enjoyed a brief period of popularity 40 years ago. The present work uses *N*-(diisopropyloxyphosphoryl)amino acids and this provided an interesting procedure for detecting racemization since *N*-protected diastereomeric peptides gave two P^{31} signals in the nmr spectrum. In the rigorous Young test, conventional methods revealed that 5-12% racemization occurred during coupling by the dialkyl phosphite route. Another novel coupling method starts with a Mannich-type condensation involving



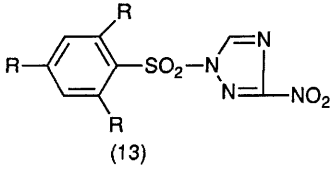
Reagents: i, NH_3/MeOH; ii, $Et_3N^+SO_2N^-CO_2Me$; iii, Et_3N (catalytic amount); iv, $ClCH_2OMe$, Pr^i_2NEt; v, Amberlyst 15, MeOH; vi, H–Gly–OMe, Et_3N

Scheme 8



Reagents: i, H^+/PhMe (Dean–Stark apparatus); ii, CN^-; iii, aq. K_2CO_3/H_2O_2

Scheme 9



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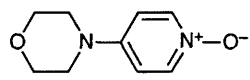

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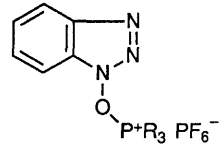


$R_3P^+Br\ PF_6^-$

(16) R = Me_2N—

(15) R = —N

a primary amide, an aldehyde and benzotriazole (Scheme 9)[93]. The direction of assembly of a peptide proceeds unusually from the *N*-terminus to the *C*-terminus but unfortunately stereoselectivity is poor. The authors aim to improve this and if successful, this method might become useful for fragment coupling.

A 1-(arenesulphonyl)-3-nitro-1,2,4-triazole (13; R=Me, Pr^i) in conjunction with *N*-methylimidazole or *N*-(1-oxopyridyl)-morpholine (14) is used in oligonucleotide synthesis. The same reagents have been used in solid-phase peptide synthesis with Fmoc chemistry[94]. The precise mechanism for the coupling has not been elucidated, but a simple tetrapeptide was obtained in 70% yield. Racemization was <0.1% in a typical case.

N-Carboxyanhydrides (oxazolid-2,5-diones) have occasionally been used in peptide synthesis, but their instability and tendency to lead to multiple couplings deterred all but a few peptide chemists. These undesirable features should be avoidable if a suitable protecting group (e.g. urethane type) could be introduced on the nitrogen atom. Such compounds have been very elusive until the appearance of a recent paper[95] which reports that they can be made by direct acylation of the *N*-carboxy-anhydrides in aprotic solvents in presence of *N*-methylmorpholine. The derivatives are stable solids that yield CO_2 as the only byproduct in coupling. Racemization is minimal. The synthesis of the peptide chemist's bête noire, acyl-carrier peptide (65-74) by a solid-phase technique in 73% overall yield should prompt further study by other groups in spite of the two-stage synthesis of the reagents.

A few papers have extended our knowledge of the phosphonium type of reagent for forming peptide bonds. BOP gave 78-92% yield in forming four of the five peptide bonds in a fragment of cyclosporin A (H-Abu-Sar-MeLeu-Val-MeLeu-Ala-OH)[96]. This peptide contains three *N*-methylamino acids as well as the sterically hindered Val side chain. Not surprisingly, the MeLeu-Val peptide bond proved to be the most difficult to forge and Fmoc-MeLeu-Cl was used for this stage. Preparation of the BOP reagent requires hexamethylphosphoric triamide which is carcinogenic. A related

reagent (PyBOP; 15) can be made from the safe starting material, tris(pyrrolidino)phosphine oxide, by the same general procedure that is used to prepare BOP[97]. It performed well in the SPPS of acyl-carrier protein fragment (65-74). As indicated above, BOP gives poor yields in coupling reactions involving *N*-methylamino acids with bulky side chains. Yet another phosphonium compound (BROP; 16) has been designed specifically for such cases[98]. Thus in the synthesis of Z-MeVal-MeVal-OMe, BOP gave only 5% yield whereas BROP gave 70% of the desired compound. It is well established that coupling of His derivatives with an N^{π}-protecting group is prone to racemization so it is encouraging that Boc-His(Tos)-OH can be coupled in SPPS using BOP with <0.5% racemization[99]. Repetitive BOP coupling can be used in SPPS with satisfactory results[100].

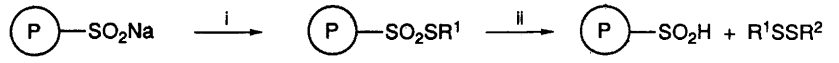
A re-examination of the claim that long chain fatty acids and azobenzene suppress racemization in coupling reactions gave negative results[101]. Finally, the enzyme-mimetic method for forming peptide bonds devised by Sasaki and his collaborators and reported on two years ago has been improved by the introduction of a methyl ester group into the crown ether template[102].

2.4 Disulphide bond formation

Aerial oxidation of H-Cys-Leu-Ala-Glu-Leu-OH gave the bis-pentapeptide[103]. Deprotection of:
Ac-Cys(Acm)-Orn-Leu-D-Phe-Pro-Val-Orn-Cys(Acm)-D-Phe-Pro-OEt
and oxidation with iodine gave the heterodetic cyclic decapeptide, an analogue of gramicidin S[104]. For the deprotection and oxidation of -Cys(Acm)- peptides, I_2 in highly acidic solution followed by extraction with CCl_4 is recommended to minimize side reactions[105]. The nature of the products formed from treatment of a mixture of a peptide containing a -Cys(Trt)- residue and another peptide containing a -Cys(Acm)- residue with I_2 is known to depend on the solvent. This method has been used to prepare unsymmetrical disulphide fragments of the ß-subunit of human choriogonadotropin[106]. Unsymmetrical disulphides have been synthesized from thiosulphonates immobilized on a

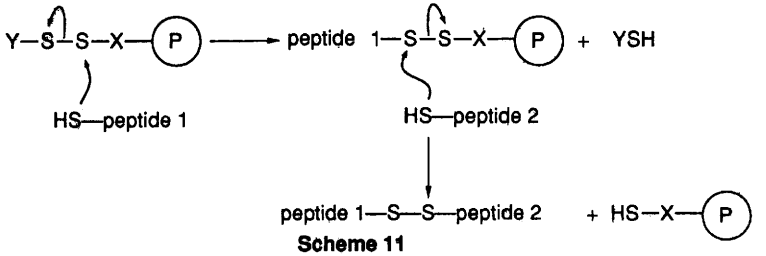
polystyrene support[107]. Polystyrene was chlorosulphonylated and then treated with Na_2SO_3 yielding a sulphinated polystyrene. Reaction of this with thionitrites formed by the interaction of thiols and nitrous acid gave immobilized thiolsulphonate esters (Scheme 10). The latter compound gave unsymmetrical disulphides when allowed to react with a thiol. The use of an insoluble support facilitates isolation of the product since the sulphinic acid formed is often difficult to separate from the disulphide in homogeneous solution. This method has yet to be applied to peptide synthesis.

The classical method of preparing unsymmetrical disulphides by exchange reactions between thiols and disulphides has been improved by using solid-phase methodology[108] (Scheme 11). The first reaction proceeds most favourably if the thiol group in HS-peptide 1 has a higher pK_a than YSH (i.e. YS^- is a better leaving group than ^-S-peptide 1). It follows that the pK_a of -XSH should be higher than the pK_a of YSH otherwise formation of the insoluble intermediate disulphide in the first stage would be disfavoured. Likewise, if the formation of the peptide disulphide is to proceed satisfactorily in the second stage, the pK_a of the thiol group in HS-peptide 2 must be higher than the pK_a of -XSH (i.e. $-XS^-$ is a better leaving group than ^-S-peptide 2). The YS group can be Nps (pK_a of conjugate acid = 2.2) while -XS- can be $-COC_6H_4S-$ (pK_a of conjugate acid $\approx$ 4.9). Good yields depend on the stoichiometric amount of HS-peptide 1 used in order to minimize the amount of symmetrical disulphides formed. The regiospecific formation of disulphide bonds in a peptide containing $\geq$4 cysteinyl residues is considerably more difficult than the synthesis of a simple unsymmetrical disulphide even though the formation of disulphide bonds is intramolecular. In order to examine the problems and evaluate a possible solution, a fragment of bovine pituitary peptide (17) has been synthesized[109]. The linear peptide containing 21 amino acid residues and including 4 cysteinyl residues was assembled by SPPS. Several possible methods for achieving regiospecific formation of two disulphide bonds were examined and the following



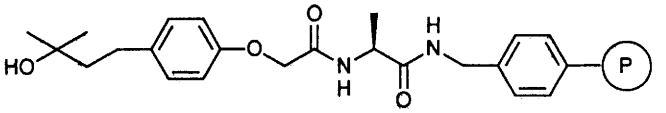
Reagents: i, $R^1S—N{=}O$; ii, R^2SH

Scheme 10



Scheme 11

(17)



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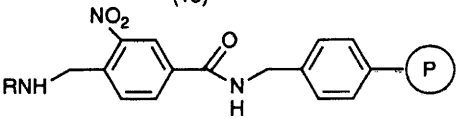

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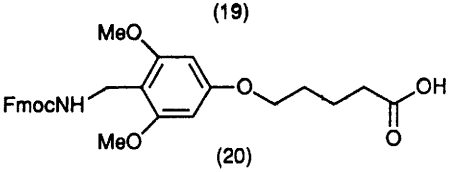

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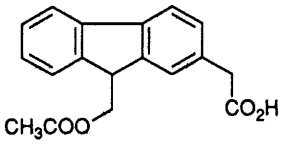

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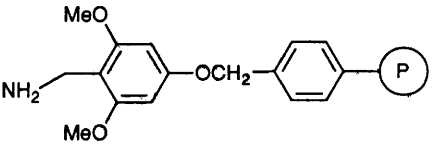


(22)

protocol was derived. The thiol groups of Cys^7 and Cys^{10} were protected with the 9-fluorenemethyl (Fm) group while the 4-methylbenzyl group was used for Cys^{13} and Cys^{15}. After assembly of the linear peptide was complete, the Fm groups were removed with piperidine - $CHONMe_2$ and the dithiol was oxidized. The peptide was cleaved from the resin and deprotected with HF. Subsequent aerial oxidation formed the other disulphide bond. Although strong acids are known to catalyse exchange reactions between thiols and disulphides, this possible side reaction did not cause significant trouble. It is important to test this synthetic strategy by synthesizing other candidate molecules, perhaps particularly a molecule containing one intrachain disulphide bond and one interchain disulphide bond. A fragment of the insulin molecule would be a suitable target.

2.5 Solid-phase peptide synthesis (SPPS)

Improved syntheses of Boc-amino acid/linker conjugates have been described[110] for both polystyrene and polyacrylamide matrices. Using 4-(halomethyl)phenylacetic acids as linkers, it was found desirable to protect the carboxyl group of the latter as the 2-trimethylsilylethyl ester. Reaction of this with the Cs salts of Boc-amino acids gave high yields of the conjugate. Removal of the 2-trimethylsilylethyl ester group was effected with Bu_4NF. A new type of t-alcohol linker (18) is recommended in order to suppress the loss of *C*-terminal dipeptide by formation of 2,6-diketopiperazines during SPPS with Fmoc-amino acids[111]. The method was tested by synthesizing bradykinin potentiator B. This peptide contains a Pro-Pro sequence at the *C*-terminus which is prone to form the 2,6-diketopiperazine at the dipeptide stage. The desired peptide was obtained in 66-68% yield.

A variety of photolytically labile linkers based on 4-aminomethyl-3-nitrobenzoic acid have been examined[112]. The Dts group was preferred for blocking the amino group of the linker during coupling to the resin. The Dts group was removed with $HSCH_2CH_2OH/Pr^i_2NEt$ and the peptide was assembled in the usual way.

Finally, photolysis produced the peptide as its amide. A photolytically detachable matrix-linker conjugate (19) has been designed specifically for the synthesis of *N*-methylamides and *N*-ethylamides of peptides[113].

The 5-(4-aminomethyl-3,5-dimethoxyphenoxy)valeric acid (PAL) linker (20) has been designed specifically for the synthesis of peptide amides[114]. For small peptides, removal of the Bu^t side chains and fission of the anchoring linkage proceeds smoothly in $CF_3CO_2H/CH_2Cl_2/Me_2S$ (14:5:1) at 25°C for 2 h. For more complex peptides, especially those containing Arg(Mtr) or Arg(Pmc) residues, final deprotection and liberation of the product is obtained in CF_3CO_2H/PhSMe/$HSCH_2CH_2SH$/PhOMe (90:5:3:2) at 25°C for 2-8 h. A side reaction was detected in the synthesis of peptides containing Trp and was attributed to alkylation of the indole ring of Trp by a carbonium ion formed when the anchoring linkage was cleaved. This problem can be circumvented in two ways. The linker moiety can be attached either to an aminoalkyl group of 'Pepsyn K' resin or to the α-amino group of an internal reference amino acid. In both cases, the bond between linker and matrix is stable to the conditions used for deprotection and peptide liberation so the byproduct remains insolubilized. Over 100 peptides have been successfully synthesized by this method.

The *C*-terminal amino acid of a peptide to be synthesized can be attached to a resin bearing a 4-alkoxybenzyl alcohol linker using the chloride of an Fmoc-amino acid[115]. The authors report that there is practically no racemization. Alternatively, Fmoc-amino acids can be esterified by free hydroxyl groups on insoluble matrices including cellulose using 2',4',6'-mesitylene-3-nitro-1,2,4-triazolide (13) in the presence of 1-methylimidazole[116]. This route is also claimed to be effectively free from racemization and not to lead to double attachment of the *C*-terminal amino acid.

Earlier difficulties experienced with a glycolamidic ester as a base-labile linker have been apparently overcome by effecting the final liberation of peptide with 1 M NaOH in

$CHONMe_2$ or Pr^iOH (70:30)[117]. Whether this report will be sufficient to overcome the reluctance of peptide chemists to use such vigorous basic conditions remains to be seen. A possible solution to the problem of cleaving peptides from a glycolamide linker, however, is already at hand[118]. $LiSCH_2CH_2OH$ in a mixture of $HSCH_2CH_2OH$ and tetrahydrofuran cleaves the glycolamide ester bond in <2 h. A linker based on 9-hydroxymethylfluorene (21) has been described and used to synthesize the *N*-terminal heptapeptide of rat transforming factor α[119]. Two acid-labile benzylamine linkers (e.g. **22**) have been designed and thymulin has been synthesized using such a support[120]. Finally in connection with linkers, a *p*-acryloxybenzhydrylamine resin can be used that permits deprotection of side chains to be carried out while the assembled peptide is still attached to the resin. The peptide is then released with $MeNH_2$ [121].

Monitoring of the completeness of removal of Boc groups during Merrifield-type SPPS is possible with the Denige reagent ($HgSO_4$ in H_2SO_4)[122]. Monitoring of the efficacy of the coupling step with picric acid has been thoroughly examined[123]. Of 1622 coupling steps tested, *ca.* 10% underwent a single low-yield step, *ca.* 10% had 2 or 3 consecutive low-yield steps and *ca.* 20% gave low yield over 4-8 steps. Almost always, a nadir in yield did not occur before the 7th step. In a comparison between the use of 1% and 2% divinylbenzene - styrene copolymers, faster reaction was observed with the lower level of crosslinking agent[124]. This is attributed to lower resistance to intraparticle diffusion. It should be noted, however, that the self-diffusion coefficients of Boc-amino acid anhydrides in preswollen polystyrene beads have been measured by an nmr pulsed-gradient spin-echo method[125]. The results indicate that diffusion of reagents is unlikely to be rate-limiting even in the fastest steps.

The use of the BOP coupling reagent in conjunction with Pr^i_2NEt rather than with *N*-methylmorpholine as tertiary base effectively suppressed the tendency for 2,6-diketopiperazines to be formed competitively at the stage where the third residue is being attached using conventional Boc chemistry and a nitro-

benzyl-type linker[126]. A hexadecapeptide derived from the conserved region of three bacterial ice nucleation proteins has been assembled on the Kaiser oxime resin using three fragments comprising 4-6 residues[127]. The BOP reagent was used in conjunction with HOBt and Pr^i_2NEt. The resin-bound peptide was cleaved with *N*-hydroxypiperidine in 2 M LiBr in anhydrous tetrahydrofuran. Two of the fragments contained a *C*-terminal Gly residue but the other had *C*-terminal Leu. The degree of racemization at this latter point was acceptably low.

A troublesome complication resulting in low coupling yields can occur if the growing peptide chain tends to form secondary structures. The latter can be detected by FTIR spectrophotometric examination of a suspension of the resin bound peptide[128]. The presence of a band at 1630 cm^{-1} is indicative of a strongly hydrogen-bonded ß-sheet structure and if an additional band is present at *ca.* 1695 cm^{-1}, an antiparallel ß-sheet is probably present. The secondary structure can be disaggregated by using 2 M LiBr in anhydrous tetrahydrofuran (see ref. 127). The use of the polar solvent 1,1,1,3,3,3-hexafluoro-2-propanol is also reported to accelerate difficult coupling steps[129].

A searching analysis of the Merrifield method of SPPS has been carried out by determining coupling yields[130]. Yields of ≤99% were considered incomplete and highly incomplete if ≤98%. After synthesizing more than 500 peptides, it was concluded that the most difficult *N*-protected amino acids to couple were His, Thr, Arg, Val, Ile and Gln. Coupling was also most difficult when the free α-amino group belonged to Gln, Leu, Ala, Arg and Ile. Not surprisingly, coupling efficiency tended to decrease with the length of the peptide. It was concluded that the formation of no peptide bond can be predicted to be complete with a single coupling step. The results point to a need for online determination of coupling efficiency so the cycle can be repeated automatically if necessary. Starting from the premise that adoption of a random coil structure in an insolubilized peptide would not tend to impede coupling whereas the presence of rigid secondary structural features would be detrimental, the

Chou-Fasman rules for predicting conformation were applied to the prediction of difficult couplings[131]. The results are in general agreement with those described above[128,130]. Thus, although the side chain of Ala is not particularly sterically demanding, its proclivity to be present in α-helices is likely to be unfavourable when the α-amino group of Ala is the nucleophile in a coupling reaction (cf. ref. 130).

A method has been described of the potential synthesis of reactive esters of peptides for fragment condensation[132]. It uses the Kaiser - DeGrado method of SPPS on an oxime resin to generate 4-methylthiophenyl esters of peptides. These are not particularly reactive *per se* and so they can be made without the occurrence of extensive racemization. Subsequent oxidation to the 4-methylsulphonylphenyl esters provides much more reactive intermediates. For example, Boc-Gly-Phe-$OC_6H_4SO_2Me$ was synthesized and then coupled to H-Leu-$NHC_6H_4NO_2$. Clearly, a more exhaustive examination of this approach is called for with quantitative determination of any racemization occurring during the coupling of the methanesulphonylphenyl esters of protected peptides.

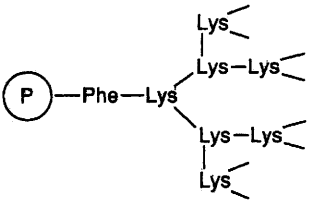
SPPS has been carried out with an acid-stable phenacyl ester linking the matrix to the growing peptide[133]. If the Boc group is used for *N*-terminal protection, intermediate deprotection can be effected with 0.5 M $MeSO_3H$ in CH_2Cl_2-dioxan (9:1).

Following SPPS with Fmoc chemistry, complications during the liberation and deprotection step can be minimized by using the following reagent:- CF_3CO_2H/PhOH/H_2O/PhSMe/$HSCH_2CH_2SH$ (82.5:5:5:5:2.5)[134]. A further difficulty encountered when using Fmoc chemistry has been overcome independently by two groups[135,136]. Attachment of Fmoc-Asn-OH or Fmoc-Gln-OH to an appropriate alkoxybenzyl type of resin is tricky. Fmoc-Asp-OBu^t or Fmoc-Glu-OBu^t, however, can be attached to an appropriate aminomethylalkoxyphenyl resin via the free carboxyl group. When peptide chain assembly has been completed, acid cleavage generates the corresponding peptides with *C*-terminal Asn or Gln

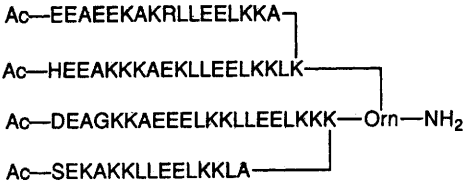
residues.

There have been further reports of the use of bromophenol blue to monitor acylation steps in SPPS[137,138]. If Fmoc chemistry is used[137], the indicator is not adsorbed by polyacrylamide resin in $CHONMe_2$. In the presence of the weakly acidic HOBt, however, binding does occur making the resin deep bluish red. This colour is discharged on acylation of free amino groups. The authors claim that no side reactions such as acylation of hydroxyl groups in the indicator occur, but no proof is given. The synthesis of 50 peptides related to HIV using a polystyrene support with monitoring by bromophenol blue has been reported[138]. The coupling efficiency was improved by using ultrasonication and an elevated temperature. A different procedure, counterion distribution, has been developed to monitor the acylation step in SPPS[139]. The anionic dyestuff, quinoline yellow, is used; it equilibrates between protonated amino groups on the resin and a soluble tertiary amine such as Pr^i_2NEt. As acylation proceeds, more dye is liberated into the solution phase and the light absorption rises to a plateau. The authors used polyacrylamide resin only, so it is not known if the dye is bound hydrophobically to polystyrene resins. Only Pfp esters have been used for coupling. The method appears not to be applicable if anhydrides are used since these liberate one molar equivalent of acid. Likewise, the use of saline coupling agents such as BOP might be expected to cause problems. Progress in the reactions during SPPS can also be monitored by measuring the conductivity of the liquid phase[140].

The requirement to synthesize antigenic peptides for possible vaccine production has prompted the introduction of technical advances. By attaching 7 Lys residues to a resin in three cycles (23), eight copies of a peptide can be assembled on the free α- and ϵ-amino groups generated from the attachment of the first Lys residue[141]. The continuous-flow technique was used without any problems arising. Multiple peptides have been produced by the Geysen method[142,143]. The formation of a 2,6-diketopiperazine moiety with simultaneous cleavage from the resin at pH7 by the *C*-terminal dipeptide permits the rapid


Lys
Lys—Lys
P
—Phe—Lys
Lys—Lys
Lys

(23)


Ac—EEAEEKAKRLLEELKKA
Ac—HEEAKKKAEKLLEELKKLK
Ac—DEAGKKAEEELKKLLEELKKK—Orn—NH2
Ac—SEKAKKLLEELKKLA

(24)


KLKELKEKLKELKEKLKELKE

(25)

testing of numerous peptides without purification. Mixtures of peptides differing at one residue only have been synthesized on a single support. After cleavage from the resin, the mixture is separated into the components by hplc[144]. In a process that resembles the production of multiple copies of a peptide[140], a four-chain peptide has been synthesized[145] that has catalytic activity resembling that of chymotrypsin (24). Three of the peptide chains had an *N*-terminal residue characteristic of the active centre of the serine proteinases (Ser, His, Asp). The three chains were separately assembled using three orthogonal protecting groups. The model catalyst strongly resembled chymotrypsin although the activity was only about 1% of that of the enzyme. In the simultaneous synthesis of multiple peptides, it is important that when different reactants are used at a particular stage they should all react within a reasonable time. Pentafluorophenyl esters have been judged to be the most suitable coupling reagents for this purpose[146].

The remaining papers on SPPS cover a variety of points. In the synthesis of phosphopeptides, phosphorylation of a hydroxyl group, which is not protected during peptide assembly, can be effected on the resin[147]. The phosphopeptide can then be deprotected and cleaved from the resin by conventional methods. Synthesis of a peptide with a *C*-terminal photoprobe was accomplished by attaching Boc-Lys(Fmoc)-OH to the resin, removing the Fmoc group and allowing the liberated ϵ-amino group to react with 4-carboxybenzophenone. The remainder of the peptide was then assembled using Boc chemistry[148]. The *N*-terminus of a peptide still attached to a matrix can be modified by attachment of a 2-(acetylthio)acetyl residue. Removal of the *S*-acetyl group with NH_2OH, for example, leaves a peptide that can be fluorometrically labelled[149]. The synthesis of cyclic peptides, especially those involving the formation of a secondary amide link between the side-chain carboxyl group of Asp or Glu and the ϵ-amino group of Lys, has been further studied[150-152]. The first of this trio of papers describes the synthesis of a novel tricyclic peptide (25).

Manual equipment for SPPS has been described and used for the synthesis of a decapeptide[153]. A side reaction during the synthesis of a His peptide occurred when the imidazole ring was protected by a tosyl group. Attempted coupling of Boc-Asn at the next stage with HOBt led to some cleavage of the tosyl group. Thereafter, coupling of Boc-Gly led to some incorporation on the imidazole ring[154]. Finally, an aminomethylated polystyrene was converted into a dithiocarbamate salt by reaction with CS_2. Reaction of this with a symmetrical anhydride of a protected amino acid gave an insoluble unsymmetrical anhydride for peptide synthesis[155]. Since the product necessarily becomes detached, this technique is not likely to find widespread use.

2.6 Enzyme-mediated synthesis and semi-synthesis

The multifarious strands contributing to this area of peptide synthesis make it difficult to present an ordered progress report. The increasing volume of literature further compounds the difficulties, especially when several aspects of the field feature in a single publication.

A number of publications are partly or mainly concerned with immobilization of proteolytic enzymes on insoluble supports[156-164]. An interesting way to produce an insolubilized enzyme comprises separate reaction of the protein and polyethylene glycol with acryloyl chloride followed by copolymerization of the products using bisacryloyl polyethylene glycol in the presence of ammonium persulphate and $Me_2NCH_2CH_2NMe_2$[156]. Obviously, the enzyme to be used must contain amino groups that can be acylated without undue impairment of catalytic activity. α-Chymotrypsin was insolubilized using this method and then used to synthesize Ac-Tyr-Leu-NH_2 from Ac-Tyr-OEt and H-Leu-NH_2 in yields of 97-99%. Trypsin has been immobilized by adsorption on a column of benzamido-'Sepharose' followed by reductive alkylation *in situ* with *n*-octanal in the presence of $NaBH_4$. This modification increased both catalytic activity and stability. Several proteinases have been coupled to polyethylene glycol and used to synthesize small peptides or polyamino acids[165,166]. There

have also been further studies on proteinases encapsulated in reversed micelles[167-170]. Some anomalous kinetic properties of proteinases thus treated have been described[167,168] including enhanced enzymic activity and the existence of a bell-shaped relationship between enzyme activity and water content.

There is little new to report on the effect of physical conditions on enzyme-catalysed peptide synthesis. In the reaction between Mal-Phe-Ala-NH_2 and H-Leu-NH_2 catalysed by papain, the use of lower temperatures favoured synthesis over hydrolysis of the peptide ester[171]. Perhaps more surprising is the observation[172] that maleyl-Tyr-OMe and a variety of amino acid derivatives in presence of α-chymotrypsin in ice at -25°C gave higher yields than when the corresponding reactions were carried out at 25°C. Even unfavourable nucleophiles such as H-Arg-OH or H-Lys-OH gave satisfactory yields in the frozen state. Similar results were obtained using papain and V8-proteinase with appropriate substrates. It should be noted, however, that the yield of product does not increase continuously with increasing concentration of nucleophile. Yields can also be enhanced by using sonication during the synthesis of a peptide in the presence of a proteinase[173]. It has been proposed that there is synergism between stereoprotonic and stereoelectronic effects in the kinetically-controlled enzyme aminolysis of specific esters by *N*-nucleophiles (Ping Pong mechanism)[174]. The detailed mechanism proposed accounts for the failure of *N*-methylamino acid derivatives to react with acylated chymotrypsin. In the light of Jakubke's results[172], however, it would be interesting to see if chymotrypsin can catalyse the synthesis of a peptide bond using an *N*-methylamino acid derivative as a nucleophile at -25°C. In another study of the effect of conditions on enzyme-mediated peptide synthesis[175], it is reported that the optimum pH of reaction depends on the nature of the amino acid side chain in the substrate serving as acyl donor. It is recommended that the ionic strength should be kept as low as possible. Temperature had only a marginal effect but no experiments were apparently carried out below 0°C. The use of a large hydrophobic *N*-

protecting group improved selectivity of catalysis.

ß-Hydroxy-α-amino acids can be resolved by the action of suitable proteinases such as chymotrypsin or subtilisin on the methyl esters of *N*-acylated derivatives[176]. Enzyme-catalysed hydrolysis of peptide esters by proteinases has been used to prepare the free acids for fragment coupling[177]. The dimethyl ester of α-dehydroglutamic acid can be selectively hydrolysed in the side chain using α-chymotrypsin A[178]. A derivative of chymotrypsin in which ε-amino groups in Lys residues had been lipoglycosylated was found to be an effective catalyst for the esterification of amino acids in polar solvents[179]. Kinetically-controlled peptide synthesis using chymotrypsin that had been immobilized on agarose is reported not to be stereospecific[180]. A comparative study has been made[181] of the stability and activity of chymotrypsin in three physically distinct forms, adsorbed on 'Celite' and suspended in isooctane, free enzyme suspended in isooctane, and a microemulsion of free enzyme. The last was preferred for some reactions, but the differences were fairly marginal. Chymotrypsin suspended in hexane with $Na_2CO_3.10H_2O$ as the only source of water effectively catalysed the synthesis of Boc-Ala-Phe-Leu-NH_2 from Boc-Ala-Phe-OMe and H-Leu-NH_2[182]. Excellent yields of product were obtained when chymotrypsin was used to effect reaction between Z-Phe-OMe and various amino acid amides in either water-miscible solvents or water-immiscible solvents containing a small amount of water[183]. When a ß-naphthylamide of an amino acid was used as a nucleophile in similar coupling reactions, the syntheses proceeded very readily[184]. It was suggested that the nucleophile had a high affinity for the acyl-enzyme intermediate. Chymotrypsin and thermolysin were used together in immobilized form to synthesize Z-Gly-Phe-Leu-NH_2 from Z-Gly-OH, Phe-OMe and Leu-NH_2 in a series plug-flow reactor[185]. Perhaps the most interesting experiment carried out with chymotrypsin was the synthesis of [D-Phe^6]-GnRH by a [3+7] fragment coupling[186]. Another potentially important paper but with few experimental details describes the direct conversion of porcine insulin into the human hormone using

trypsin that had been immobilized on spherical macroporous bead cellulose[187]. Not surprisingly, recombinant DNA technology has been used to obtain a mutant form of subtilisin that displays enhanced stability in organic solvents and gives high yields in simple coupling experiments[188]. This probably heralds a flood of similar publications. A conjugate of subtilisin and polyethylene glycol adsorbed on to a support or used as a suspension gave only moderate yields of coupled product under the conditions used due to competing hydrolysis of ester substrate[189]. The kinetics of transesterification reactions catalysed by the same forms of enzyme were consistent with the Ping Pong mechanism with competing hydrolysis.

Several papers have appeared in which papain has been used to catalyse peptide synthesis and related reactions[190-199]. Some are concerned with the synthesis of peptides derived from a single amino acid[190-193]. There is the first report of the use of free amino acids as nucleophiles in the synthesis of dipeptide derivatives by papain[194]. An α-aminophosphonic acid ester can act as a nucleophile and the coupling is stereospecific[195]. Not surprisingly, thio acids can serve as acyl donors in papain-catalysed reactions[196]. *C*-Terminal fragments of CCK with the sulphate ester in position on the Tyr residue have been synthesized[197]. Clostripain catalyses transpeptidation reactions between Bz-Arg-OEt and a variety of amino acid and peptide derivatives at pH 9.2[200].

Thermolysin has featured in two interesting papers, once as a substrate and once as a catalyst. When thermolysin was autolysed, cleavage of the 196-197 and 204-205 peptide bonds occurred[201]. The 205-316 fragment was isolated and hydrolysed with *S. aureus* V8 proteinase cleaving the Glu^{302}-Val^{303} bond. The two fragments, one of which was synthesized, were coupled using the same enzyme at pH 6.0 in 50% glycerol with yields of up to 90%. Thermolysin catalysed the synthesis of Z-Phe-Phe-OMe in EtOAc/Tris buffer, pH 7.5, but difficulties were experienced because of the low solubilities of substrates and the tendency of the enzyme to be inactivated at the interface[202].

Carboxypeptidases Y and C have been used to synthesize simple peptide derivatives[203,204].

The partitioning of an acyl enzyme in a Ping Pong reaction between nucleophilic attack by an amine and by water has been characterized by the partition constant[205]. This is defined as the concentration of amine which results in the competing reactions having equal velocities. A method is reported, based on the integrated rate equation, for calculating the partition constant from the product ratio.

Although not yet applied to the peptide field, the hydrazinolysis of ethyl acetate is catalysed by lipases[206]. This could be a useful component of the Curtius method for fragment coupling. Another new development with a promising future involves the removal of an amide group from the *C*-terminal residue of a synthetic peptide[207] made by either solid-phase methodology or enzyme-catalysed synthesis. The method uses an peptide amidase isolated from the flavedo of oranges. The enzyme does not act on amino acid amides so it can be present during enzyme-catalysed coupling to force the position of equilibrium further towards product.

A carboxypeptidase and an aminopeptidase have been employed to catalyse the synthesis of peptides containing D-alanine[208,209]. Dipeptidyl peptidase is another unusual enzyme in the field of peptide synthesis[210]. It is particularly useful because it will accept free amino acids as nucleophilic substrates. Finally, and perhaps unexpectedly, immobilized baker's yeast cells in reverse micelles can be used to effect peptide synthesis[211]. For example, Z-Tyr-Gly-Gly-Phe-Leu-NH_2 was synthesized from Z-Tyr-OMe and H-Gly-Gly-Phe-Leu-NH_2.

2.7 Miscellaneous reactions relating to peptide synthesis

Peptides containing a residue of Tyr(Bzl) can be directly fluorinated in the 3'-position using a solution of acetyl hypofluorite prepared from F_2 and $CH_3CO_2^-Na^+$ in $CFCl_3$[212]. When the phenolic hydroxyl group was unprotected, a mixture of products was formed. Complexation of acyclic dehydrodipeptides with

certain metal ions, permits hydrogenation to proceed in a diastereoselective manner[213]. A rather recondite side reaction was noted during the synthesis of a peptide containing Arg(Tos) and a residue of pyrenylalanine[214]. Examination of fluorescence and nmr spectra indicated that attempted cleavage of the tosyl group by acid led to byproducts in which the tosyl group was present in the pyrenyl group. The authors suggest that this side reaction may be due to an electrophilic attack of tosyl cations on the electron-rich pyrenyl group in presence of HF. When Ser or Thr is at the *N*-terminus of a synthetic peptide and it bears a urethane protecting group on the α-amino group but the ß-hydroxyl group is unprotected, exposure to alkali can lead to cyclization to an oxazolid-2-one derivative[215]. When Et_3N is used as neutralizing base during the assembly of a peptide containing Asp, the latter is likely to be converted in part to aspartimide. It is safer to use Pr^i_2NEt or *N*-methylmorpholine[216]. A general method has been described for the modification of the ϵ-amino group of Lys during SPPS[217]. For example, treatment of a resin-bound peptide of lysine with $(PhO)_2C{=}NCN$ in $CHONMe_2$ followed by reaction with a primary amine affords a derivative of homo-arginine, -Lys[C(NHR)=NCN]-. Finally, an impurity was detected in the synthesis of thymopentin after using Pd as a catalyst for deprotection[218]. This impurity was shown to be a 1:1 complex of thymopentin and Pd. It is surprising that this phenomenon is not more common.

3. Selected examples of peptide synthesis

Only three examples are reserved for this section, not because there is a dearth of suitable papers to quote, but rather because there is a plethora of syntheses that would have been unthinkable a few years ago. Many examples that could easily have been selected for special mention are to be found in the appendix.

The synthesis of the insulin-like growth factor has not been chosen because it is the longest peptide synthesized. It contains 70 amino acid residues and the main point of interest

is the successful formation of three disulphide bonds. This was accomplished by oxidation of the hexathiol with oxidized glutathione[219]. The product had 70% of the potency of the natural peptide, a result that suggests that the reduced peptide had already folded into a conformation that strongly favoured the correct pairing of thiol groups when subjected to oxidation. The second example is the synthesis by conventional solid-phase methodology of the *C*-terminal peptide containing 101 amino acid residues derived from a HIV protein[220]. The coupling efficiency was monitored and was 99.5%; it was calculated that 60% of the molecules present should have the correct sequence. Finally, ubiquitin has been made by SPPS using Fmoc chemistry and with protection of the Arg residues by the Pmc group[221].

4. Appendix. A list of syntheses

The syntheses are listed under the name of the peptide/protein to which they relate, but no arrangement is attempted under the subheading. In some cases, closely related peptides are listed together e.g. oxytocin and vasopressin are listed under posterior pituitary hormones.

Peptide/protein	Ref.
4.1 Natural peptides, proteins, analogues and fragments	
Acyl carrier peptide	
Synthesis of fragment (65-74)	95,97
Alemethicin	
Analogues containing *C*-terminal amino alcohol	222
ß-Amanitin	
Conjugate with poly-L-Orn and EGF	223
Aminoacyl-tRNA synthetase	
Synthesis of fragment (366-385)	224
Amyloid ß-protein	
Synthesis of 2 fragments (26-33 and 34-42)	225
Angiotensin	
Various analogues	226-228
Heterodetic cyclic analogue containing -SS- link	229

4.3 Enzyme substrates and inhibitors

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

BY J.S. DAVIES

1. Introduction

Perceptive readers of these Reports over recent years will have appreciated that with the expansion in the field of conformationally restricted peptides, *via* cyclisation, isostere replacement or the substitution of unusual amino acids in the peptide backbone, there has been an increasing amount of overlap between the subject coverage of Chapters 3 and 4. This year authorship of both Chapters has been placed in the lap of the same reviewer, so some rationalisation of these areas has been possible. In broad terms the structure of this Chapter remains as in previous years except for the section on O-phosphorylated and glycosylated derivatives which now has been subsumed into Chapter 4. Cyclic peptides constructed as conformationally-constrained analogues are covered in this Chapter, while naturally-occurring cyclic peptides in all their rich variety of structures are covered exclusively this year in Chapter 4.

Mainstream primary journals in the Bio-Organic area and Chemical Abstracts (up to June 1991) were again the main sources, with papers more widely distributed than recent years. A trend towards short papers in the rapid publication media was evident. Overall, industry contributed 25% of the papers, the others coming from academia and research institutions. No attempt has been made this year again to cover the patent literature.

It was something of a quiet year for peptide bond surrogates, but constraining peptides into cyclic analogues remains a popular field of endeavour. However, the buoyant area of current interest with quite an explosion in the number of synthetic routes to statine analogues, is the design of renin inhibitors. If success is synonymous with effort then we can all look forward to a future with our blood pressure under control.

A comprehensive report[1] on the European Peptide Society's biennial meeting held at Platja d'Aro in Spain contains a great deal of relevance to this Chapter. However, no attempt was made to review the large number of short papers published in these proceedings.

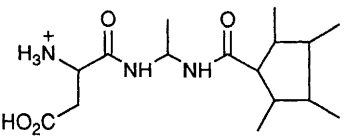
2. Peptide-backbone Modifications

A variety of the modifications discussed under this section have been subjected to structural studies using tandem mass spectrometry[2]. In this technique, after fast

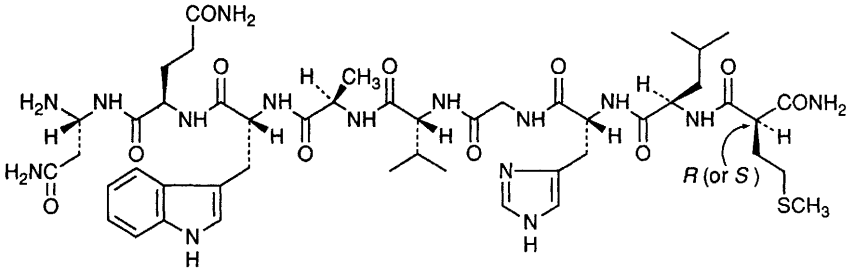
atom bombardment followed by collision-activation, unmodified linear peptide fragments give N-terminal ions as the most abundant, but ψ[CH_2NH] and ψ[CH_2S] modified linear peptides gave prominent C-terminal sequence ions. Both N- and C-terminal fragmentation occurred when a ψ[CH_2SO] had been inserted.

2.1 ψ[CSNH]-Analogues - Thionation of amides, peptides and lactams has been carried out successfully[3] using a 1:1 ratio of P_2S_5 and Na_2CO_3, but the Lawesson reagent remains the reagent of choice in all the other reports under this category. The latter was used[4] to convert Boc-Phe-NH_2 into its corresponding thioamide before inclusion into the pentagastrin analogue Boc-β-Ala-Trp-Met-Asp-Pheψ[$CSNH_2$]. This compound showed similar activity to pentagastrin during *in vivo* stimulation of HCl secretion and *in vitro* stimulation of amylase release in isolated rat pancreatic acini. The chiroptical properties of thionated N-acyl dipeptide N-methylamide models have been reported[5]. The optical activity of the thioamide chromophore is dominated by the chiral contributions of perturbants attached to the C_α at the NH side. Peptide sequences with alternating thioamide-amide-thioamide sequences tend to adopt a 1←4 H-bonded β-conformation . Similar models when subjected to ir, ^{13}C- and ^1H-nmr studies[6], reveal that the conformation of thiopeptides is determined by two factors, the H-bond donating and accepting ability of the CSNH group and the repulsion between the sulfur atom and the side chains of the neighbouring amino acid residues. The conformation of the chemotactic tripeptide analogue HCO-Metψ[CSNH]Leu-Phe-OCH_3 has been considered in detail[7] on the basis of an X-ray structure. Although the thionated peptide showed no chemotactic activity there was no obvious difference in its crystal conformation from that of the amide bond original structure. However from molecular free energy calculations it was shown that the main result of the CSNH substitution was to prevent the existence of C_{eq}^7 conformations, so that more H-bonding to the formyl CO group became prevalent. This emphasises the importance of the latter in giving the biological response.

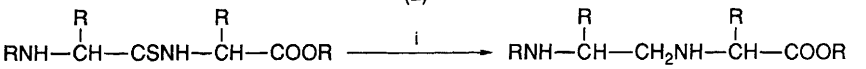
2.2 ψ[NHCO]-Retro-Inverso Analogues - X-ray studies[8] on both the L-*(R)* and L-*(S)* forms of the retro-inverso aspartame analogue (1) have further confirmed the postulate that sweeteness is associated with an 'L-shape' profile initially deduced from molecular mechanics and nmr studies. The C-terminal nonapeptide H-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 has been shown to retain the full agonist action of bombesin. An end group modified retro-inverso analogue (2) has been synthesised[9] in two diastereoisomeric form. The analogue with the *(S)*-configuration at the malonamic acid analogue of the methionyl residue was essentially inactive as an agonist while the *(R)*-form had weak agonist activity. Neither had any bombesin antagonist activity.



(1)

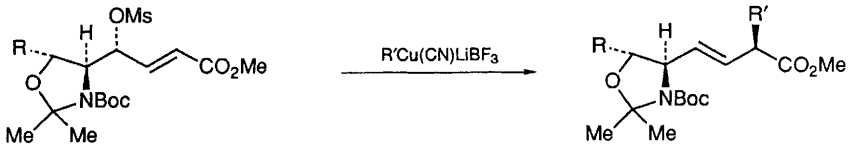


(2)



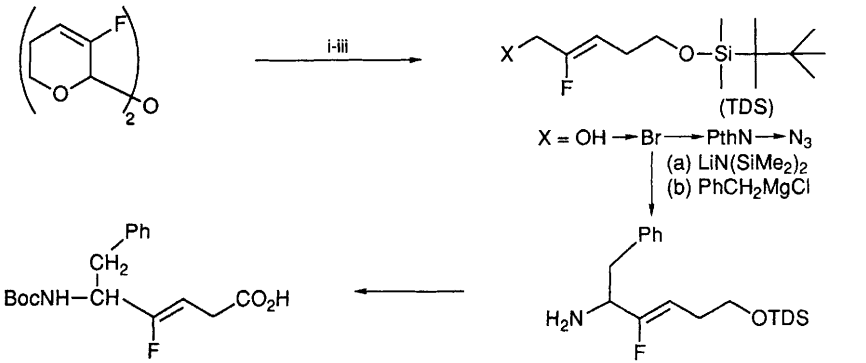
Reagent: i, Nickel boride

Scheme 1



Ms = mesylate, R′ = Me, $CHMe_2$, CH_2Ph

Scheme 2



Reagents: i, aq.HCl/reflux; ii, TDS–Cl; iii, DIBAH, –70 °C

Scheme 3

2.3 ψ[CH_2NH] Amino Methylene Analogues (and Retro-Forms) - The ψ[CH_2NH] isostere has been incorporated[10] into many positions at the N-terminal end of the growth hormone-releasing factor (1-29) amide [GRF(1-29)NH_2] using reductive alkylation of a preformed amino aldehyde and $NaBH_3CN$ in each case. Agonists with about 0.1% of natural activity were obtained from the isosteres at 1/2, 2/3 and 6/7 positions with activities of 0.39 and 1.6% achievable by incorporation at 10/11 and 3/4, respectively. Antagonistic activity at the 10 μM level compared with 1 nM for GRF was achieved for [Ser^9,ψ[CH_2NH]Tyr^{10}]GRF(1-29)NH_2. In an interesting new development which will no doubt in general supercede the older Mozingo reaction of desulfurisation with Raney Nickel, it is now possible using nickel boride to convert endothiopeptides into aminomethylene analogues[11] as summarised in Scheme 1 in high yield. The conformational space available to residues with aminomethylene isosteres is very similar[12] to that for the native peptide bond, but geometry minimisations of a grid of values on the retro-reduced equivalent when inserted into helices and sheets is complicated by mismatching of H-bonds.

2.4 ψ[CH=CH] and ψ[CF=CH] - Ethylenic Isosteres - *(E)*-Alkene isostere syntheses can be carried out[13] stereospecifically *via* organocyanocopper-boron trifluoride involvement in the key step from the precursor mesylates summarised in Scheme 2. Although it had been speculated by Abraham and Thomas sometime ago that ψ[CF=CH] is an even better isostere for an amide than its ethylene analogue, it is only now that a reasonable synthesis has evolved[14]. Scheme 3 summarises the main stages of the approach to Glyψ[CF=CH]Gly and racemic Pheψ[CF=CH]Gly. But in a modified synthetic approach[15], enantioselective synthesis of both antipodes of the latter analogue has been achieved by adding optically active ester enolates to the α-fluoro-α, β-unsaturated aldehydes in Scheme 3 followed by an S_N2' substitution of the allylic hydroxyl formed with a trichloacetamido group. Insertion of the isostere into a substance P C-terminal hexapeptide sequence gave receptor binding results as recorded in Table 1 which refers to the formula below. The IC_{50} results should be compared with the value of 1.3 nM for native substance P.

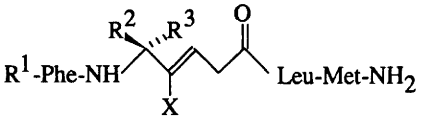


Table 1

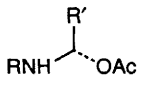
R^1	R^2	R^3	X	IC_{50}
Arg-Pro-Lys-Pro-Gln-Gln	CH_2Ph	H	F	2 nM
"	H	CH_2Ph	F	20 nM
pyroGlu	CH_2Ph	H	F	0.8 μM
"	H	CH_2Ph	F	10 μM
"	CH_2Ph	H	H	>10 μM

2.5 Phosphono-Peptides - Dipeptides and cyclodipeptides containing structures such as Z-NH-CH(R)-P(=O)OEt-NHCH(R)-COMe have been prepared[16] and analysed using the usual physical methods. Oxidative decarboxylation of α-amino acids with lead tetracetate has provided[17] the starting material (3) which can be converted readily by $(MeO)_3P/TiCl_4$ to (4). Compounds of structural type (5) have been coupled under mixed anhydride coupling conditions[18] to give a series of phosphono dipeptides. Synthetic techniques used to prepare 3-amino-phosphonocardicinic acids can also be adapted for use with peptides[19].

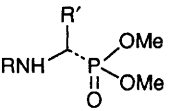
2.6 ψ[CH_2CH_2] - Carba Analogues - An unequivocal method[20] for preparing stereochemically precise carbadipeptides utilises stable and inexpensive reagents and is outlined in Scheme 4. Other alternatives synthesised were the diastereoisomer Boc-Leuψ[CH_2CH_2]-L or D-Phe-OH.

2.7 ψ[CH_2O] - Methyleneoxy Analogues - Cyclisation of bromo derivative (6) to give a δ-lactam intermediate not only provides a high yielding route (Scheme 5) to methyleneoxy isosteres[21], but the intermediates allow the absolute configuration to be determined by nmr techniques. In a strategy whereby each methyleneoxy pseudopeptide was obtained as a racemate, hplc separations enabled the diastereoisomers to be resolved. In this way non-glycosylated isosteres of the immunostimulating N-acetylmuramyl dipeptide were prepared. Two configurational forms of the pseudopenta- or hexapeptides of general formula R-Glyψ[CH_2O]-D(L)-Ala-Ala-D-Glu[Lys(R^1)-NHEt]-NH_2, also with Gly replaced by Ser, were synthesised in this work[22].

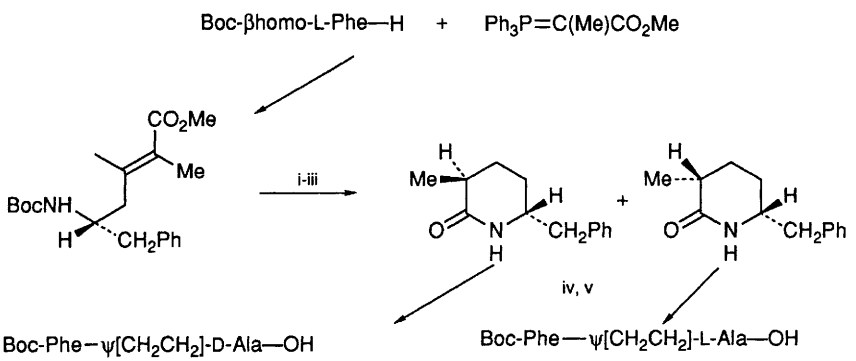
2.8 ψ[COO]-Depsipeptides - Depsipeptide links occur widely in nature (Chapter 4) but there was only one report this year of the insertion of the ester link as an isostere[23]. The conformations of sequences based on the repeating peptides of elastin, such as sequences Val-Pro-Gly-Hiv-Gly and Val-Ala-Pro-Gly-Hiv-Gly where Hiv = *S* α-hydroxyvaleric acid, have been compared with their corresponding all amide sequences. While the latter tend to exist as an equilibrium between a γ-turn and a β-turn structure in the Pro-Gly segment, in the depsipeptide a β-turn cannot



(3)

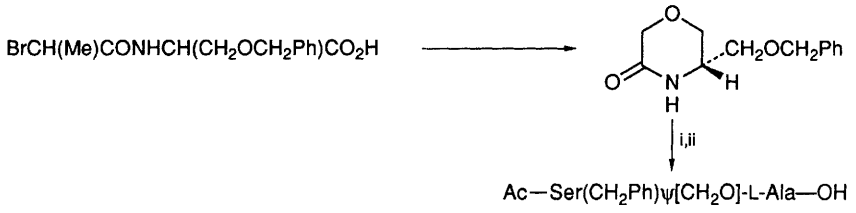


(4) R = Z
(5) R = H

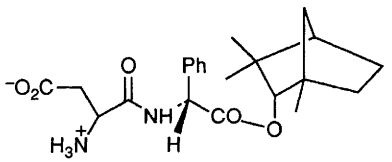


Reagents: i, H_2/Pd/C; ii, TFA; iii, Δ/pyridine; iv, HCl; v, Boc_2O

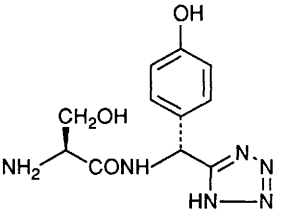
Scheme 4



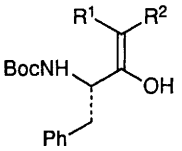
Scheme 5



(–) α-ester
(7)



(8)



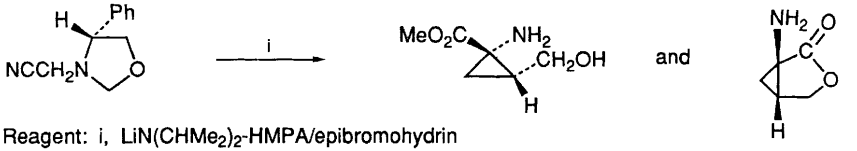
(9) $R^1 = R^2$ = CN, CO_2Me or C_6H_4–NO_2(*p*)

occur and only a γ-turn was seen. In polydepsipeptides based on the same repeating units the major conformational feature was found to be a type I β-turn involving Gly^{5}NH and Pro CO.

2.9 Replacement of L- by D-residues - The enzyme, muramoyl pentapeptide carboxypeptidase (E.C. 3.4.17.8), has shown stereospecificity in favour of D-residues[24]. When Ac-Lys(Ac)-D-Ala-D-LacOH or Ac-D-Ala-OMe were used as acyl components only D-forms of neutral, basic and hydrophobic amino acids were incorporated to give D-D dipeptide units. Even enzymes such as chymotrypsin will now entertain fragment coupling analysis involving D-residues. The enzyme catalysed a high yield coupling[25] of p-Glu-His-TrpOEt with H-Ser-Tyr-D-Phe-Leu-Arg-Pro-GlyNH_2 to give D-Phe^6-GnRH without racemisation. The conditions were made favourable by the presence of the D-Phe^6 residue inhibiting chymotryptic cleavage at the Tyr-CO position. It was something of a surprise to find[26] L-Asp-D-PhGly-(α) and (β) fenchyl esters (7) showing sweeteness potencies of 1200 and 3600 times that of sucrose, respectively, since the Ph group in this configuration is a much larger group than has previously been accommodated at this position in the 'sweeteness model'.

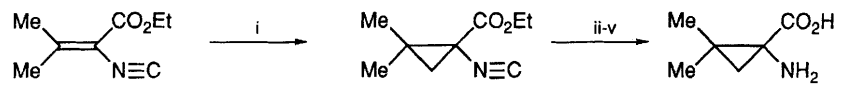
2.10 Miscellaneous Modifications - C-Terminal tetrazolyl groups when incorporated in peptides such as (8) have a close resemblance to the COOH group in terms of pK_a values steric and electronic qualities[27]. A series of novel α-chymotrypsin dipeptide substrates have undergone successful electron-withdrawing functionalisation[28] which include derivatives such as (9) obtained from the corresponding carboxylic acids using 1,1′-carbonyldiimidazole as an activating agent.

2.11 α,α-Di-Alkylated Glycine Analogues - The wide-ranging applications of cyclopropane amino acids in analogue studies have been reviewed[29] authoritatively by a leading practitioner in this 'small ring' field. Scheme 6 outlines a plausible general method for the chiral synthesis of cyclopropane amino acids[30], which depends a great deal on chromatographic separation of the predominant diastereoisomeric forms. The three step reactions[31] summarised in Scheme 7 yields racemic substituted cyclopropane amino acids, which are then resolved using (-)-quinine. An intramolecular carbenoid reaction of a malonate precursor followed by a Curtius rearrangement has yielded 2,3-methanohomoserine lactone (10) in high yield[32]. Tritium gas over Pd/C in dioxane has been added[33] to a cyclopropene derivative to give the tritiated analogue (11), useful as a ligand for the glycine B receptor. Stepwise solution phase coupling has enabled 1-amino-1-cyclopropane carboxylic acid (Acc) to be inserted[34] into the tuftsin analogues (12) - (14). It has been shown[35] that 2,4-methanoproline confers bulkiness and rigidity to the



Reagent: i, $LiN(CHMe_2)_2$-HMPA/epibromohydrin

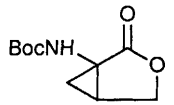
Scheme 6



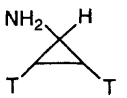
(–) + (+) forms

Reagents: i, $Me_3S^+I^-$/NaH; ii, 90% AcOH; iii, OH^-; iv, quinine salt; v, HCl

Scheme 7



(10)

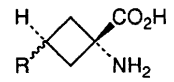


(11)

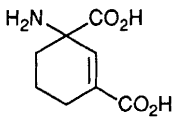
H—Acc-Lys-Acc-Arg—OH (12)

H—Acc-Lys-Pro-Arg—OH (13)

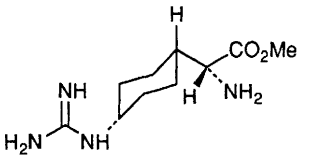
H—Thr-Lys-Acc-Arg—OH (14)



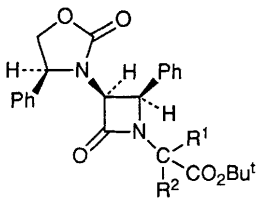
(15) R = CO_2H
(16) R = CH_2CO_2H
(17) R = $(CH_2)_n P(O)OH_2$
n = 1 or 3



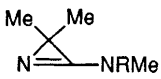
(18)



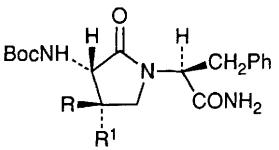
(19)



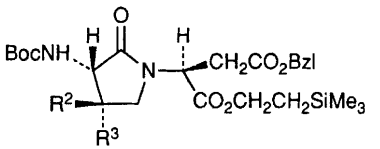
(20) $R^1 = R^2 = H$
(21) R^1 = Me, $R^2 = CH_2—CH{=}CH_2$ or CH_2Ph



(22)



(23a)



(23b)

C-terminus of [2,4-MePro^3]-TRH, but does not mimic exactly all the conformations of proline in the native hormone.

trans-1-Aminocyclobutane-1,3-dicarboxylic acid, and analogues containing phosphonic acid and carboxyl substituents (15) - (17) have been prepared[36] for evaluation as agonists or antagonists at the N-methyl-D-aspartic acid (NMDA) receptors. Best result was achieved for *trans*-(15) which turned out to be 20 times more active at the receptor than NMDA itself, while its *cis* counterpart was only one-third as potent. A conformationally-rigid analogue (18) of glutamic acid has been synthesised[37] to study vitamin K-dependent carboxylation of glutamic acid-containing substrates. The Bucherer-Bergs reaction starting with 3-carboxy-4-cyclohexenone was the source of racemic (18) but on coupling with L-leucine the diastereoisomeric dipeptides could be resolved to give both isomers as confirmed by c.d. measurements. The synthesis of both *cis*- and *trans*-forms of the rather interesting rigid analogue (19) of D-arginine methyl ester has been reported[38].

α,α-Dialkylated glycine containing peptides in general are quite difficult to synthesise, and increasing the steric bulk of the α-substituents causes further problems. It is not therefore surprising that a four-component condensation reaction[39] (the Ugi method) required high pressure (9 kbar) to succeed. Moderate yields of tripeptides based on Z-Val-X-GlyOMe, where X varied from α,α-diisopropylglycine to α,α-diphenylglycine were obtained. The β-lactam ring has been used successfully[40] to serve as a chiral auxiliary to direct incoming alkylations at a neighbouring site. Thus sequential treatment of the derivative (20) with alkylating agents can give rise to (21) (or its diastereoisomer if the order of alkylation is reversed). Deprotection of (21) with trifluoroacetic acid followed by reductive ring cleavage (Li/NH_3) gave H-Phe-NH-$CR^1R^2CO_2H$ with R^1 and R^2 the same as for (21). The previously reported azirine/oxazolone method has been applied successfully[41] to the synthesis of the Aib (α-aminoisobutyric acid) residues within the 12-20 nonapeptide fragment (Z-Leu-Aib-Pro-Val-Aib-Aib-Glu(Bzl)-Gln-L-NHCH(CH_2Ph)CH_2OH) of the ionophore alamethicin. Key synthons for these insertions were the intermediate 3-amino-2,2-dimethyl-2H-azirines (22) with R = Me or Ph.

The conformational effects derived from having α,α-disubstituted residues present in peptides have been explored using a number of physical techniques. Energy calculations[42] on right-handed helical structures of L-Ala and α-MeAla oligomers revealed a new 3.6_{10}-helix structure which has relevance to the formation of voltage-sensitive ion-channels. X-ray diffraction studies[43] on Boc-(L-Leu-$Aib_2)_2$-OBzl show the presence of two 3_{10}-helical forms A and B in the unit cell. Molecule A folds into a right-handed helix while B folds into a left-handed one. Comparisons made from X-ray data[44] on crystals of Boc-Aib-Ala-Leu-Ala-Aib-Aib-Leu-Ala-Leu-Aib-OMe, and Boc-Aib-Ala-Aib-Ala-Leu-Ala-Leu-Aib-Leu-Aib-OMe show both to be predominantly α-helical. Residue exchanges of Aib

with Ala and Aib with Leu therefore do not offer any changes in conformation. The helical decapeptide Boc-Aib-Ala-Leu-Ala-Leu-Aib-Leu-Ala-Leu-Aib-OMe seems to be able to exist in three crystalline polymorphs[45], depending on whether crystals are grown from methanol, isopropanol or ethylene glycol/ethanol mixtures. The main differences found was that antiparallel helix aggregation occurred in crystals from methanol while parallel packing was observed in the other crystals. A short segment of 3_{10}-helix at the N-terminus was found[46] to be present in Boc-(Val-Ala-Leu-Aib)$_4$-OMe but Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-(Val-Ala-Leu-Aib)$_2$-OMe has been shown to be entirely α-helical. There is also evidence[47] that the nature of the N-terminal protecting group affects the aggregation properties. The N-Boc derivative of -Trp-Ile-Ala-Aib-Ile-Val-Aib-Leu-Aib-Pro-OMe crystallised as a parallel aggregate while the N-acyl derivative settled in an antiparallel fashion.

As seen already in this section the stereochemically constrained Aib residues are very capable of supporting extended α-helical conformations. The introduction of a strong β-turn promoting segment into these peptides has been monitored[48] using the synthesised peptide Boc-Val-Val-Aib-Pro-Val-Val-Val-OMe. Nmr studies on this heptapeptide showed significant solvent dependence. In chloroform, as in the crystal a 3_{10}-helical structure was favoured, while in D_6-DMSO an Aib-Pro β-turn was indicated. X-ray and theoretical methods have also confirmed[49] that Z-Ala-Aib-Aib-OH adopts a consecutive type III β-turn which characterises a right-handed 3_{10}-helix.

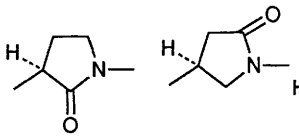
Synthesis of α,α-diphenylglycine derivatives and corresponding dipeptides has been carried out[50] *via* the 5(4H)-oxazolone method. Ir and nmr studies on the compounds were in agreement with energy calculations worked out for these conformationally restricted molecules. Similarly, studies[51] on Ac-NHC(CH_2Ph)$_2$CO-NHMe, and as dipeptide derivatives such as CF_3CO-NHC(CH_2Ph)$_2$CO-Gly-NHN(Bzl)$_2$ have confirmed the same pattern, with the minimum energy conformation falling in the fully-extended (C_5) region. Two α-helix forming sequential peptides, *p*BrBz-(Aib-Ala)$_5$-OMe and *p*BrBz-(Aib-Ala)$_6$-OMe have been studied[52] by X-ray diffraction, and found to be basically α-helical with 1←5 H-bonds but with some deviation towards 1←4 and 1←6 type H-bonds at their C-termini.

3. Conformationally Restricted Cyclic and Bridged Analogues

Much of the ethos and justification for restructuring the multiple, rapidly-changing conformational forms of most biologically active peptides has been the subject of recent reviews[53-55].

3.1 Rings and Bridges formed *via* Amide Bonds - The conformational constraints imposed by γ-lactams in peptides have been studied[56] using valence force field energy calculations and flexible geometry maps. γ-Lactams (23a) and (23b) designed with orthogonal protecting groups suitable for incorporation into larger peptides have been synthesised[57] efficiently starting from commercially available Boc-Asp(Bzl)-OH. The intermediate $BzlO_2CCH(CHO)$-$CH(NHBoc)CO_2CH_2CH_2$-$SiMe_3$ served as a common precursor to both (23a) and (23b). The relative stereochemistry of the substituents on the rings was established by ^1H nmr techniques. Previous publications by Friedinger *et al.* have established the imide (24) as a conformational constraint. Its γ-lactam analogue (25) has now been synthesised[58], as yet only as a racemate, and inserted into human growth hormone (7-13) analogue (26) using solid phase techniques. Analogue (26) was longer lasting in activity than its imide analogue due possibly to the greater stability of the γ-lactam to physiological degradation.

A bicyclic conformational constraint as depicted in (27) has been incorporated[59] into a substance P(SP)-related sequence culminating in a competitive antagonist GR71251 with high affinity ($pK_B = 7.7$) and selectivity for NK-1 receptors. Previous work had established the C-terminal hexapeptide analogue [Ava^6]-SP(6-11) as the active core for modification (Ava = δ-aminovaleryl). Analogue (28), the (*R*)-spirolactam, was a full agonist at NK-1 receptors but nine-fold less potent than the parent Ava-Phe-Phe-Gly-Leu-Met-NH_2. The (*S*)-form showed no agonist activity but acted as an antagonist of substance P methyl ester. Replacement of the C-terminal Gly residue in glutathione by β-Ala or γ-aminobutyric acid (GABA) has provided[60] the opportunity of making the cyclic analogues, *cyclo*(Glu[Cys-β-Ala]-OH) and *cyclo*(Glu[Cys-GABA-]-OH) using the pentafluorophenyl ester for cyclisation. Only low cytotoxic activities against three types of human tumour cell lines were achieved by the compounds. A β-Ala-β-Ala dipeptide has been inserted[61] as a putative cyclisation arm into *cyclo*-(Pro-Phe-β-Ala-β-Ala-) to force the two α-residues into a β-turn conformation. A crystal structure of this cyclic analogue confirmed that there was an intramolecular H-bond between the β-Ala^4CO and the β-Ala^3 residue which stabilised a β-turn incorporating the other residues. Bridging strategically-placed lysine residues with the succinic acid moiety has produced[62] highly selective cholecystokinin analogues (29)-(32). When compared to potent CCK analogues Boc[$Nle^{28,31}$]-CCK-7 and Boc-Trp-Leu-Asp-NH_2 (33), the receptor specificity between pancreatic receptors and their brain counterparts was approx. 10,000 for (32) and 2000 for (31) with affinities comparable to that of (33). By first of all studying[63] the molecular mechanics of the 'bare' low-energy ring structures of a series of nine cyclic constrained dermorphin analogues (34)-(42) only four low-energy ring conformers were found in each. When the Tyr^1 moiety was then incorporated, only the analogues (34)-(38) which are known to have high μ-receptor affinity showed a



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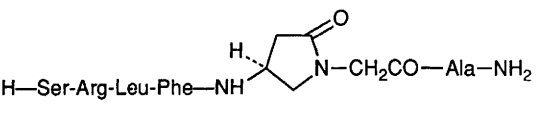

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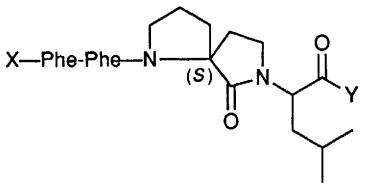

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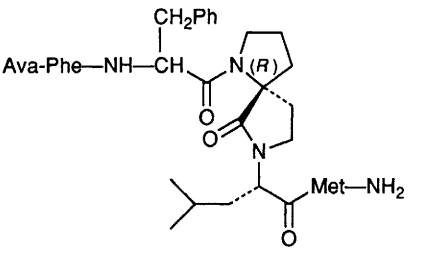

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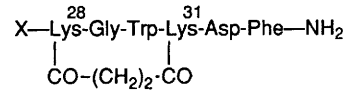
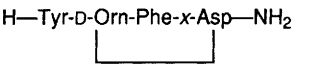
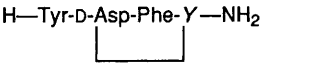


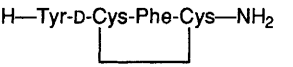
(29) X = Ac—$TyrSO_3H$ (31) X = H
(30) X = Ac—Tyr (32) X = Ac



(34) *x* = L (35) *x* = D



(36) *Y* = Orn (37) *Y* = A_2bu



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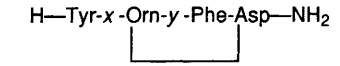
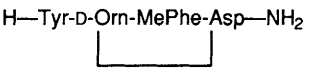


(39) *x* = L, *y* = L (40) *x* = D, *y* = D



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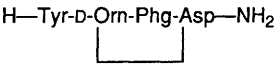
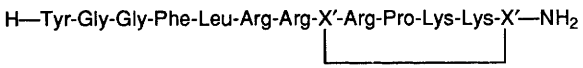
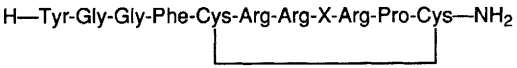


(42) Phg = phenylglycine



(43) X′ = L- and D-Cys



(44) X = Ile, D-Ala

tilted stacking between the Tyr[1] and Phe[3] aromatic rings in the low energy conformers. The analogues (39)-(42) with poor affinity for the μ-receptor showed no stacking. Cyclic tetrapeptides (34) and (36) have also been studied[64] in more conformational detail using ^{1}H nmr, molecular dynamics and energy minimisation. It is implied that the constrained ring maintains the relative orientation of the exocyclic Tyr and Phe aromatic rings which is conducive to μ-receptor affinity at the expense of the δ-opioid activity.

Residues 127-132 of murine tumour necrosis factor have been constrained[65] into a cyclic hexapeptide *cyclo*(Lys-Gly-Asp-Gln-Leu-Ser) using the pentafluorophenyl ester as the linear precursor. The cyclic analogue displayed weak cytotoxic activity on 3 of 4 human tumour cell lines. The superactive somatostatin cyclic peptide (Veber *et al*) *cyclo*(Pro-Phe-D-Trp-Lys-Thr-Phe) which inhibits release of growth hormone has itself been the focus of analogue studies[66]. As seen in Table 2 analogue (W) was designed to replace Pro with the thiazolidine-4-carboxylic acid residue (Thz) to test the effect of reducing *cis-trans* isomerism. The others (X, Y and Z) are retro-inverso analogues, and the preliminary results of the biological tests are listed in Table 2.

Table 2

Relative molar potencies in inhibition of release of growth hormone *in vitro*

Analogue	Relative Molar Potency	
Native somatostatin	1.0	
cyclo(Pro-Phe-D-Trp-Lys-Thr-Phe)	0.21	
cyclo(Thz-Phe-D-Trp-Lys-Thr-Phe) (W)	0.41	
cyclo(gSar-*R,S*-mPhe-D-Trp-Lys-Thr) (X)	0.11	(*S*-form, *R*-inactive)
cyclo(Pro-Phe-D-Trp-Lys-gVal-*R,S*-mPhe) (Y)	Inactive	
cyclo(*R,S*-mAla-Phe-D-Trp-Lys-Thr-gPhe) (Z)	Inactive	

Conformational analysis using ^{1}H nmr and molecular dynamics on the analogues revealed[67] in most cases the presence of a βII′-turn about D-Trp-Lys usually postulated as a requirement for biological activity. This turn apparently maintains the proper orientation of the Phe, Trp and Lys side-chains. Cyclisation of the linear precursor to H – Tyr – D – Lys – Gly – NH – $CH(CH_2 - Ph - pNO_2)$CO – Leu was carried out[68] using diphenylphosphoryl azide. Uv and fluorescence spectral methods indicated that the substituted phenyl ring within the bridge was further away from the tyrosyl side-chain in the analogue than it was in the linear precursor. Diphenylphosphoryl azide in the presence of K_2HPO_4 was also the reagent of choice

in the synthesis[69] of cyclic analogues of the active 3-7 fragment of the serum thymic factor giving *cyclo*(Gly-Lys-Ser-Gln(or Pro)-Gly-Gly) and Lys – Ser – Gln(or Pro) – Gly – Gly – Gly.

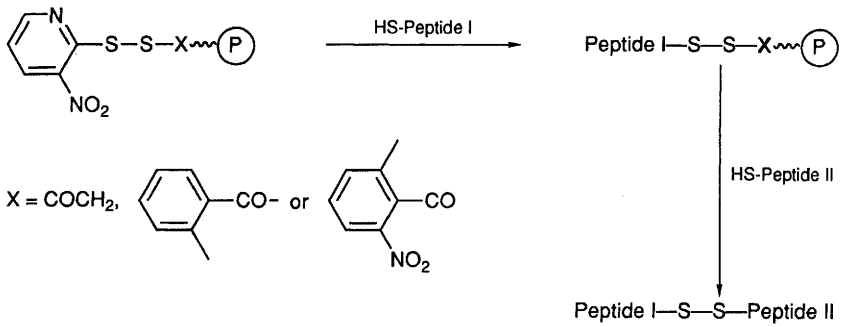
3.2 Bridges formed by Disulfide Bonds - A systematic study[70] of the effects of conformationally restraining angiotensin II(AII), H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH has been reported using homocysteine (Hcy) residues at residues 3 and 5 to provide the disulfide bridge. [$Hcy^{3,5}$]-AII was shown to have high contractile activity ($pD_2 = 8.48$ compared to 8.81 for AII) and an excellent binding affinity with an IC_{50} value of 2.1 nM (AII 2.2 nM). [Sar^1, $Hcy^{3,5}$, Ile^8]AII with a $pA_2 = 9.09$ and an IC_{50} of 0.9 nM proved to be a highly potent antagonist. Cyclic dynorphin analogues (43) and (44) were designed[71] to investigate the effect of conformational restraints on the putative address segment. Analogue (43) possessed high κ and μ opioid affinities centrally (guinea pig brain) but only weak activity at the peripheral κ and μ opioid receptors (guinea pig ileum). Cyclic compound (44) showed the reverse phenomenon and may suggest the existence of distinct κ and μ opioid receptor subtypes for the central and peripheral nervous systems. Substitution of L- or D-penicillinamine or D-cysteine instead of Cys residues at positions 7 and 23 in α-human atrial natriuretic peptide (α-hANP) followed by disulfide linking[72] did not seem to alter the receptor binding activity. Accumulation of cyclic guanosine monophosphate and vasorelaxant activity was observed, but the accumulation alone did not always promote the vasorelaxation in all the analogues tested. With enkephalin analogue H – Tyr – D – Pen – Gly – Phe – D – Pen – OH (DPDPE) recognised as one of the most selective δ-opioid receptor agonists known it is not surprising that similar ring analogues have been subjected to computational studies[73]. Thus model compounds D – Pen – Gly – Ala – D – Pen – OH, D – Cys – Gly – Ala – D – Cys – OH, D – Cys – Gly – Ala – D – Pen – OH and their C-terminal L-analogues have been 'generated' with the RNGCFM programme and energy minimised with the AMBER programme. Conformations with a positive dihedral angle of the disulfide bond were seen for the sequence which is similar to DPDPE and agrees with previous proposals on the conformation associated with δ-receptor selectivity. The determination and description of models of μ- and δ-receptor bound cyclic enkephalin analogues with a Phe residue at position 4 has been carried out[74] by comparing the geometrical similarity amongst low-energy structures for [D-Cys^2,Cys^5]-, [D-Cys^2,D-Cys^5]-, [D-Pen^2,L-Pen^5]- and [D-Pen^2,-D-Pen^5]-enkephalinamide. The results indicated a μ-receptor bound β-I bend centred on the Gly^3-Phe^4 region. More extended conformations seemed prominent for δ–receptor bound conformations. Superactive octapeptide cyclic analogues of somatostatin involved in growth hormone inhibition are also more potent than somatostatin-14 in suppressing gastric acid secretion. Analogues tested in this way

were[75] H – D – Phe – Cys – Tyr – D – Trp – Lys – Val – Cys – Trp(or Thr) – NH_2 (RC-160) and D – Trp – Cys – Phe – D – Trp – Lys – Thr – Cys – Thr – NH_2 (RC-121).

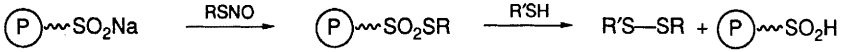
It has now become easier to consider synthesis of disulfide bonds in the solid phase synthetic context. By careful selection of the relative "acidity" of the thiol components it has been possible[76] to apply a routine based on Scheme 8. Thiosulphonates functioning as immobilised reagents on a polystyrene support have been used[77] in the effective synthesis of mixed disulfides, as outlined in Scheme 9. A mixed disulfide of glutathione and 2-mercaptoethane sulfonic acid were generated in this manner. Synthesis of porcine brain natriuretic peptide-32 utilised[78] silver tetrafluoroborate as a new protecting group for S-trimethylacetamidomethyl and S-acetamidomethyl cysteine residues. Although not strictly a disulfide linker bridge quite an interesting constrained peptide (45) has been prepared *via* a bis-thioether link between two histidine imidazole rings.

3.3 Miscellaneous Bridges and β-Turn Mimetics - As a means of checking the importance of *cis-trans* proline isomerisation in the biological activity of morphiceptin H-Tyr-Pro-Phe-Pro-NH_2, a selective agonist for the μ-receptor, the synthesis has been reported[80] of analogues containing 2-amino-cyclopentane carboxylic acid residues (46) (β-Ac^5c) at the Pro^2 position. The analogue containing *R,S*-β-Ac^5c was active at both the μ- and δ-receptors, while (*S,R*),(*S,S*) and (*R,R*) analogues showed minimal activity at the μ-receptor and are inactive at the δ-receptor. *Cis* and *Trans*-3-Substituted proline residues have also been synthesised[81] in optically pure forms. 5,5-Dimethylthiazolidine-4-carboxylic acid residue (Dtc) (47) has been developed[82] as a conformationally-restricted analogue of Pro. Spectroscopic evidence derived from Boc-Dtc-Ile-OMe showed that two slowly exchanging *cis-trans* isomers were present with both forms showing amide proton resonances upfield at δ6.67 and 6.76 corresponding to the non-hydrogen bonded Ile-NH. X-ray data showed that only *cis*-Boc-Dtc methane amide bond was present but two Dtc ring conformations were seen, one with the β-C atom *anti* to COOH, the other with the γ-S-atom *anti*. With the availability of a highly stereoselective synthesis[83] of 1,2,3-trisubstituted cyclopropanes (48) and (49), their influence on the conformation of constrained analogues can be explored. The four diastereoisomers of D-3,4′-cyclopropylglutamate (50) have been synthesised[84] using medium pressure liquid chromatography to separate the diastereoisomers. The (2*R*,3*S*,4*R*) configuration proved the most potent and selective NMDA receptor ligand.

A β-turn mimetic design has been based[85] on a nine-membered ring lactam system. The model dipeptide mimetic (51) has a *trans* amide bond with groups at neighbouring positions corresponding closely to the side-chain positions of residues

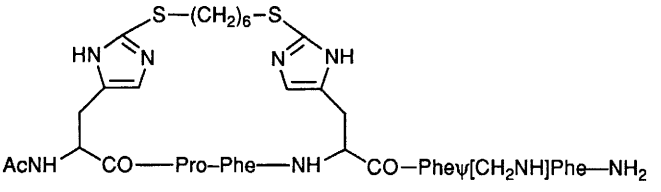


Scheme 8



R = $CH_2CH_2SO_2Na$ or $CH_2CH_2SO_3Na$ or Ac—Cys—OH
R′ = Ac—Cys—OH, H—Cys—OH or reduced glutathione

Scheme 9



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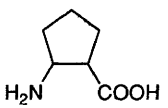

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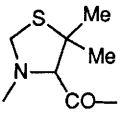

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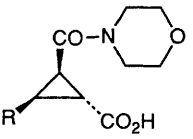

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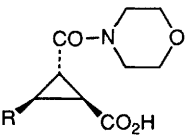

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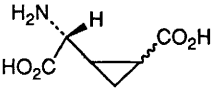
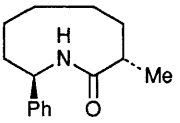


(50)



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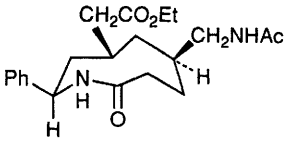


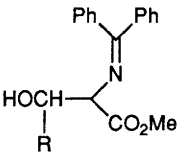
(52)

i+1 and i+2 of a classical type II β-turn. In the model (52) all four side-chains are well matched in the low energy *trans* amide conformer to their peptide counterparts.

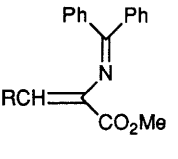
4. Dehydroamino Acid Analogues

Synthesis of dehydroalanine residues from diphenylphosphonoserine derivatives NH-CH[$CH_2OP(=O)(OPh)_2$]-CO- can be carried out[86] by application of triethylamine when the serine derivative is C-terminal but the base DABCO is needed for an internal or N-terminal residue. Inexpensive β-hydroxy amino acid derivatives such as (53) readily lose the elements of water in the presence of diisopropylcarbodiimide copper(I) chloride to give (54) with absolute geometric selectivity[87]. L-Forms of Thr and Phe lead to (*E*)-isomers of (54), but the (*Z*) isomers can be obtained by heating the (*E*) isomer (145°C) or using piperidine at 60°C. Various kinds of N-benzyloxycarbonyl-protected dehydro amino acid esters have been obtained[88] *via* the condensation of α-oxocarboxylic acids with $PhCH_2OCONH_2$, or by the Wittig-Horner reaction of aldehydes with $(EtO)_2P(=O)CH(NHZ)CO_2Me$. Dehydroamino acid analogues have been used[89] to gain insight into the structural requirements for the inhibition of N-acetylated α-linked acidic dipeptidase (NAALA dipeptidase). The most active inhibitors studied were (*E*)-$HO_2CCH{=}CH$-CO-Glu-OH, $HO_2CCH_2CH_2CO$-Glu-OH, and (*Z*)-$HO_2CCH{=}C(NAc)CO$-Glu-OH with K_i values of 0.9, 0.4 and 1.4 μM, respectively. The relative spacing between side-chain and α-carboxyls appears to be important for binding to the active site.

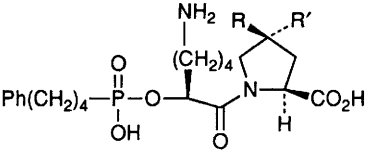
The dehydro-residues remain popular as structural design units in X-ray investigations. The results of X-ray studies[90] on 19 such residues have been analysed and all confirm that the dehydro units are essentially planar. A type II β-turn forms if the Δ-residue is placed either at the (i+1) or at the (i+2) corners of a β-turn. Where there are consecutive Δ-residues the backbone folds into an alternating right- and left-handed α-helix. Similar types of β-turns have been found in Boc-Phe-ΔLeu-Val-OMe[91] and Boc-Phe-Pro-ΔPhe-Gly-OH[92] but Boc-D-Ala-ΔPhe-Gly-ΔPhe-D-Ala-OMe showed in an X-ray study[93] the presence of two type III β-turns. The peptide adopts a left-handed 3_{10}-helical conformation due to the D-Ala and the two Δ-residues are located in the i+1 position of the first β-turn, and in the i+2 position of the second β-turn. ^1H Nmr studies[94] at 500 mHz on a heptapeptide with Δ-residues separated by three amino-acid residues, Boc-Gly-ΔPhe-Ala-Phe-Leu-ΔPhe-Ala-NHMe, have indicated that in chloroform there is a significant population of folded and α-helical structures. In $(CD_3)_2SO$ there was evidence of conformational heterogeneity, although the major component was helical. The full complement of physical methods has been applied[95] to Boc-X-ΔAla-$NHCH_3$ where X = Ala, Val or Phe. N.O.e. studies indicated an inverse



(53)



(54)



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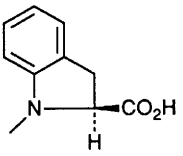

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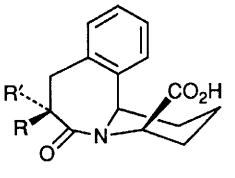

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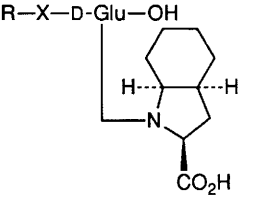
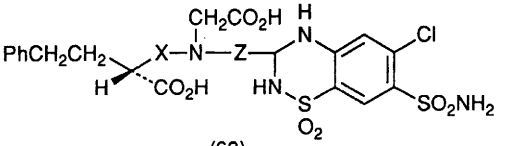


(58) R = Z
(59) R = aryl, aryloxy or cycloalkoxy; X = Lys



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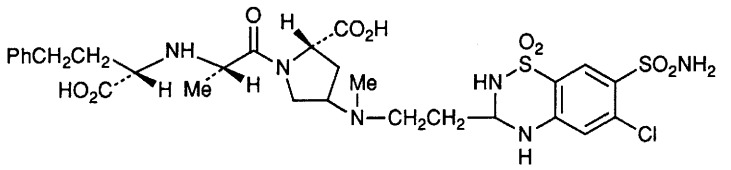
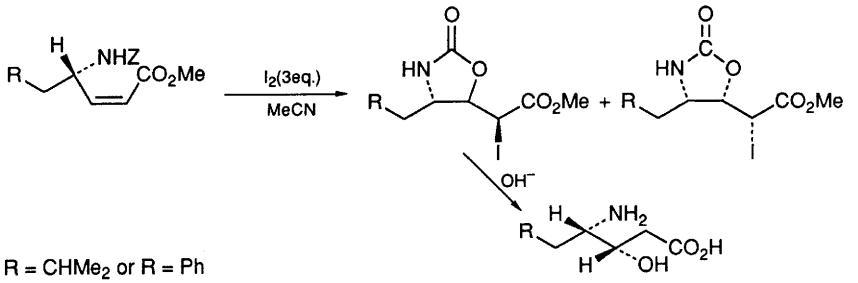


(61)



R = $CHMe_2$ or R = Ph

Scheme 10[105]

γ-turn conformation, so that ΔAla seems to be different from ΔPhe and ΔLeu which tend to stabilise β-turns.

Hydrogenation of dehydrodipeptides of structure PhCH=C(NHAc)CO-Pro-OH in the presence of various metal ions gave a variable set of diastereoisomeric excesses. Amongst the best[96] was hydrogenation over $CaCl_2$ and a Pd-containing polymer which gave a diastereoisomeric excess of 88%.

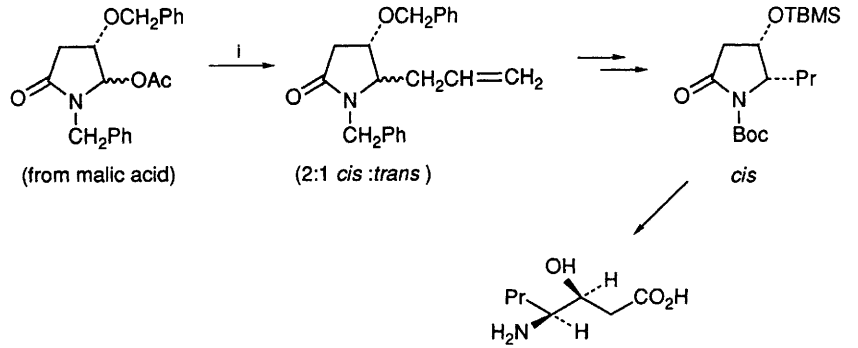
5. Enzyme Inhibitors

A useful reference source for enzyme inhibitors has been published[97].

5.1 Angiotensin Converting Enzyme (ACE) Inhibitors - X-ray studies[98] on two potent ACE inhibitors, *(5S)*-5-benzamido-6-phenylhexanoyl-L-Pro-OH and *(1S,2R)* 1-[(2-benzoylthio)cyclopentyl carbonyl]-L-Pro-OH showed interatomic distances similar to captopril and the enzyme substrate hippuryl-L-His-L-Leu-OH. However, the co-ordination distances and bond angles seem different from assumed values for the ACE active site, and provide an alternative model for the interaction of ligands. Analogues of (55) R=R^1=H (SQ 29,852), in which the terminal Pro residue has in turn been substituted by a variety of substituted prolines, N-aryl glycines and bicyclic amino acids have been synthesised and tested[99]. Addition of lipophilic substituents to the Pro^4-position resulted in substantial increases to the *in vitro* activity, but only modest *in vivo* increases. The indoline replacement (56) for proline was by far the best on i.v. administration to normotensive rats. Activities comparable to captopril have been achieved[100] for the conformationally restricted ACE inhibitors of general structure (57) where R^1 and R represent CH_2SH as a pair of antipodes. A number of D-γ-glutamyl tripeptides have been synthesised[101] and tested for ACE inhibition. Introduction of Lys or Orn into the P_1 position (X in structure (58) provided the most potent inhibitors, some of which exhibited oral antihypertensive activity. These results have obviously led[102] to a new series of inhibitors based on structure (59). *In vitro* inhibitory activity at the nanomolar level and antihypertensive potency at an oral dose of 10 mg/Kg was achieved with compounds in the (59) series, the best being (59) R = 2-$ClC_6H_4CH_2O$, 2-$FC_6H_4CH_2O$, 4-FC_6H_4, 4-HOC_6H_4 or 3-pyridyl, which were as long lasting in their effect as enalapril. An attempt has been made[103] to combine into the same molecule the diuretic effect of sulfonamide moieties with the antihypertensive properties of ACE inhibitor molecules. Structures (60)[103] and (61)[104] represent the typical approaches. IC_{50} Values for ACE inhibition as low as 7 nM were observed and discernible diuretic activity was seen for several hydrochlorothiazide-based moieties. Compound (61) has been chosen for further development.

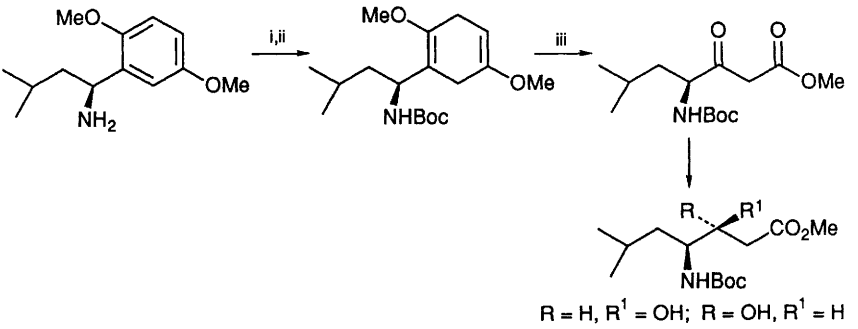
5.2 Renin Inhibitors - This section has seen the biggest growth in activity during the year. There are already several syntheses of statine and its analogues reported over the last 3 or 4 years, but a number this year concentrate on the stereoselectivity achieved. Stereo-control has been achieved in various ways and the chemistry underlying the many routes has been summarised in Schemes 10 → 16 (References 105-111). Stereochemical control has also been achieved *via* a β-lactam ring (62)[112] which readily opens to give renin inhibitor precursors such as (63) or by means of the chiral imine (64)[113] which in the presence of $CeCl_3$ gave (65). Dihydroxyethylene isostere (66) has been synthesised[114] using a Sharpless epoxidation as a key step in its formation. γ-Keto-δ-amino acid unit (67) has been synthesised[115] as a precursor molecule for enzyme inhibitors, and used in another report[116] for further derivatisation to a Leu-Val isostere following Scheme 17. Using a very similar synthetic plan to that summarised in Scheme 13 statine, ketomethylene and hydroxyethylene dipeptide isosteres have been produced[117] from norleucine and lysine aldehydes of general formula (*S*)-Boc-NHCH(R)CHO where R = Bu or $(CH_2)_4NH$-Z, and $Me_3SiCH_2CH{=}CH_2$ in the presence of titanium chloride. Boron-mediated hydroxylation followed by $RuCl_3/NaIO_4$ gave (68) which on alkylation gave (69) in three stages. By starting with the aldehydes Z-D-Phe-H or Z-D-Leu-H in the presence of Me_3SiCN followed by hydrolysis, a one-pot procedure[118] yielded *(2S,3R)*-$H_2NCH(CH_2Ph)CH(OH)CO_2H$ and *(2S,3R)*-$H_2NCH(CH_2CHMe_2)CH(OH)CO_2H$. Suitably protected aldehydes under phase transfer conditions[119] were converted diastereoselectively to cyanohydrin acetates which then yielded *(2R,3S)*- and *(2S,3R)*-3-amino carboxylic acids.

The intensive search within the pharmaceutical industry for renin inhibitors has certainly spawned a number of publications this year. Abbott Laboratories's successful incorporation of (70) has been followed up with a six-step construction of the unit in 36% yield[120], while a dihydroxy difluoromethylene dipeptide mimic (71) has been reported[121] as a potent human renin inhibitor (IC_{50} = 6.5 x 10^{-10}M). An efficient stereospecific synthesis of the related ketodifluoromethylene analogue (72) has also been reported[122]. The angiotensinogen transition state mimic (73) has been shown[123] to be an orally potent human renin inhibitor (10-20 mmHg drop in mean blood pressure and a reduction of plasma renin level for 5 hr in monkeys). Its interactions with the protease has been studied by modelling techniques which have drawn the conclusions, (i) the cyclohexyl and naphthyl groups are accommodated in large hydrophobic subsites S_1 and S_3 and the histidine imidazole was H-bonded to the CH_2OH of Ser-233, (ii) the cyclohexylnorstatine iso-propyl ester residue was accommodated in S_1-S_1'. The stereochemistry represented in (71) had to be present for maximal potency. A simple one-pot synthesis of (73) has been presented[124], and stages to the synthesis capable of being carried out on a large scale have also been reported[125,126]. The requirement for the N-terminal component interaction with a hydrophobic subsite in renin has been explored[127] *via* the analogues (74). Inhibition



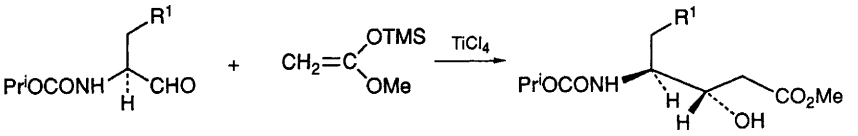
Reagent: i, allylsilane/$SnCl_4$

Scheme 11[106]

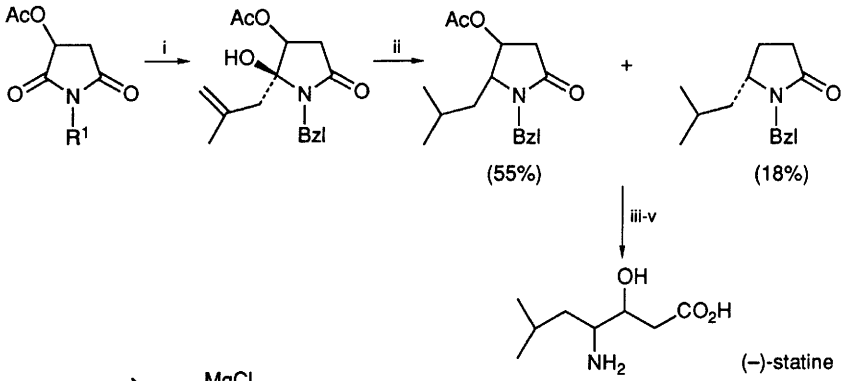


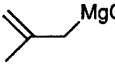
Reagents: i, $(Boc)_2O$; ii, Na/NH_3; iii, O_3/reduction

Scheme 12[107]

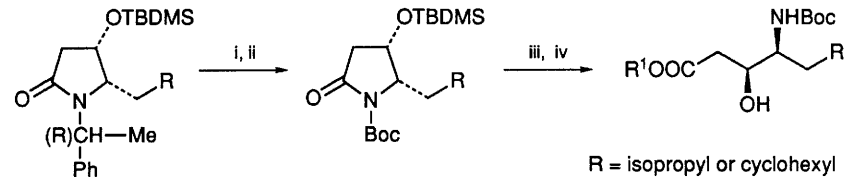


Scheme 13[108]



Reagents: i,  ; ii, H_2/10%Pd-C; iii, HCl/MeOH/60°C;
iv; Na/NH_3,–78°C; v, 6M HCl/110°C

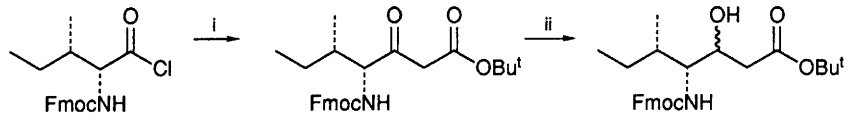
Scheme 14[109]



R = isopropyl or cyclohexyl

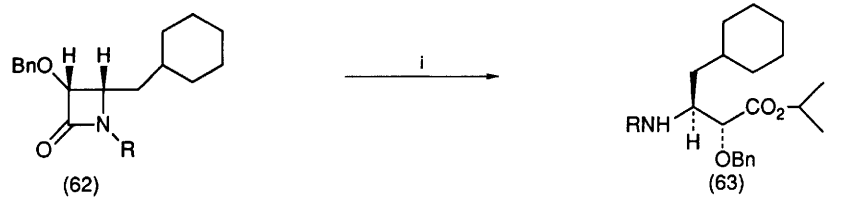
Reagents: i, Na/NH_3; ii, Boc_2O; iii, NaOMe; iv, $Bu^n{}_4NF$

Scheme 15[110]

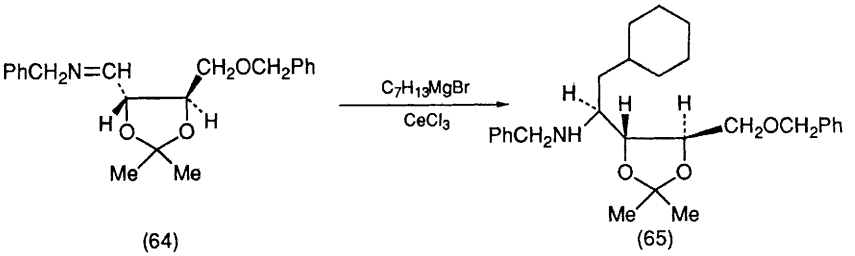


Reagents: i, $Li^+{}^-CH_2CO_2Bu^t$/–80°C/THF; ii, KBH_4/EtOH, 0°C, Purified to
(3*S*, 4*R*, 5*S*) form

Scheme 16[111]

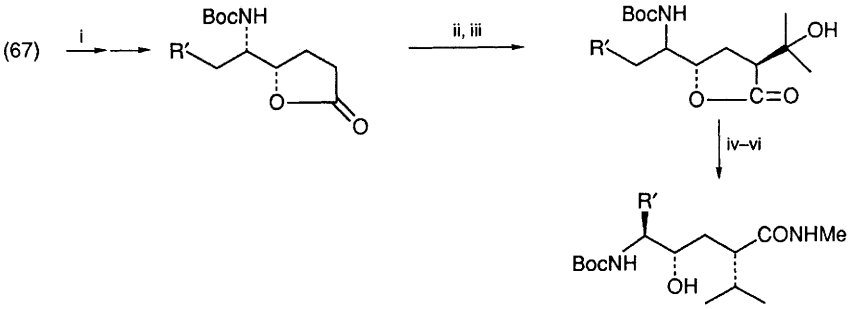


Reagent: i, HCl/Pr^{i}OH



(64) (65)

(66) (67) R = Me, or Et

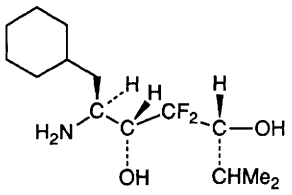


Reagents: i, $RuBr_2$[(*R*)-binap]; ii, LDA; iii, Me_2CO; iv, MeOCOCOCl; v, $Bu^n{}_3SnH$; vi, $MeNH_2$/MeOH

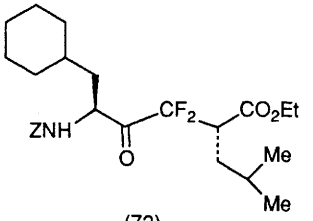
Scheme 17

(68) R = H
(69) R = alkyl

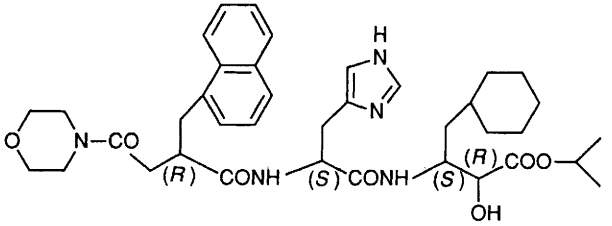
(70)



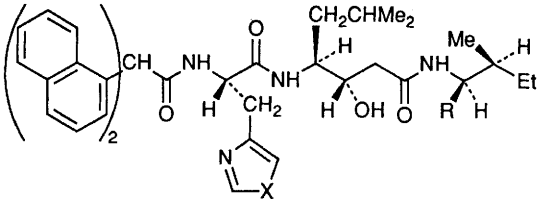
(71)



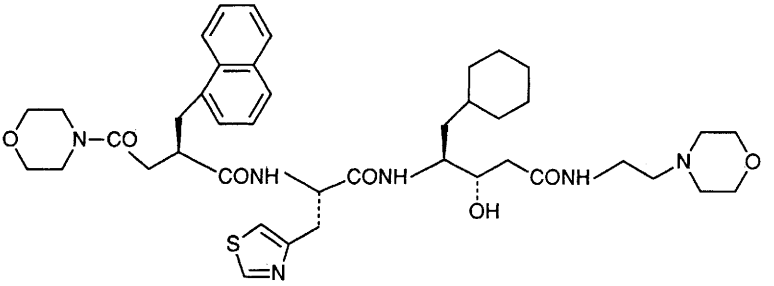
(72)



(73)



(74) X = NH, S, R = H; X = S, R = CH_2OH



(75)

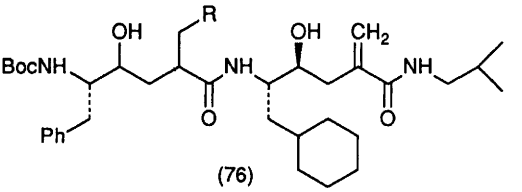
values obtained were: X = NH, IC_{50} = 9.2 nmol dm^{-3}; X = S, IC_{50} = 0.7 and 1.3 nmol dm^{-3}. A highly potent inhibitor was also obtained[128] from the related structure (75) which had an IC_{50} value of 4.6 x 10^{-9}M and at 3 mg/Kg inhibits the plasma renin of marmosets by more than 80% after 1 hr. Analogues based on the hydroxyethylene isostere (76) with variations at the P_2 site have led[129] to the conclusion that when R = $CHMe_2$ 4-fold less activity was observed, while R = $CH_2CH_2CH{=}CH_2$ improved activity 9-fold.

In a very comprehensive study[130] whereby 13 different isosteric replacements were made at the P_3-P_2 position (Phe-His) in the potent renin inhibitor Boc-Phe-His-Sta-Leu-$NHCH_2Ph$ in the hope of improving stability towards enzymes, it was shown that all replacements aided stability with the hydroxyethylene showing the best results. Boc-Pheψ[$CHOHCH_2$]Gly-ACHPA*-Leu-$NHCH_2Ph$ (IC_{50} = 61 nM) and Boc-Pheψ[$CHOHCH_2$]Gly-ACHPA-Leu-$NHCH_2$(mPh)-CH_2NH_2 (IC_{50} = 22 nM) were the best but neither showed a tendency for blood pressure lowering.

Introduction of a retro-inverso amide bond at the acyl residue has given[131] an analogue (77) which has oral activity. Modification of the N-terminal Phe region of inhibitors by an azaPhe has already been shown to give rise to less inhibition than the parent Boc-Phe-Gly-ACHPA-Ile-3-pyridylmethylamide, but on replacing[132] the Gly with azaGly quite a specific inhibitor of renin (IC_{50} = 2.45 x 10^{-7}M) was obtained, though still less active than the parent peptide. Conformationally restricted cyclopropyl analogues such as (78), suitable for investigation of the P_1-P_1' transition state requirements have been synthesised[133] stereochemically pure. Non-peptidic replacement for the P_4-P_2(Pro-Phe-His) of the natural substrate angiotensinogen has been investigated[134] *via* the pyrazine derivatives (79). Potent inhibition was found with (79a) R = cyclohexyl, $CHMe_2$, R^1 = $CH_2C_6H_4CH_2NH_2$-3; R = cyclohexyl, R^1 = (*S*)-$(CH_2)_4CH(NH_2CO_2H)$ with IC_{50} values of 1.7, 6.8 and 3.7 nM, respectively, and these lowered blood pressure by i.v. administration but not orally, probably due to poor adsorption. Analogue (79b) with an IC_{50} = 0.2 nM showed activity consistent with it binding to the S_4-S_2' human renin sites. Rigidity has also been incorporated[135] into analogues such as (80) where R represented a range of heterocycles such as 4-imidazolylmethyl and n = 1 or 2. A β-aspartyl residue was introduced[136] into the inhibitor (81) as a replacement for His to take advantage of the Thr-84 interaction on the flap region of the enzyme. Compound (81) had an IC_{50} of 5.2 nM. A homostatine-containing inhibitor with a sulfonomethylene isostere at its N-terminus revealed[137] an IC_{50} value of 0.17 nM. This isostere together with other non-standard amino acid residues as in (82) have produced[138] almost a non-peptide orally active renin inhibitor at a level of IC_{50} = 3.3 nM.

Delivery of the many inhibitors to their site of action *in vivo* is still apparently a great problem in drug development, and explains the many attempts at improving

* ACHPA = 4(*S*)-amino-3(*S*)-hydroxy-5-cyclohexylpentanoic acid.



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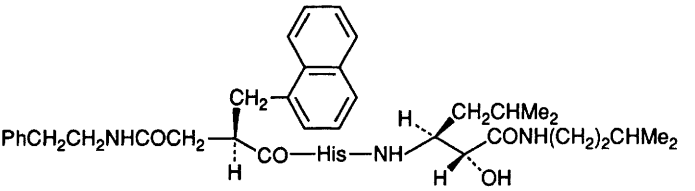

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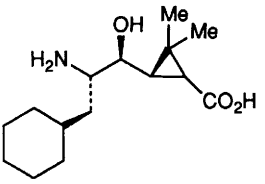
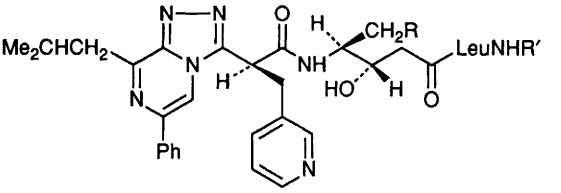


(78) and diastereoisomer



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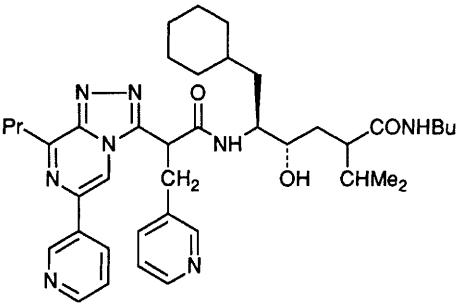

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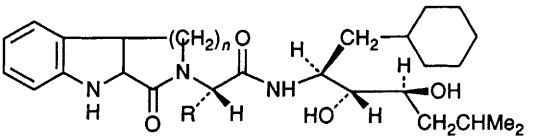

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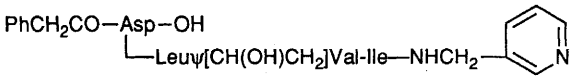

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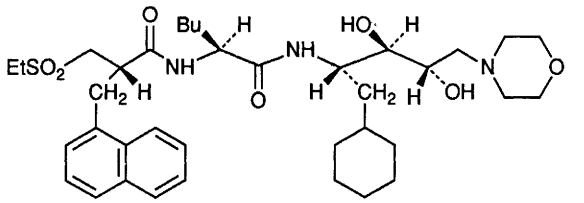

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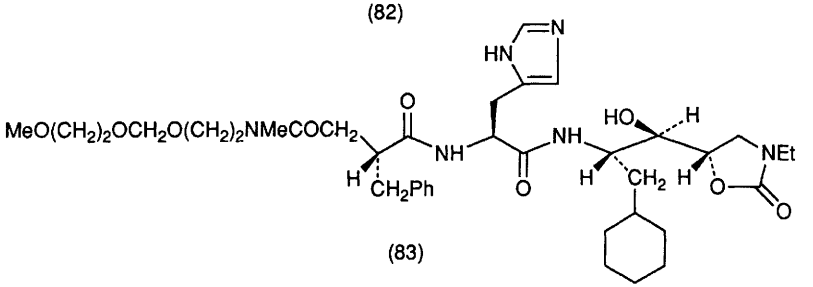

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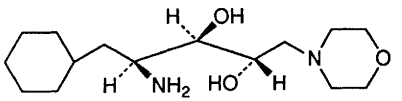

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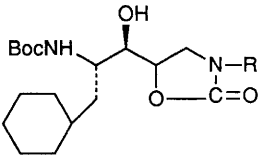

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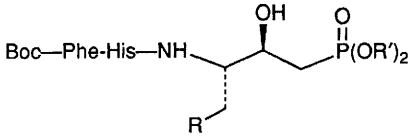

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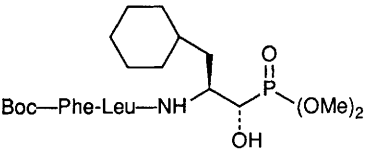

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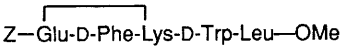

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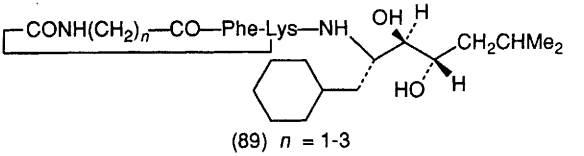


(89) n = 1-3

transport. Prolonged duration of action was achieved[139] with the water-soluble inhibitor (83) upon i.v. administration but again extensive liver action limited its bioavailability. Maybe the answer to delivery problems could come from much simpler inhibitors such as (84)[140] and (85)[141]. The former was synthesised stereospecifically from a protected sugar, while the latter's design was based on modelling studies which had indicated that the oxygen in the heterocyclic ring could H-bond with the flap region of renin. From two independent studies, the potential of using phosphostatine analogues can be gleaned. Thus the Leu^{10}-Val^{11} replacement (86)[142] provided a 130-fold boost in potency over the parent compound, with average potency in the 20-50 nM range for IC_{50} values of the analogues tested. Good inhibition at the IC_{50} 10 nM level was obtained for (87)[143].

Some workers have diversified away from modification of the angiotensinogen tetradecapeptide as a source of inspiration and have concentrated on a major effort to modify the known renin inhibitor Boc-D-Phe-Cys(Acm)-D-Trp-Leu-OMe. In a report[144] on 83 analogues of this sequence the rationale was based on (a) residue replacement, (b) effect of α-aza, α-methyl N-Me and α,β-dehydro group insertion and (c) a study of the binding between substrate and enzyme with residues being added at the N-terminus. Table 3 lists the most potent analogues to

Table 3

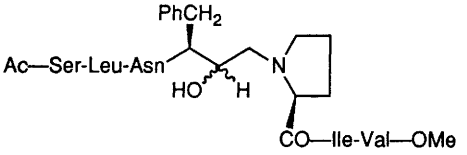
Analogue	Inhibition of human renin IC_{50}/μM
Boc-D-Phe-Cys(Acm)-D-Trp-Leu-OMe	40
Boc-D-Phe-Cys(Acm)-D-Trp-Leu-NHEt	8.5
Boc-D-Phe-Cys(Acm)-D-Trp-Leu-Pro-OMe	5.2
Z-D-Phe-Cys(Acm)-D-Trp-Leu-Ser-OMe	3.2
Boc-D-Phe-Cys(Acm)-D-Trp-Leu-Val-OMe	10.0
Boc-D-Phe-Cys(Acm)-D-Trp-Leu-IleO-Me	6.5
Boc-D-MePhe-Cys(Acm)-D-Trp-Leu-OMe	10.0

give a flavour of what was achieved. Cyclic analogues based on the same sequence were also studied[145]. The general conclusions were that in a series based on (88), the size of the ring (15-membered) could not be varied and the best potency was obtained from an analogue where Z in (88) was replaced by Me_3CCH_2 and LeuOMe replaced by $NHCH_2CH_2CHMe_2$ when the IC_{50} value reached 6.3×10^{-8}M. Cyclic peptides based on D-Phe-Lys-D-Trp only, gave reduced potency. When the previously reported renin inhibitor Boc-Pro-Phe-MeHis-Leuψ[$CHOHCH_2$]-Val-Ile-Amp (Amp = 2-aminomethylpyridine) was modified[146] by placing hydrophilic groups at either end, e.g., tris(hydroxymethyl)aminomethane or glucosamine at the N-terminus and the tris(hydroxymethyl)amido methane or aminopyridine N-oxide at

the C-terminus, good activity was maintained on oral or i.v. administration in a rat model. Cyclisation[147] has also been attempted as a means of stabilising renin inhibitors towards chymotrypsin degradation. The structures (89) were cyclised using phosgene. The renin inhibitors based on the sequence Boc-D-Phe-His-D-Phe-D-Leu-NH$(CH_2)_5CO_2Me$ have also been found[148] to be resistant to proteolytic enzymes.

5.3 Inhibitors of Other Enzymes - Expertise derived from renin and related enzymes is also being ably applied to the discovery of HIV protease inhibitors. Hydroxyethylamine mimics of the tetrahedral intermediate for the hydrolysis of the Tyr-Pro bond have been incorporated[149] into (90) which gave a tight binding at the level K_i = 0.66 nM. Sensitive continuous measurement of HIV protease activity can now be carried out[150] using the fluorescence intensity that is released when HIV protease hydrolyses compound (91) at the Tyr-Pro bond. This assay is believed to have the highest sensitivity of any method so far. A number of isosteric bonds have been introduced[151] into H-Ser-Asn-Val-Phe-Ala-OBzl the smallest inhibitory peptide of myosin light chain kinase, for the study of potency. Reversal of stereochemistry at individual αCH-centres caused loss of potency but the startling result found was that inversion of all centres does not diminish enzyme inhibition. The ψ$[CH_2NH]$ at various positions gave unconvincing results and it was the ψ[CH=CH] insertion that proved to be the best inhibitor with structure (92) having an IC_{50} value of 0.6 μM.

Phosphinic peptide derivatives have been evaluated[152] as inhibitors of the aspartic proteases. The most potent of those studied was compound (93) which gave K_i values of 0.26 and 0.19 nM, respectively, for pepsin and penicillopepsin. A series of N-(monoethylphosphonyl) peptides have been synthesised[153] and their inhibition of purified human skin fibroblast collagenase examined. The most potent, (EtO)(OK)P())-Ile-TrpNHMe was nearly 100 times stronger than (EtO)(OK)P(O)-Ile-Ala-Gly-OK which has the $P_1'P_2'P_3'$ sequence of the α_1I-chain of collagen. Trypsin, plasmin and kallikrein are three enzymes which have been used to study[154] the inhibition properties of a series of leupeptin, Ac-Leu-Leu-DL-Arg-H, analogues. Z-Leu-Leu-Arg-H had reduced potency and Z-Leu-Leu-Lys-H, was less effective than leupeptin with trypsin and plasmin. Z-Leu-Leu-Orn-H showed significant inhibition of kallikrein activity only. The best affinity-labelled inhibitor of calpain has turned out[155] to be H-Leu-Leu-Cys(Npys)-NH_2 with an $IC_{50} = 1.8 \times 10^{-7}$M. Calpain representing a cysteine protease, together with α-chymotrypsin as a serine protease have been subjected to inhibitor studies[156] using α-diketo amino acid derivatives as a novel class of electron deficient CO containing inhibitors. Z-Val-NHCH(CH_2Ph)COCOMe was found to be a potent inhibitor of both types of enzymes. Similar differentially protected keto esters based on lysine have proved[157] to be useful trypsin inhibitors with the formula Ac-Ala-Lys-CO_2CH_3. The same research group have also explored[158] peptidyl fluoromethyl as well as α-keto esters



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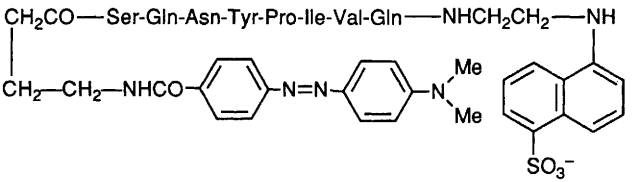

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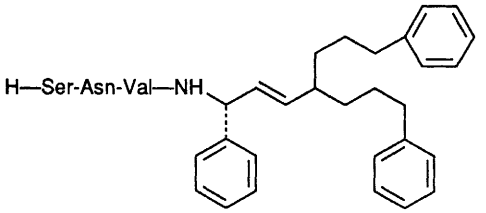

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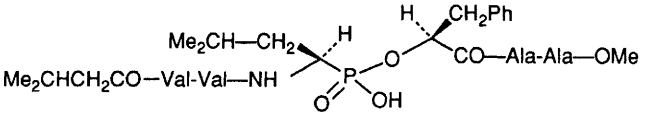

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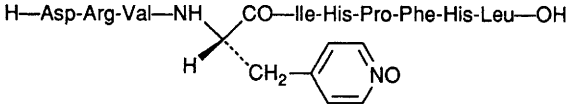

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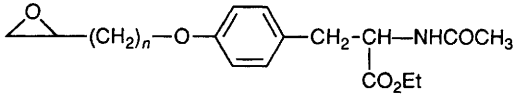


(95)

in elastase and cathepsin G inhibition. In general the keto esters were more potent than their trifluoromethyl ketone equivalent. The most potent elastase inhibitor was found to be N^{α}-Ad-SO_2-N^{ε}-(MeOSucc)-Lys-Pro-Val-CF_3 which has a $K_i = 0.58$ nM. The already patented route to fluorinated ketone analogues of the Phe, Lys and p-guanidino-Phe has now been supported by a report[159] on the experimental details. Conformational studies[160] on (2*S*,3*R*),2,3-methanoPhe-LeuOMe, a serine protease inhibitor have been made using 250 MHz nmr techniques. (*R*)-Homo-β-Pro was more potent than its (*S*)-enantiomer as an inhibitor[161] of $GABA_A$ receptor whereas the $GABA_B$ receptor affinity of homo-β-proline resided exclusively in (*S*)-homo-β–proline.

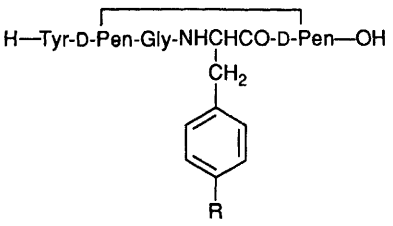
An N-oxide substituent in position 4 of angiotensin I as illustrated by (94) did not produce much inhibitory activity towards protein-tyrosine kinases although it could be synthesised[162] asymmetrically. Better success was obtained in the inhibition of chymotrypsin by using the irreversible inactivator (95) which was only active in the L-form. The thrombin inhibitor and anticoagulant H-D-Phe-Pro-Arg-H tends to cyclise readily in neutral aqueous solution at higher temperature causing deactivation. It was reasoned[164] that N-methylation of the N-terminal position would reduce the cyclisation tendency. A successful selective anticoagulant was achieved with D-MePhe-Pro-Arg-H.

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

A similar level of productivity to last year has been achieved in the work described under this section. Diversity of structure has again made division into suitable sub-headings a difficult task, but after some thought the broad guidelines used last year seemed appropriate again. Subject matter featuring in more than four papers was deemed to have 'earned' a sub-heading. A 'quantitative structure-activity relationships' (QSAR) study[165] does have implications for bioactive peptides under more than one heading. QSAR has relied in the main on the L-forms of the amino acids in defining its descriptor scales z_1z_2 and z_3 (hydrophobicity, bulk and electronic effects). In the present work[165], substance P, enkephalins and bradykinins have been used to establish the effect on the z-scales of changing the chirality of residues within the compounds. It was concluded that the descriptive availability of the models were improved by the introduction of a qualitative chirality variable.

6.1 Peptides with 'Opioid Characteristics' - When the NH_2-terminal position of [D-Ala^2,Leu^5]-enkephalin was quaternized with $CH_3I/KHCO_3$ the product [Me_3^+-Tyr^1,D-Ala^2,Leu^5]-enkephalin and its amide showed[166] only a slight reduction

in potency in the guinea pig ileum (gpi) test. However, the pentamethyl derivative with all amide bonds methylated (prepared from CH_3I/Ag_2O on a protected enkephalin) showed no activity in the gpi test or receptor binding assay. The 'Schwyzer' membrane compartment concept has been brought to bear on a study[167] of enkephalin analogues carrying artificial 'address' peptides designed to investigate the role of membrane affinity in opiate receptor binding. The three peptides investigated were based on H-Tyr-Gly-Gly-Phe-Leu-Gly-Pro-R with R representing -(Lys-Sar-Sar-Sar)$_2$-OMe (96), -(Lys-Pro-Pro-Pro)$_2$-OMe (97), and -(Lys-Aib-Lys-Aib)$_2$-OMe (98). Opioid receptor affinities of (96) and (97) were similar to [Leu]-enkephalinamide indicating that the C-terminal additions had no effect. On the other hand, δ- and μ-receptor affinities of (98) were about a twentieth and a sixth of those of [Leu]-enkephalinamide, thus giving (98) a higher selectivity towards the μ-receptor. The latter molecule was shown to have an amphiphilic α-helical structure and was the best distributed into the lipid bilayer membrane. Analogues with lengthened sequences[168] such as H-X-D-Met-Gly-MePhe[NH(CH$_2$)$_5$CO]$_2$-OEt with X = Tyr or MeTyr have been prepared by solution methods but did not show any opiate receptor agonistic activity. Phenylalanine-substituted analogues of DPDPE (99, R = H) have been prepared[169] by solid phase methods and their potency assessed. All four analogues, (99, R = F, Cl, Br or I) possessed greater δ-receptor affinity than DPDPE in the mouse vas deferens (mvd) assay and in radioreceptor assays. The chloro analogue had $IC_{50}\mu/IC_{50}\delta = 574$, i.e., about 5-fold more δ-opioid receptor selective than DPDPE in the radioligand binding assays whereas the iodo analogue was more selective in the classical bioassays (mvd and gpi). The importance of the Phe side-chain in binding is therefore confirmed. Single residue modification[170] in (99, R = H) have been explored conformationally and pharmacologically. It was concluded that diallyltyrosine at position 1 and phenylglycine at position 4 each create their differences in biological activity due to localised differences in conformation in the vicinity of the inserted residue. Enhancement in μ-receptor-binding due to an amide at the C-terminal appears to be due solely to electronic differences with no change in conformation. The Aib-residue at position 3 showed similar *in vitro* opioid behaviour to (99, R = H) but nmr showed a totally different conformation. Energy calculation studies[171] have aided the search for conformational features responsible for binding cyclic enkephalin analogues to opioid receptors, taking as models [D-Cys^2,D(or L)-Cys^5]-enkephalin amides and Tyr – D – L$\overline{\text{ys} - \text{Gly} - \text{Phe}}$ which have a preference for μ-receptors, and [D-Pen^2,D(or L)-Pen^5]-enkephalins as δ-selective compounds. It is concluded that the μ-receptor-bound conformation resembles a β-I bend centred on the Gly^3-Phe^4 region, while the δ-receptor favours two slightly different models which include a γ-turn (or a γ-like turn) on the Gly^3 residue. [2,6-^{3}H$_2$-Tyr^1]-Leu-enkephalin with a specific radioactivity of 1.37 TBq/mmol has been synthesised[172] by tritiation of a dibromotyrosine precursor, while 4,5-didehydro-Leu containing



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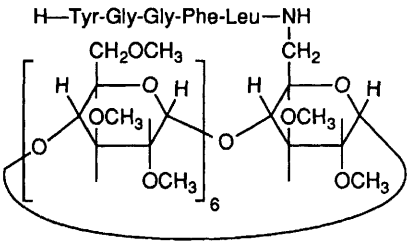

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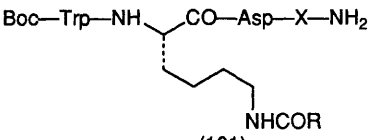

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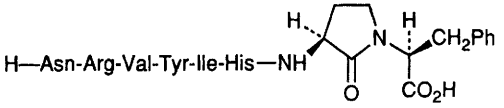

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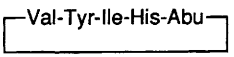

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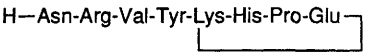

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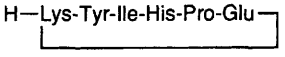

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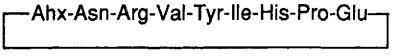

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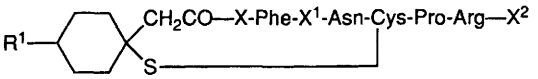


(107) X = Tyr, Tyr(Me), D-Tyr(Et); R^1 = NH_2, NBu_2, NHEt, NEt_2

(108) X = D-Tyr(Et), X^1 = Val, X^2 = NH_2, R = H

precursors have yielded[173] [D-Ala^2]- and [D-Ala^2,D-Leu^5]-Leu enkephalins having tritium-labelled leucine with specific activities of 5.35 and 5.45 TBq/mmol^{-1}, respectively. A series of dimeric opioids derived from μ-selective monomers, e.g., (H-Tyr-D-Ala-Phe-X-$NHCH_2$)$_2$ where X = Gly, Gly-Phg, Gly-Tyr-Pro-Ser and Gly-D-Phg was synthesised[174] to investigate whether μ- and δ-receptors co-exist. Some compounds turned out to display preferential selectivity for δ-receptors. The first example[175] of a neuropeptide (Leu-enkephalin) grafted onto mono-6-amino permethyl β-cyclodextrin giving (100), seems an interesting idea to assess the bioavailability/enzymic stability of this new class of peptide 'transporter'.

Variations at the C-terminus of the analgesic tripeptide Tyr-D-Arg-Phe-X have included[176] X = $NHCH_2CF_3$, Sar, $NHCH_2CH_2CN$, taurine NH_2, $NHCH_2CF_2CF_3$, $NHNHCH_2CF_3$, $NH(CH_2)_3OMe$ and $NH(CH_2)_4OH$. The first three were potent in the gpi assays and had high affinity for the μ-receptor. The taurine-NH_2 analogue had a four-fold higher μ-receptor selectivity than that of [D-Ala^2,MePhe^4,Gly-ol^5]-enkephalin. Glycosylation of the Glu^6 position in [Glu,Pro^9] substance P(6-11) enhanced the solubility and produced[177] an analogue 100 times more selective than substance P for the same receptor. Of the four tetrapeptides synthesised[178] as possible opioid agonists and can be produced by degradation of human β-casein, only H-Tyr-Pro-Phe-Val-NH_2 was active showing a 60% increased activity for the μ-receptor as compared with morphiceptin. Phenylalanine at position 3 was essential to elicit binding to the μ-receptor.

The best κ-opioid activity in the rat vas deferens assay was achieved[179] by H-MeTyr-Gly-Gly-Phe-Leu-Arg-MeArg-D-LeuNHEt during a synthetic/biological activity survey on a number of [MeTyr^1,MeArg^7]-dynorphin A analogues. The D-Leu NHEt analogue had a similar receptor selectivity to that of dynorphin A, but a MeIle residue at the C-terminus showed a 2-fold improvement in analgesic effect. Lipophilic residues at position 8 and an unchanged 7-8 amide bond seem to be essential for κ-opioid activity. In a related study[180] [MeTyr^1,MeArg^7, D-Leu^8] dynorphin A(1-9)-NHEt and [D-Cys^2-Cys^5,MeArg^7,D-Leu] dynorphin A(1-9)-NH_2 have been assessed. Twenty analogues of dynorphin A(1-8)-NH_2 have been synthesised[181] by the solution phase approach. Introduction of MeArg in position 7 protected the Arg^6-Arg^7 bond from enzymic degradation without loss of potency and selectivity. [MeTyr^1,MeArg^7,D-Leu^8]-Dyn(1-8)-NHEt was similar to dynorphin A in most assays but had a relatively high κ-receptor selectivity with a 2.5 fold more potent analgesic effect than morphine. An even more potent effect (40- to 60-fold increase in selectivity and a 2.5-fold more potent analgesic effect than morphine) was achieved by [D-Cys^2-Cys^5,MeArg^7,D-Leu^8]-Dyn(1-8)-NHEt. Replacement[182] of the enkephalin sequence at the N-terminal end of dynorphin, H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-OH by the dermorphin and dermorphin(1-5) sequence (Dermorphin = H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) caused a remarkable increase in analgesic potency and a 3-6-fold increase

in potency binding against [^{3}H]-dihydromorphine. Based on the relative potencies obtained from all assays, it was evident that the N-terminal dermorphin moiety and not the C-terminal dynorphin fragment dominated opioid activity and receptor preference.

Improvements in the enzymic stability were the main reasons[183] given for the analgesic potencies of a series hexapeptides related to dermorphin. N-Methyl and D-residue substitution was included in the survey on H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-OH analogues. Two retro-inverso analogues, H-Tyr-D-Ala-Phe-Glyψ[NHCO]-X-Pro-OH (X = Tyr,Phe) were also amongst the twelve new compounds studied. Direct fluorination of the N-terminal tyrosine in a dermorphin 1-4 sequence has been successfully achieved[184]. Acetylhypofluorite (CH_3COOF) obtained from F_2/NaOAc inserted the fluorine atom into the position next to tyrosine hydroxyl, a technique which should prove useful for incorporation of ^{18}F. Solid phase methodologies using a benzhydrylamino polymer have proved useful[185] in the synthesis of a series of dermorphin pentapeptide analogues, e.g., H-Tyr-D-Ala-Tyr-Tyr-Tyr-NH_2 or with Phe replacing the C-terminal tyrosines. A series based on H-Tyr-Y-Phe-Arg-Tyr-NH_2 (Y = D-Arg,Arg,D-Ala) were also prepared. α-Methoxyglycine has been synthesised[186] by chlorination and methanolysis of Z-Gly-OMe. Catalytic hydrogenation of Z-DL-NHCH(OMe)CO_2Me in the presence of Boc-Tyr(Bu^t)-$OCO_2CH_2CHMe_2$ gave a diastereoisomeric dipeptide unit which was used to make the dermorphin analogues, H-L-Tyr-D-(and L)-NHCH(OMe)CO-Phe-Gly-NH_2.

A theoretical analysis[187] on analogues of the opiate H-Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu-OH showed the existence of 14 low-energy conformers. The tritium labelled highly potent mast cell-degranulating substance P analogue, H-Arg-Pro-Lys(3,4-^{3}H-Pro)-NH-$C_{12}H_{25}$ has been prepared[188] with a specific activity of 1.07 TBq/mmol.

6.2 Cholecystokinin Analogues - A series of novel glutamic acid-derived cholecystokinin(CCK) receptor ligands have been designed and synthesised[189] to test the structural analogy between glutamic acid and the recently reported benzodiazepine CCK antagonists. Thus a number of glutamic acid dialkyl amides based on (*R*), (*S*), or (*RS*)-$R^3CONHCH(CH_2CH_2(COR^2)CONR^1_2$ were tested for their receptor selectivity but none of the compounds were brain CCK/gastrin selective. However the (*R*)-form in which R^1 = n-pentyl, R^2 = pyrrolidino and R^3 = 3-$MeOC_6H_4NH$ was potent and selective in the pancreas binding assay. Positions 28 and 31 in CCK(26-33) have been replaced[190] by MeNle residues and gave highly potent and selective receptor affinities. The pancreas to brain cortex binding affinity ratio for [MeNle^{28,31}] CCK(26-33) was found to be 5100 in the rat model with high potency (IC_{50} = 0.13 nM). Nmr studies indicated that high selectivity might be due to the *cis/trans* rotational isomerism about the MeNle

residues. Since the tripeptide derivative Boc-Trp-Orn(Z)-Asp-NH_2 has been shown to have the same affinity $K_i = 2.0 \times 10^{-7}$M and the same antagonist activity ($pA_2 = 6.63$) as Boc[Nle^{28},$Orn(Z)^{31}$] CCK(27-33) it has spawned an investigation[191] into other analogues of the tripeptide. Replacement of the Z-group of the side-chain by a Boc-group slightly decreased the affinities of the analogues, while deletion at the N-terminus or the Phe residue did not play a key role in recognition. The Orn(Z) analogues competitively antagonised the stimulation produced by CCK-8. A series of tetrapeptides have been reported[192] as representing a dramatic departure from what is currently known about the structural requirements for agonist response at cholecystokinin-A receptors. In the tetrapeptides, of which (101) is representative, the X-residue was mainly Phe and they do not require an acidic moiety for potency as do the longer peptides, but they are 1000-fold more selective for CCK-A receptors than CCK-8. A series of N-alkylcarbamates representing dipeptoid analogues of CCK(30-33) (or CCK4) have been synthesised[193]. The analogues, based on α-MeTrp-Phe-NH_2 and their arylethylamine counterparts have micromolar affinity for CCK-B receptors. Bulky substituents at the N-terminus, e.g., Boc, *t*-amyloxycarbonyl(Amoc), adamantyloxycarbonyl(Adoc) and trichloro-*t*-butoxycarbonyl(TcBoc) were preferred, together with a D-MeTrp and L-Phe combination of configurations. These small, sometimes non-peptidal models such as Boc-DL-MeTrp-CH_2CH_2Ph have comparable receptor affinities to certain tetrapeptide CCK-4 analogues.

6.3 Angiotensin and Analogues - [Asn^1]-Angiotensin analogue (102) has been synthesised[194] using azide coupling techniques. In (102) the Pro^7-Phe^8 position has been replaced but the product had no agonistic or antagonistic activity in myotropic, histamine releasing and pressoric tests. A ^{1}H nmr study[195] on [$MeAib^1$,$Tyr(Me)^4$]- and [$MeAib^1$,$Tyr(Me)^4$,Ile^8]-angiotensin II in $^2H_6$DMSO confirmed the presence of restricted rotation around His-Pro with the ratio between *cis-trans* rotamers being 1:6 in favour of *trans*. Residue substitution in the recently reported angiotensin II antagonist, [Sar^1]-angiotension II(1-7)NH_2 has been expedited[196] using solid phase techniques. Antagonistic activity seems to rely on having Sar at position 1, and Pro at position 7. However, no antagonistic activity was found[197] in [des-Arg^2,ε-Ahx^1]- or [des-His^6]angiotensin II (ε-Ahx = ε-aminohexanoic acid). The analogues did show strong and weak agonistic activity (70 and 30%, respectively, of the contractile activity of angiotensinamide). Studies[198] on the N-terminus modifications of [Ile^8]-angiotensin, a known angiotensin antagonist, have revealed that replacement of up to the first three residues by non-peptidic fragments featuring amine or guanidine groups does not interfere with their antagonistic activity but all compounds were devoid of agonist activity. $H_2N(CH_2)_3CO$-Val-Tyr-Ile-His-Pro-Ile-OH antagonised the angiotensin II-induced blood pressure effect in anaesthetized rat when infused at 30 μg/Kg/min. Cyclisation of a series of angiotensin analogues using

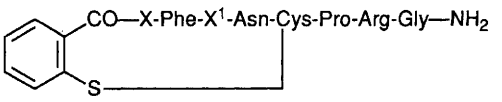
pentafluorophenyl ester activation of linear precursors, proved to be synthetically successful but the compounds (103-105) were devoid of angiotensin-like activity. Cyclic peptide (106) showed decreased pressor effects as compared to angiotensin. Biotinylated and photoreactive probes for use in purifying and studying angiotensin receptors have been developed[200]. Several improved and unequivocal pathways have been formulated to make the three analogues biotinyl-aminohexanoyl-[Tyr(3I)^4,Phe(4N$_3$)^8]-, iminobiotinyl-Gly-aminohexanoyl-[Ala^1,Tyr(3I)^4,Phe-(4N$_3$)^8]-, and biotinyl-ethyl-1,3′-dithiopropionyl-[Ala^1,Tyr(3I)^4,Phe(4N$_3$)^8]-angiotensin II. All three showed an affinity of the order of 10^{-9} mol dm^{-3} for angiotensin II receptors, and after introduction of the photoactivatable group a mean yield of 25% of covalent bonding was achieved.

6.4 Vasopressin Analogues - Fluorescent, photoreactive and biotinylated vasopressin analogues have been prepared[201] by attachments either at positions 4 or 7. Two biologically active parent analogues, [1-desamino,Lys^4]- and [1-desamino,aminoproline^7]-arginine vasopressin were taken as the core units and their side-chain amino groups acylated with the appropriate probe molecules. Two non-selective antagonists of the vasopressor (V_1) and antidiuretic (V_2) responses to [Arg]-vasopressin (AVP) with the sequence Aaa-D-Tyr(Et)-Phe-Val-Asn-Abu-Pro-Arg-Arg-NH$_2$ where Aaa = adamantylacetyl and its desArg^9 analogue have been reported already. Twenty one new analogues of these compounds have now been reported[202] using solid phase techniques. Phenylacetic acid (Phaa) and t-butylacetyl at position 1 and substitutions such as D-Tyr^2, D-Tyr(Me)^2, Gln^2, Arg^6, Lys^6, Orn^6 and MeAla^7 were amongst the modifications assessed. The first AVP antagonist with a mean pA$_2$ value >9 (9.05±0.09) was obtained when the desArg^9 sequence was substituted as [Phaa^{1}Gln^4,Lys^6]. Non-coded amino acid D-homoarginine in position 8 and p-substituted D- or L-Phe at position 2 in vasopressin gave analogues[203] with very low antidiuretic and pressor activities. All analogues substituted in position 2 were uterotonic inhibitors, the most potent being [D-Phe(Et)^2,D-Har^8]-vasopressin with pA$_2$ 8.15. Bulky and lipophilic substituents (R = Bu^t,Ph) in the cyclohexyl ring of (107) in combination with X^1 = Abu(aminobutyric acid) has led to a decrease[204] of antivasopressor potency and a strong decrease of the antiuterotonic potency. Alkylation of any type of carboxamide group at positions 4(X^1) and 9(X^2) reduced biological potency in all tests. The compound (108) is a known antagonist of antidiuretic (V_2) and the vasopressor (V_1) receptors and has been synthesised by solid phase techniques by Manning *et al* (1987). A solution phase method particularly adaptable to a large scale process has now been reported[205]. The molecule was assembled by a 3+4 coupling *via* the azide method, the disulfide bridge link was made by iodine treatment of the bis(acetamidomethyl) protected thiols and the terminal arginine amide added by a 7+1 coupling. Gram quantities with an overall yield at the

13% level, but with 98% purity, were made using this strategy. It appears[206] that incorporation of a rigid thiosalicylic acid residue at position 1 as in (109) was not conducive to enhancement of activity. All analogues based on (109) with X = Tyr, X^1 = Glu; X = D-Phe, X^1 = Glu,Ile, suffered from severely depressed pressor and antidiuretic activity and were devoid of antagonistic effects. Very similar negligible activities were also the results obtained[207] from insertion of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) into positions X and X^1 in vasopressin analogue (110). The solid phase synthesis and pharmacological properties have been reported[208] on naturally occurring vasotocin type compounds and their analogues. These included the AVP-like factor F_1[Leu^2,Thr^4]-AVT from subesophageal and thoracic ganglia of *Locusta migratoria*, Arg-conopressin-S ([Ile^2,Arg^4]-AVT) and Lys-conopressin-G([Phe^2,Arg^4]-LVT), both isolated from fish-hunting *Conus* marine snails.

6.5 Luteinising Hormone-Releasing Hormone (LHRH) - Twenty new analogues of LHRH, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 have been synthesised[209] and from this crop some have potencies superior to the known antagonist [NAc-D-2-Nal^1,D-4FPhe^2,D-Trp^3,D-Arg^6]-LHRH known as antide which is presently undergoing clinical evaluation. These new analogues featured acylated aminocyclohexylalanines and lysines at positions 5 and 6. The two most potent analogues turned out to be [N-Ac-2-Nal^1,D-pClPhe^2,D-3-Pal^3,$PicLys^5$,D-PzAcAla^6,-Val^7,$ILys^8$,D-Ala^{10}]-, and [N-Ac-D-2-Nal^1,D-pClPhe^2,D-3-Pal^3,$PicLys^5$,D-Pic-Lys^6,Abu^7,D-Ala^{10}]-LHRH with 100% at 1 μg and 50% at 0.25 μg of antiovolutary activity, respectively [ILys = N^{ε}-isopropyllysine, 3-Pal = 3-(3-pyridyl) alanine, PicLys = N^{δ}picolyl Lys and PzAcAla = 3-(4-pyrazinylcarbonylaminocyclohexyl)-alanine]. Analogues of LHRH with D-Ala^{10}, Sar^{10}, D-Ser^{10}, (desGly^{10}NHEt), D-Abu^{10}, Gly^{10} and with substitutions in positions 5, 6 and 8 have also been assayed [210]. D-Ala at position 10 seemed to favour high antiovulatory activity. Unnatural amino acid residues inserted at position 6 have also been a source[211] of new LHRH antagonists. The best effect in the rat antiovulatory assay came from [NAc-D-2-Nal^1,D-4ClPhe^2,D-3-Pal^3,Arg^5,D-A2^6,D-Ala^{10}]-LHRH which inhibited ovulation completely at 1 μg/rat. [D-A2 = structure (111).] A new photolytically detachable linker to polystyrene resin, [3-nitro-4(alkylaminomethyl)benzamido)-methyl] has been used[212] to produce N-Me and N-Et amides of LH-RH.

It has been suggested that LH-RH and its 'anti-sense' peptide H-Ser-Arg-Ala-Gln-Ser-Ile-Gly-Pro-Val-Leu may be interacting, so further conformational studies[213] have been carried out on the 'anti-sense' form as well as its reverse sequence. 2D-Nmr techniques have revealed that the 'anti-sense' peptide exists as one complete 3_{10}-helical turn followed by an extended conformation. The reverse of this sequence has more β-turn character, although these are present only in minor concentrations.



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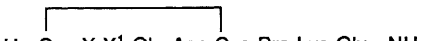
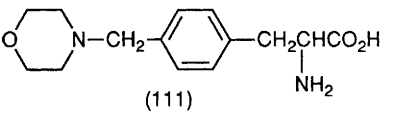


(110) X = Tic, X^1 = Phe; X = Tyr, X^1 = Tic



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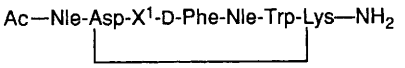

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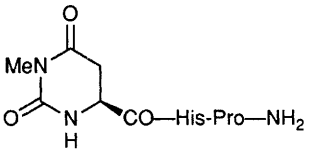

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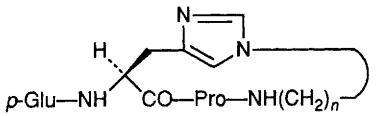
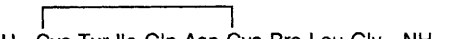


(114) n = 5,6



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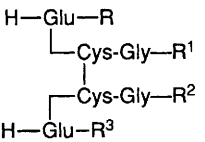
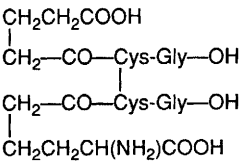


(116) R = R^3 = NH_2, R^1 = R^2 = OH, R = R^3 = OH, R^1 = R^2 = NH_2
R = NH_2, R^1 = R^3 = OH, R = R^3 = OH, R^1 = NH_2



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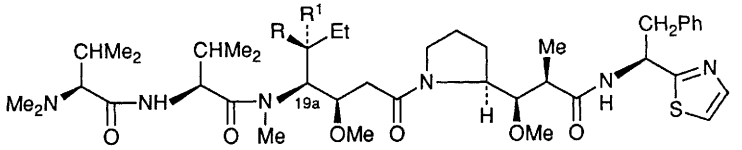


(118)

6.6 Miscellaneous Examples - The search for highly potent α-MSH antagonists has been explored[214] *via* the synthesis of analogues of the agonist Ac-Nle-Asp-His-D-Phe-Arg-Trp-Lys-NH_2. The molecule that showed the best inhibitory action against α-MSH on melanocyte stimulation was Ac-Nle-Asp-Trp-D-Phe-Nle-Trp-Lys-NH_2. But many such analogues showed complete loss of antagonistic activity, as happened in the case of lactam (112) which functioned as a full agonist. In order to obtain information about the chemical character of the melanotropin receptor new sources of alkylation centres have been incorporated[215] into α-MSH analogues. Because of the instability of the previously used N-(2-chloroethyl)-N-nitrosocarbamoyl peptide derivatives, use has been made of the phenylalanine mustard (melphalan) residue inserted at several positions in the α-MSH sequence. The peptides were produced in the solution phase and the only special care necessary was to avoid unnecessary basic conditions. Alkylating peptides with melphalan in place of Arg, Phe or Met possessed prolonged biological activity and were inhibitors of α-melanotropin, suggesting an irreversible binding to reactive nucleophiles in the Met-Glu-His-Phe-Arg segment. When Leu was substituted for Phe in the MSH release-inhibiting hormone H-Pro-Phe-Gly-NH_2, its antidepressant activity was not changed[216], but NO_2, NH_2, OH or $NHCO(CH_2)_3NH_2$ groups substituted in the *para* position of the Phe ring resulted in decrease or loss of antidepressant activity.

Residue insertions into positions 1 and 2 of TRH (p-Glu-His-Pro-NH_2) have been evaluated from the point of view of feasibility of synthesis, as well as, in the case of the N-terminal position[217] whether the changes influenced locomotor activity, antagonistic effect on reserpine-induced hypothermia or pentobarbital anaesthesia. Analogue (113) based on (*S*)-4,5-dihydroorotic acid showed the most potent activities which were 30-90 times greater than those of TRH. The TSH-releasing activity of (113) was 50 times weaker than TRH. The strategy and tactics used in the cyclic analogues (114) evolved[218] into (a) a regiospecific introduction of the τ substituent and, (b) acceptance that the His-Pro bond needed to be formed before cyclisation. π-Phenacyl protection was used on the imidazole nucleus, and the safety catch principle previously developed using 2-hydroxyphenyl esters proved successful in the cyclisation step. When photochemical trifluoromethylation (CF_3I/hν/MeOH/Et_3N) was attempted on TRH both the 2- and 4(5)-CF_3Im-TRH were formed[219] which were separated by reversed phase hplc and characterised. A search for the true role of the imidazolyl side-chain inTRH's biological activity can now be continued with the replacement[220] of His by L-2-furylmethyl, L-2-thienylalanine, and D- and L-2-pyrrolylmethyl. In the context of the latter the LLL- and LDL-diastereoisomers were separated at the end of the synthesis.

Three oxytocin (115) analogues with $HSCH_2COOH$ in position 1 and β-homotyrosine, O-methyl β-homotyrosine or β-homophenylalanine have been synthesised[221] and their potencies and binding affinities determined. The reaction

between metallic sodium/liq NH_3 and Z-Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 to yield oxytocin (115) has been optimised[222] with the aid of a combined hplc/electrochemical (conductivity) monitoring system. Analogues of oxytocin (115) with tetrahydroisoquinoline carboxylic acid (D- and L-Tic) in position 2 have been synthesised[223] and found to be *in vitro* uterotonic inhibitors. Although the D-Tic[2] analogue increased inhibitory activity its conformation was similar to [D-Phe[2]] oxytocin which suggests a conformation conducive to interaction with the receptor. Substitution by L-Tic led to a different conformation, coinciding with poor receptor binding as well.

Further fine tuning of the solution conformation of [des-Trp[1],Nle[12]]-human minigastrin has been made possible[224] by the synthesis of α-deuterated glutamate residues introduced at positions 6, 7 and 5 and 7 of H-Leu-$(Glu)_5$-Ala-Tyr-Gly-Nle-Asp-PheNH_2. Certain details still remain to be resolved, so that the "hairpin" conformation with two helical segments and a bend in the middle of the molecule can still be considered tentative. The presence of a shielded amide NH in the middle of the Glu^5 sequence remains unresolved, together with the fact that a strong H-bond around the C-terminus favoured by the 'hairpin' conformation seems to be ruled out in the present set of results. In order to ascertain the minimum structural requirement for a C-terminal gastrin antagonists, conventional solution phase synthesis[225] has been used to make analogues of Boc-Trp-Met-Asp-NH_2 a known antagonist. The range of structures investigated were based on R-Leu-β-Ala-OH with R = Boc-Trp,PhCH_2CH_2CO and indole-3-propionyl. PhCH_2CH_2CO-MeLeu-β-Ala-OH and PhCH_2CH_2CO-Leu-NMeCH_2CH_2COOH were also prepared. The minimum requirement appears to be in agreement with Morley's previous hypothesis - an indole ring and a β-carboxylic acid separated by a hydrophobic spacer residue such as leucine. As part of a project where more than 300 octapeptide analogues of somatostatin have been assessed[226], several of the superactive analogues have been tested *in vivo* for their effects on gastric acid response to various exogeneous and endogenous stimulants. Analogues of somatostatin such as D – Phe – $\overline{Cys - Tyr - D - Trp - Lys - Val - Cys}$ – Trp – NH_2 and its threonine equivalent, D – Phe – $\overline{Cys - Tyr - D - Trp - Lys - Val - Cys}$ – Thr – NH_2, superactive in tests on suppression of growth hormone were 4-5 times more potent than somatostatin in inhibiting desglugastrin-stimulated gastric acid secretion. In general octapeptide analogues which are superactive in growth hormone-inhibition tests are also more potent in suppressing gastric acid secretion. Solid phase technology[227] has produced 37 new analogues of human growth hormone-releasing hormone (GH-RH). Most contained a sequence of 27 amino acids, desamino Tyr(Dat) at the N-terminus, 4-guanidinobutylamine(Agm) at the C-terminus and Ala^{15} and Nle^{27} substitutions. [Dat[1],Ala^{15},Nle^{27}]GH-RH(1-28)Agm was the most active analogue. *In vitro* it had GH-releasing potency 10.5 times higher than that of GH-RH(1-29)NH_2, *in vivo* it

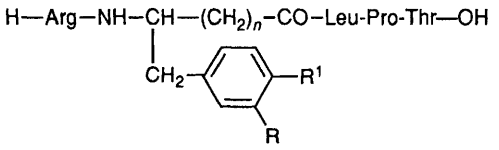
was 4-5 times more active than standard. The agmatine(Agm) at the C-terminal seemed important for potency.

Neuropeptide Y(NPY), a 36-amino acid peptide, is believed to adopt a polyproline-type II helix for residues 1-8, followed by a β-turn in positions 9-14, an amphipathic α-helical from position 15-32. The N-terminal portion has been probed[228] more closely from information received from Ala substitutions made in positions 1-10, the analogues being prepared using solid phase couplings using the BOP reagent. Results suggest that the polyproline-type II structure is involved in both potency and affinity as important losses of activity occurred when Ala was placed instead of Pro at positions 2, 5 or 8. A critical loss of potency also occurred when Tyr^1 was replaced by Ala. A related study on NPY analogues[229] indicated that N-terminal substitutions did not induce dramatic decreases in affinity, and that the C-terminal tetrapeptide Arg-Gln-Arg-Tyr-NH_2 directly binds to the NPY receptor in rabbit kidney membrane. Fmoc strategy using BOP activation in a multiple peptide synthesis approach was used to make the analogues. Peptide YY(PYY) is known to have distinct sequence homology with NPY and avian pancreatic polypeptide(APP) and exhibits both NPY- and APP-like biological activities. Two analogues of PYY with modified N-terminal regions (1-8) have been synthesised[230] and assessed by comparison with PYY (13-16) and PYY itself. Residues introduced to the PYY N-terminus included Gly^1, Ser^3, Gln^4, Thr^6, Tyr^7 or Glu^3, Lys^7 were chosen to increase stability of α-helical structure in this region. C.d studies and an assay using rat vas deferens indicated that the amphiphilic α-helical structures are stabilised by intramolecular hydrophobic interactions within the N-terminal regions and potentiate the activities of the analogues.

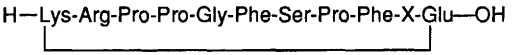
Atrial natriuretic factor (ANF) analogues with modifications to the disulfide bridge and lacking an exocyclic N-terminus have been synthesised[231] using segment condensation. Deaminocarba, β,β-dimethylcarba, and dehydrodicarba spanner units bridging positions 106 and 120 retained high affinity for ANF receptors in bovine adrenal zona glomerulosa cells and were found to be potent antihypertensive and diuretic agents. In another series of analogues[232] the disulfide ring has been retained and Tyr substituted in position 116 of ANF(101-126) and [3-Mpr^{105}]-ANF(105-126) where 3-Mpr = 3-mercaptopropionic acid, did not alter the biological activity profile. The analogue [3-Mpr^{105},Nva^{109}]-ANF(105-126) showed very low affinity in receptor binding. A novel gonadotropin hormone releasing hormone [D-Phe^6,Gln^8,desGly^{10}]-GnRH ethylamide has been found[233] to be one of the most effective analogues so far reported for artificial propagation of fish. Mixed anhydride couplings followed by disulfide couplings of S-tritylglutathione with iodine methanol have produced[234] compounds (116) and (117) for the study of enzyme-ligand interactions in glutathione reductase. The need for expedient methylation times to enable $^{11}CH_3I$ to be introduced into the ACTH fragment analogue H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH *via* its homocystine-

containing precursor has been discussed and accomplished[235]. As with many other peptide hormone antagonists, selective substitutions with Trp have led to highly potent parathyroid hormone (PTH) antagonists. The result of placing hydrophobic substitutions at position 18 in PTH was to confirm[236] that receptor binding can tolerate substitution here. The most active antagonist on renal receptors was [Nle^8,D-$Trp^{12,18}$,Tyr^{34}]PTH(7-34)NH_2 (K_b = 4 nM, K_i = 30 nM). A simple octapeptide H-Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr-OH (peptide T) seems to inhibit[237] HIV infectivity and to activate human monocyte chemotaxis. The peptide T, and shorter analogues T(3-8)OH and T(4-8)OH have been prepared and displayed potent bioactivity (chemotactic activity in the range 10^{-11} to 10^{-10}M). Abu^4 substituted for Thr^4 could be tolerated, but the same change in Thr^5 or Thr^8 was detrimental. The C-terminal pentapeptide and residues 5 and 8 seem to play a crucial biological role. Dolastatin 10 (118, R = Me,R^1 = H) from the Indian Ocean sea hare *Dolabella auricularia* has proved to be a potent antineoplastic substance, and to elucidate its chiral and structural parameters several dolastatin isomers have been synthesised[238]. One of these (118, R = H, R^1 = Me) 19a(*R*) isodolastatin was more cytostatic than dolastatin 10 providing an ED_{50} of 4.9 x 10^{-5} μg/mL. Enhancement in antiarrhythmic activity of antiarrhythmic peptide (AAP) has been studied[239] *via* synthesis and testing of analogues. Insertions into the AAP sequence H-Gly-X-X^1-Gly-Ala-Gly-OH by X-X^1 = Sar-Pro,Pro-Sar or Sar-Sar showed that X-X^1 = Sar-Sar was more active than AAP where X-X^1 = Pro-Hyp. This becomes equipotent to the antiarrhythmic drug quinidine so far as delay in the onset of ventricular tachycordia, fibrillation and cardiac arrest are concerned. In a series of analogues[240] of H-Pro-Leu-Gly-NH_2 which is capable of modulating dopamine receptors, two of the analogues Pro-Ahx-Gly-NH_2, and Pro-Phe-Gly-NH_2 enhanced binding of 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene(ADTN) to striatal dopamine receptors by 16% at 0.1 μM and 31% at 1 μM, respectively.

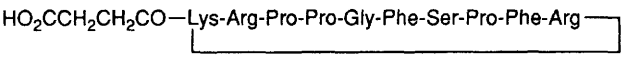
Further studies have been carried out on proctolin (119) analogues[241] where position 2 has been modified by insertion of tyrosyl and *para*-substituted-Phe analogues. At physiological concentrations (10^{-9}–10^{-7}M) three analogues (120) - (122) stimulated the heart action of the insect *P.americana*, but only (121) was active in *T.molitor*. Thymopoietin II (32-36) H-Arg-Lys-Glu-Val-TyrOH has been the subject[242] of Pro insertions at various positions at its C-terminal and it is concluded that the Tyr residue does not seem to have a fundamental role in activity. Cyclic analogues (123) and (124) based on bradykinin have been synthesised by classical techniques[243]. Compound (124) does not possess myotropic histamine-releasing or hypotensive activity, but compound (123) (X = Arg) did elicit histamine release in isolated rat mast cells, altered arterial pressure following i.v. administration and affect the heart rate of cats and dogs.



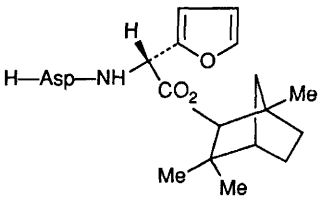
(119) Proctolin; R^1 = OH, R = H, n = 0
(120) R^1 = OEt, R = H, n = 0
(121) R^1 = OH, R = NH_2, n = 0
(122) R^1 = OH, R = NO_2, n = 0



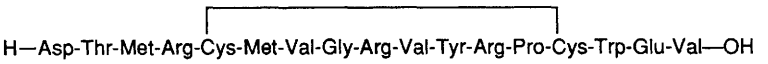
(123)



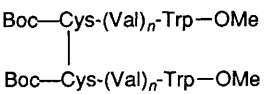
(124)



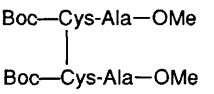
(125)



(126)



(127)



(128)

The 44 residue A chain of the sweet protein monellin has been synthesised[244] as an analogue [Asn^{22},Gln^{25},Asn^{26}]-A chain using solid phase techniques and the Fmoc strategy. This analogue when linked to a [Asn^{49},Glu^{50}]-B chain of 50-residues produced a monellin analogue which was 550 times sweeter than sucrose. The best sweetness value obtained from a series of inverted-aspartame type sweetener[245] was only 50 times the sucrose sweetness. The compound giving this value, $PhCOCH_2$-Gly-Lys-OH, is useful as it lacks an ester function which increases stability and lowers toxicity. In a series of L-aspartyl dipeptide derivatives[246] derived from heterocyclic glycine esters, (+)-fenchyl ester (125) was found to be the most potent (16,500 times the sweetness of sucrose).

7. Conformational Information derived from Physical Methods

It is probably a reflection on the maturity reached in the application of many modern physical techniques that many papers tend now to combine synthesis and conformational studies. So one major reason for fewer papers being cited under this section is that they have been discussed under other sub-headings in this Chapter. Nmr techniques continue to flourish into very high technology, producing a wealth of information. The combination of computationally-derived distance parameters linked to nOe studies seem to be very useful for the fine-tuning of conformational models.

7.1 Nuclear Magnetic Resonance and Related Techniques - Although the bradykinin molecule H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH is flexible and non-structured in solution, nmr studies[247] at 500 mHz coupled to distance geometry and restrained molecular mechanics on bradykinin in the presence of sodium dodecyl sulphate (SDS) micelles show β-turn like characteristics at residues 6-9. In a separate investigation[248] under similar conditions using cd, ^1H, ^{13}C and ^{19}F nmr, spectra for [^{13}C-2-Gly^6]-bradykinin at pH 8.3 confirmed the high *cis/trans* ratio about the 6th bond. Addition of 5.2 mM SDS broadened both *cis* and *trans* ^{13}C-resonances but only shifted the *trans*. The *cis/trans* ratio increased substantially, so that the *cis* form must be enhanced. ^{19}F Nmr of [Gly^6,*p*F-Phe^8]-bradykinin also sensed the *cis/trans* isomers of Pro^7. Strong interactions between monomeric SDS and bradykinin were reflected in broadening of all ^1H signals. Recent experimental evidence has suggested that vertebrates may possess separate receptors for melanin concentrating hormone MCH (126- as in the salmon) and its hormonal counterpart αMSH. The question of a structural link between MCH and MSH has led to an nmr study[249] of MCH using all the latest 2D tools - NOESY, COSY, TOCSY and (DQF)COSY. A type I β-turn region was identified in the region 7-10, together with a transannular effect of the Tyr^{11} side chain moiety. A range of nmr

techniques combined with cd data[250] applied to the azetidine carboxylic acid-containing tetrapeptides, Boc-(Pro)$_3$-Aze-OC$_6$Cl$_5$ and Boc-(Aze-Pro)$_2$-OC$_6$Cl$_5$ showed the latter, in CF_3CH_2OH, had an all *cis* left-handed helix conformation. *Cis* and *trans* bonds were identified in the former. While the solid state crystal structure of Boc-Pro-Ser-NHMe indicated[251] a *cis* urethane tertiary amide, in $CDCl_3$ nmr data showed two conformers differing in the rotameric state of the tertiary amide bond. In the *trans* form a type I β-turn was implied, while the *cis*-rotamer seen in the crystal as a β-pleated backbone was probably due to packing forces. For the symmetrical cystine peptides (127) with n = 1, 1; 2,2 or 3,3, there was evidence[252] in d_6DMSO solutions that the ValNH protons were solvent inaccessible, and $J_{NHC\alpha H}$ values and nOe supported extended β-strand structures. Intramolecular antiparallel β-sheet conformations have been interpreted[253] as the best explanation of data accumulated by X-ray, cd and nmr techniques for the cystine peptide (128).

High resolution ^{15}N nmr in the solid state has been applied[254] to oligopeptides (X-Gly-Gly) with known X-ray structures, and to polypeptides containing ^{15}N-labelled L-Ala residues[255]. A variety of ^{15}N-labelled co-polypeptides were prepared by polymerisation of ^{15}N-Ala-N-carboxyanhydride with the other amino acid residue to give $[^{15}N\text{-Ala-X}]_n$ X being Gly, Ala, D-Ala, Leu, Val, Ile, Sar or β-Bzl-Asp. Conformation dependent ^{13}C chemical shifts in the ^{13}C cross-polarisation-magic-angle-spinning (CP-MAS) were used as data. Spin-lattice relaxation times T_1 of $H_2{}^{17}O$ have been measured[256] for aqueous solutions of 11 apolar amino acids and five glycine peptides.

Biosynthetically directed fractional isotope labelling with ^{13}C starting from glucose has yielded cyclosporin A and two globular proteins with sufficient ^{13}C enrichment to enable[257] diastereotopic methyl groups of valyl and leucyl side chain to be studied by ^1H and ^{13}C nmr. 2D Nmr and distance geometry calculations have been used[258] on bleomycin-FeII-CO complexes and show the active participation of 5 Fe-binding sites in the bleomycin molecule, identified as the β-amino-Ala fragment, the aromatic pyrimidine, the amide and imidazole of β-HOHis and the carbamoyl group of the mannose sugar. In this way several acceptable model structures could be generated. Measurement[259] of the relative intensities of cross peaks in pure phase absorption NOESY spectra has enabled the corresponding interproton distances to be determined for a number of amino acids. The model compound used for the study was cyclic bradykinin, ⌊Lys – Pro – Pro – Gly – Phe – Gly – Pro – Phe – Arg⌋.

The potential of reducing the acquisition time needed to record 3D nmr spectra has been explored[260]. The technique used was a DEPT-TOCSY experiment to restrict the spectral width in a 3D spectrum, and without isotopic labelling recording the spectrum of *cyclo*-(-D-Ala-Phe-Trp-Lys(Z)-Val-Phe) in DMSO only took 8.5 hr with comparable resolution to 2D spectra. The spatial structure of gramicidin A in SDS micelles has been determined[261] by 2D nmr. The head to head dimer structure of 2 right-handed $\pi_{LD}6.3$ helices differed in handedness from the

accepted channel model proposed by Urry. Surfactant micelles around the heptadeca peptide bombolitin, Ile-Lys-Ile-Thr-Thr-Met-Leu-Ala-Lys-Leu-Gly-Lys-Val-Leu-Ala-His-Val-NH_2 from nmr/nOe studies[262] cause the Ile^1-Lys^2 amide to be *cis*. In aqueous solution the peptide is random in conformation and all *trans*. The peptide in the presence of SDS micelles adopts an amphiphilic α-helix extending from 3-14 and probably accounts for the 1-2 *cis* amide.

7.2 X-Ray Crystallography - The crystal structure[263] of L-Pro-L-Leu·H_2O showed that the peptide linkage was *trans*, and the pyrrolidine ring adopts an envelope conformation. Boc-L-Asn-L-Pro-OBzl and its dehydration side product Boc-β-cyano-Ala-Pro-OBzl crystallised[264] with a similar extended conformation, with the Asn-Pro *trans* in the former case.

7.3 Circular Dichroism(cd), Theoretical and Computational Methods - Molecular dynamics simulation[265] on the transmembrane antibiotic alamethicin have been performed and compared with previous results from cd, X-ray, nmr and theoretical computations on fragment analogues. Evidence was found for the first segment to be substantially α-helix, with a certain amount of 3_{10} helix in the terminal segment. The backbone conformational requirements for α-pyroglutamic acid have been found[266] by conformational energy calculations to be similar to those associated with D-amino acid residues. Two blocked peptides, Ac-Ala-Ala-NHMe and Ac-Pro-AlaNHMe have been subjected[267] to molecular dynamics simulations using specialised sampling techniques free energy surfaces as functions of a conformation coordinate. The results demonstrated that reverse turns in blocked dipeptides are intrinsically unstable in water. Previous 4-21G *ab initio* geometry optimisations of conformations of model dipeptides based on N-acetyl, N-methyl amides of Gly and Ala have been used[268] to refine the empirical force constants in the CHARMM force field for peptides. Significant improvement from previous force field results was achieved for *cyclo*-(Ala-Pro-D-Phe)$_2$. A series of conformational energy computations[269] have been carried out on the effect of an L-azetidine-2-carboxylic acid residue on the conformation of dipeptides, homopolymers and copolymers and collagen-like poly(tripeptides). It has been deduced that peptides containing Aze would be more flexible than their Pro counterparts because of a decrease in constraints caused by repulsive non-covalent interactions of the ring atoms with neighbouring residues. Polymeric structures also showed increased flexibility when Aze was considered instead of Pro, but although the most stable triple helix formed by poly(Gly-Pro-Aze) was shown to be collagen-like, all low-energy triple helices that can be formed by poly(Gly-Aze-Pro) and poly(Gly-Aze-Aze) were very different from collagen. A computer graphics model for endothelin has been produced[270] using the 3D structure of apamin as a starting point. The model shows a single α-helix involving residues 9-15, giving a helix content of 33% comparable to figures obtained from cd studies.

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4
Cyclic, Modified, and Conjugated Peptides

BY J.S. DAVIES

1. Introduction

Twenty two years ago I cut my first reporting teeth on this Chapter, and the understanding I had with the Senior Reporter at the time, Dr. G.T. Young was that I should review 'Peptides with Abnormal Structure', sometimes interpreted as 'Funny (Unusual) Peptides'. On revisiting that inaugural Chapter again I note that the themes were, cyclic peptides (especially peptide antibiotics), depsipeptides, peptides with thioether linkages, peptides involving β or γ-linkages and those conjugated to lipids, carbohydrates and nucleotides. Has anything changed therefore over almost a quarter of a century? The topics might be the same but the early Chapters in this series concentrated heavily on structural elucidation, and synthesis and had an 'academic flavour' about them. Perusal of the literature for 1990 however leaves one in no doubt that the thrust for researching cyclic peptides, novel peptide antibiotics nowadays comes from their immense clinical potential, e.g., as immunosuppressive reagents, and as templates of biological control molecules which could be mimicked as pharmaceutical compounds. The molecules are also being conformationally investigated nowadays using physical methods with a precision that almost could not have been anticipated twenty or more years ago.

With the change of authorship of the Chapter this year, after Dr. Paul Hardy's decade of reporting, overlap sections with Chapter 3 have been reduced so that the 'modified' part of the title now refers to those peptides which nature itself has seen fit to 'modify'. The man-made modification of peptides which is currently a very active field,. e.g., conformational constriction by forming cyclic analogues and the use of surrogate peptide bonds, etc., has this year been incorporated almost entirely into Chapter 3. This inevitably makes for a shorter treatise, but regular readers will find that the sub-divisions of the Chapter follows the format established over recent years. In the absence of a detailed index, recognisable sub-headings have been deemed to be a useful focus for retrieval of information by the reader.

This year again there has been ample evidence of intense activity in this area, with many analogues of naturally occurring compounds being synthesised. Advanced nmr techniques have been brought to bear on a number of structures and theoretical computation of likely conformational forms are actively pursued. Papers have been retrieved from a wide variety of sources but the focus of the retrieval is the section on Amino acids, Peptides and Proteins in Chemical Abstracts up to the June 1991 issue.

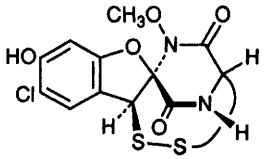
No attempt has been made to retrieve information from the patents literature, and the reporting has concentrated on refereed papers, so although the symposium proceedings[1] of the 21st European Peptide symposium at Platja d'Aro Spain has a wealth of short papers of great interest to this Chapter, they have been left to mature and await report when they appear as full papers.

2. Cyclic Peptides

2.1 Naturally occurring Dioxopiperazine (Cyclic Dipeptides) - Aspirochlorine A30641(1) is a unique epidithiapiperazine-dione first isolated from *Aspergillus tomarii* in 1976 and present in other Aspergillus species. Its structure has proved a synthetic challenge, but a report[2] of the synthesis of the model structure (2) confirms that a full synthesis must be near to completion. Although stretching the structural definition of this sub-heading away from dioxopiperazines, it is noted that the cyclic lactam (3) can be biosynthesised[3] from MeVal-Tryptophanol. The lactam represents the basic ring structure of tumour promoters teleocidins.

2.2 Other Dioxopiperazines - To overcome the rapid inactivation *in vivo* of the cytostatic agent chlamydocin *cyclo*-[(*S*)Aoe-Aib-(*S*)Phe-(*R*)Pro], protected analogues of the 2-amino-8-oxo-9,10-epoxydecanoic acid (Aoe) have been prepared[4] and incorporated into dioxopiperazines such as (4). *p*-Nitrophenyl ester activated salicoyl dipeptides Sal-Xaa-Pro-ONp (where Xaa = Phe, Gly, Aib) when treated with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) in benzene, all gave[5] oxacyclols of the structural type (5), tautomeric forms of the corresponding 10-membered cyclodepsitripeptides. No tendency was shown to isomerise to the corresponding macrocyclic lactones. Partially cyclised dimeric products (6) were also detected and isolated. Nmr studies[6] on dioxopiperazine (7) containing two non-identical L-aromatic acids have shown that there is (within the nmr time scale) a fast equilibrium between a folded form(F) (both aromatic systems sharing the space above the dioxopiperazine ring) and an extended (E) conformer. Conformational and configurational evidence has deduced[7] that the *cis* (8) and *trans* (9) forms were produced when Z-Phe-*(3R,5S)*-tetrahydro-1,4-thiazine 3,5 dicarboxylate esters were hydrogenolysed on palladium. Solid state X-ray determination and nmr studies[8] have given a contrasting picture of the dioxopiperazine ring conformation of (10). In the crystal the ring is a flattened chair conformation, while in solution the ring assumes a boat-like shape typical of Pro cyclodipeptides. Condensed prolyl-type cyclodipeptides have also been subject[9] to factor analysis (abstract factor analysis or principal-component analysis).

The kinetics and mechanism of the facile cyclisation of His-Pro-NH_2 to *cyclo*(His-Pro) in aqueous solution at pH 2-10 show a maximal rate[10] at pH 6-7. It is presumed that the imidazole group of histidine would be involved in



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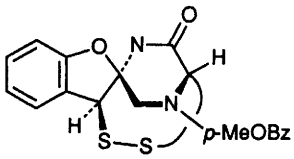

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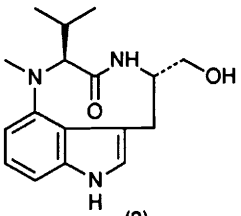

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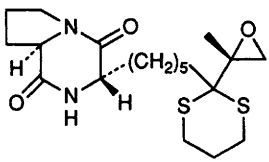

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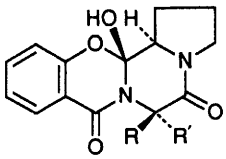

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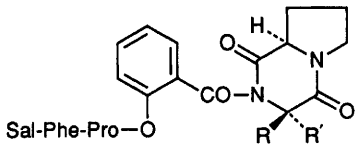

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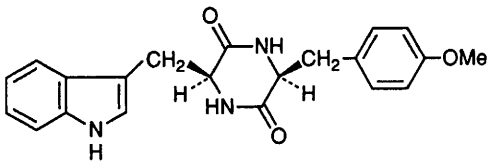

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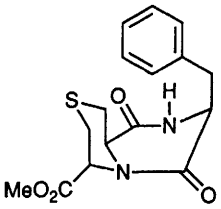
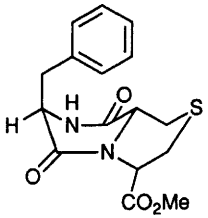


(8) *cis*

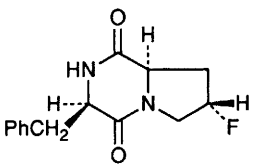


(9) *trans*

intramolecular general acid catalysis at this pH. In the presence of human plasma enzymic hydrolysis competed with cyclisation. The imidazole group has also been implicated[11] in the catalysis of asymmetric addition of HCN to aldehydes in the presence of *cyclo*[(*S*)-Phe-(*S*)-His]. Enantiomeric excesses of 97% in the resulting (*R*)-mandelonitrile formed from benzaldehyde were reported and the mechanism portrayed as in diagram 1. A synthetic route[12] to 3-alkylidene-2,5-piperazinediones (11) has involved condensation of stabilised phosphorus ylides with 5-substituted-2,3,6-piperazinetriones. Thus reaction of $NCCH{=}PPh_3$ with a piperazinetrione gave (11) R = Ph or $PhCH_2$ in 50-64% yield.

2.3 Cyclic Tetrapeptides - The original structures proposed for fenestin A (12) and fenestin (B) (13) in 1988 have been questioned[13], on the basis that the original δ values of the α-protons were outside the δ 3.9 to 4.09 usually associated with cyclopeptides, and the surprise that the ring in (12) had been designated all-*(S)*. When Z-Leu-Ile-Pro-Pro-OC_6F_5 was treated with catalytic hydrogen and cyclised only a very low yield (<5%) of (12) was obtained, but a dimeric cyclic product was produced in 18% yield. Fenestin (B) (13) however was obtained from cyclisation of Z-Leu-Ile-Pro-Val-Pro-OC_6F_5, but the product was not identical with the natural product. Large differences were found between the nmr and mass spectra of fenestin B and the synthetic product and the nmr of the latter as expected showed α-H signals in the region of δ 4.0 - 4.65. So what are the correct structures - read this space next year?

2.4 Cyclic Pentapeptides - In a paper[14] which is both a review of past work, as well as a study of new results on *cyclo*(Gly-Pro-Ala-D-Phe-Pro) and *cyclo*-(D-Ala-Pro-Asn-Gly-Pro), there is further confirmation of the range of conformations possible for the ring system, and how these are dependent on sequence chirality. It was anticipated that the two cyclic pentapeptides above would contain an inverse γ- and a β-turn which would consist of either Gly-Pro-Ala-D-Phe or D-Ala-Pro-Asn-Gly in positions i to i+3. Quantitative nOe measurements in combination with molecular dynamics simulation and energy minimisations have shown that both type I and type II β-turns were present in equilibrium. Presence of Asn at i+2 shifted the equilibrium towards type II. The β- and γ-turn combination of reverse turns has also been observed by X-ray and nmr studies to be present in the typical example *cyclo*-(D-Phe-Pro-Gly-Pro-Gly). An analysis[15] of a pseudopeptide of this type of sequence but containing a thiomethylene insert as in (14) (X = CH_2S, Y = O) has also been carried out. Solid phase synthesis was used for the linear precursor and cyclisation between C-terminal Gly and N-terminal D-Phe was achieved using DPPA/HOBt/DMAP in 70% yield. The CH_2S group replaced the amide associated with the γ-turn in the parent molecule, so when a ^1H and ^{13}C nmr analysis was carried out on the analogue, it was shown that the all-*trans*



(10)

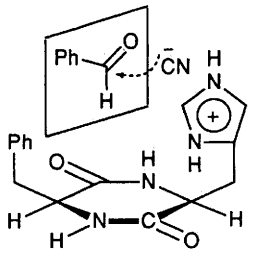
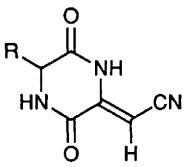


Diagram 1



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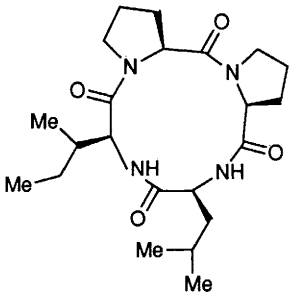

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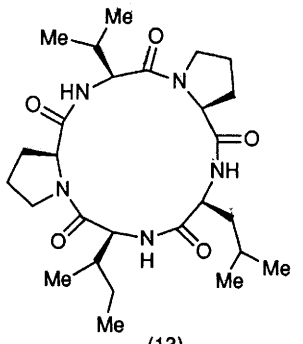

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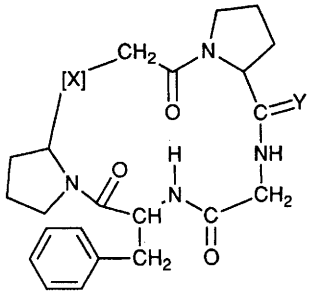

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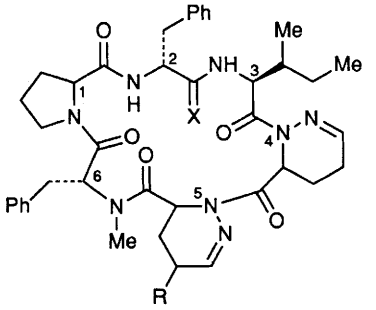
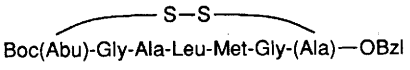


(15) X = O, R = H
(16) X = O, R = $CH_2N(CH_3)_2$; X = S, R = H



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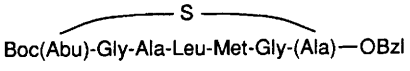


(18)

amide conformation was preserved in $CDCl_3$ but the H-bond to maintain the β-turn appeared weaker. In d_6DMSO another minor conformer could also be detected which was characterised as being due to a *cis* amide at Gly^1-Pro^2. The compatibility of thioamide bonds with the conformational subtleties of reverse turns have been investigated[16] using the two thioamide analogues (14) (X = CSNH, Y = O) and (14) (X = CONH, Y = S). Specific incorporation of the CSNH bonds were made at the linear precursor stage with Lawesson's reagent, and cyclisation carried out using the DPPA procedure. β- and γ-Turns like the parent molecule were seen by nmr analysis of (14) (X = CONH, Y = S) in both $CDCl_3$ and d_6DMSO, while these were seen in (14) (X = CSNH, Y = O) only in $CDCl_3$ solution. In d_6DMSO the latter showed two conformations in the ratio 2:1, the minor component being the *cis* amide rotamer at Gly^1-Pro^2, while the major component was the all-*trans* equivalent.

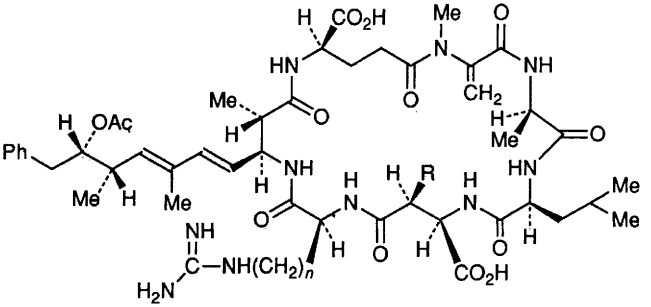
Of general interest to cyclic peptide enthusiasts, not necessarily cyclopentapeptides only, is the application[17] of the distance geometry algorithm for conformation searches. The effectiveness of the procedure in sampling conformational space for cyclic peptides was measured by the ability of the programmes to recover, from a set of 500 structures, conformations similar to those experimentally observed for six cyclic peptides containing from 8 to 20 rotatable backbone bonds. The method worked for structures up to the 16-bond case.

2.5 Cyclic Hexapeptides - A cyclic bouvardin analogue *cyclo*-(-Pro-MeTyr-Ala-MeTyr-MeTyr-D-Ala) has also been determined[18] by distance geometry calculation and restrained energy minimisation from nmr data. A new software package GEOM was used to carry out the calculations on 500 different initial structures and two different strategies led to a unique backbone conformation which consisted of two β-turns, a β-II turn at Pro^1-$MeTyr^2$ and a β-VI turn at $MeTyr^4$-$MeTyr^5$. Two β-turns were also deduced[19] to be the crystal conformation of cleromyrine II *cyclo*(Gly-Tyr-Tyr-Gly-Pro-Leu-Pro) isolated from *Clerodendrum myricoides*. The β-turns include the Pro residues and there is one central short antiparallel sheet stabilised by H-bonds.

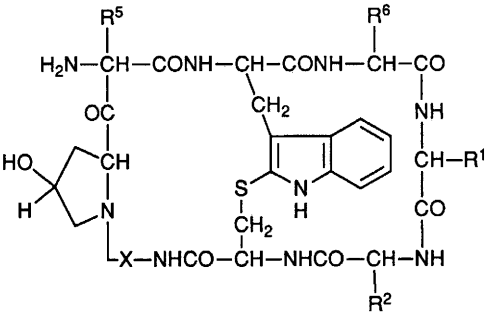
The new structural class of oxytocin antagonists identified as cyclic hexapeptides from *Streptomyces silvenis* and modified to give L-365,209 [structure (15)] have been further modified[20] for structure/activity testing. The impetus came from the poor solubility of (15) for intravenous administration. The difficult Δ-piperazic acids in the sequence were replaced by D- and L-pipecolic acid, but these residues required acid chloride activation for coupling. Oxytocin receptor affinity and selectivity was achieved by substitutions at positions 2 and 4. Basic groups at positions 5 and 6 also improved aqueous solubility, good enough for i.v. administration. Peptide backbone modifications in L-365,209 have also been

investigated[21] by insertion of a thioamide bond at position 2 (16, X = S, R = H) which could also be converted to a CH_2NH surrogate by use of Raney nickel. Analogue (16) (X = O, R = $CH_2N(CH_3)_2$) was characterised as a functional oxytocin antagonist similar to its non-substituted analogue, but had no agonist activity in stimulating phosphatidyl inositol turnover *in vitro*. An X-ray study was also carried out on (16) X = S, R = H which was shown to have a high affinity (K_i = 1.1 nM) for the oxytocin receptor. *Cyclo*-[D-Ala-Phe-Val-Lys(Z)-Trp-Phe], originally derived[22] from analogues of somatostatin and antanamide has been shown to be a very potent inhibitor of the bile acid transport system in hepatocytes. Nmr studies[22] and restraint molecular dynamics calculations have shown that the cyclopeptide shows two conformations (94:6) in slow exchange on the nmr time scale. The dominant form was proved to have all-*trans* peptide bonds forming a βII, βII′ backbone conformation, whereas the minor conformation has a *cis* amide between $Lys(Z)^4$ and Trp^5 forming a βVI turn about these residues. Cyclic hexapeptides have featured[23] successfully in the partial synthesis of the antibiotic nisin. The principle involved is exemplified by (17) leading to a one step desulfurisation to yield (18) by using $P(NEt_2)_3$.

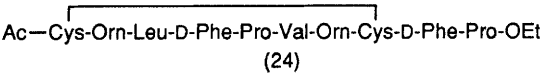
2.6 Cyclic Hepta and Octapeptides - A further three new hepatotoxic cyclic heptapeptides have been characterised[24] from the cyanobacterium (blue-green alga) *Nostoc sp.* strain 152. ^1H and ^{13}C Nmr spectra, chiral analysis of the components on a g.c. chiral capillary column proved the structures to be (19) - (21) with all three toxins having the 9-acetoxy-3-amino-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid residue, instead of the 9-methoxy derivative found in the microcystins. The hydrogenation of the diene bonds in this residue destroys the hepatoxicity. The Western Pacific Ocean sponge *Hymeniacidon sp.* has been found to contain[25] the cyclooctapeptide, *cyclo*[Pro-Pro-Tyr-Val-Leu-Ile-Ile] which is active against the P.388 leukemia cell line (ED_{50} 3.5 μg/mL). Again structural determination was aided by chiral g.c., FAB-MS and 400 MHz nmr techniques. The amatoxin analogue (22) *S*-deoxo-Abu^1, Ile^3-amaninamide, has an L-α-aminobutyric acid residue instead of L-Asn and has been shown to be inactive. It does not inhibit the eukaryotic DNA-dependent RNA polymerase form II in concentrations up to 10^{-4}M whereas 50% inhibition was exerted by the Asn analogue (22) X = -$NHCH(CH_2CONH_2)CO$- in 10^{-6}M solution. The Abu^1 analogue (22) has now been synthesised[26] and nmr studies showed that the striking difference in activity of analogues can be traced to a relatively small conformational change in the Ile region. In the Asn analogue the side-chain $CONH_2$ H-bonds to Trp and Ile NH's, but this is not possible when Abu is present. Modern nmr technology and restrained molecular dynamics have been used[27] on the phallotoxins, as represented by phallacidin (23). Earlier studies on these go back to 1973, so the extra precision now possible has justified a re-look. An assignment of most of the resonances has now been achieved



(19) R = Me, n = 3 (20) R = Me, n = 4 (21) R = H, n = 3



(22) R^1 = CHMeEt, R^2 = H, X = NHCHEtCO, R^5 = $CHMe_2$, R^6 = H
(23) R^1 = $CHMe_2$, R^2 = $CH(OH)CO_2H$, X = null, R^5 = Me, R^6 = $CH_2C(OH)(Me)CH_2OH$



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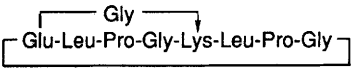


(25)

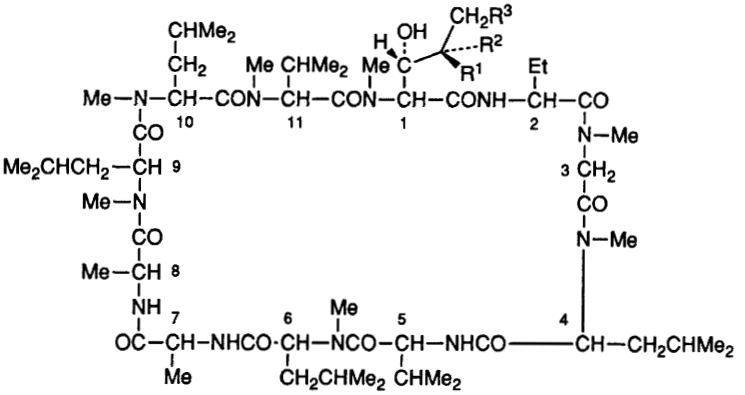
and internuclear distances obtained from rotating frame nOe experiments. Several conformations were detected and the molecule is likely to be equilibrating through at least four forms. The groups essential to toxicity, i.e., Ala side-chain, OH in Pro, the S atom and Trp NH, did not change their conformation in the simulations.

The linear analogue of gramicidin S, Ac-Cys(Acm)-Orn-Leu-D-Phe-Pro-Val-Orn-Sys(Acm)-D-Phe-Pro-OEt has been synthesised[28] using solution techniques and the disulfide ring analogue (24) formed using iodine treatment. The cyclic analogue was 8-16 times less active than gramicidin S, but the linear analogue was completely inactive, so it shows conformational restriction by the S-S link improves potency. The solid state conformational analysis[29] of (25) has been carried out by X-ray techniques. The all-*trans*-amide compound has 3 H-bonds to stabilise its conformations. A type II β-turn, a mixed type I-type III β-turn and a pseudo γ-turn involving Glu have been identified. This solid state conformation is rather different from the solution conformation proposed for the free and Ca^{2+} complexed form previously reported. The hydrophilic cavity with a hydrophobic exterior required for ionophoric molecules have been reproduced[30] using cyclic octa and nonapeptides. Solid phase techniques have enabled linear precursor sequences for *cyclo*-$[Gly_n\text{-}Lys(Z)]_m$ (where n = 3, m = 2 or n = 2, m = 3) to be prepared. A TFA-labile p-alkoxymethyl solid support and an Fmoc-based strategy was used to synthesise the two precursors, H-$(Gly)_2$-Lys(Z)-$(Gly)_3$-Lys(Z)-Gly-OH and H-Gly-Lys(Z)-$(Gly)_2$-Lys(Z)-$(Gly)_2$Lys(Z)-GlyOH. An interesting comparison was made of the yields obtained from the reagents used in the cyclisation steps. As can be seen in Table 1, the first three reagents listed were confirmed as the most efficient but because of the highly toxic nature of DEPC, the authors recommend DPPA and BOP.

Table 1

Reagent	Yield %	Recovery %
Diethylphosphorocyanidate(DEPC)	>99	65
DPPA	>99	70
BOP	>99	68
Woodward K	30	15
HOBt/DCC	65	20
HONSu/DCC	65	25

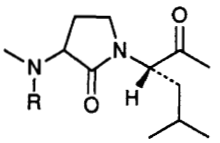
2.7 Higher Cyclic Peptides - Three analogues of the immunosuppressive agent cyclosporin A (26) (R^1 = H, R^2 = CH_2-CH=CH-CH_3, R^3 = H) have been synthesised[31]. The analogues (27) were variants of the MeBmt amino acid at position 1, and when biologically tested showed lower immunosuppressive activity than the parent (26). Nmr methods were used to determine each analogue's conformation in $CDCl_3$, and showed that the 33-membered ring of each analogue



(26) cyclosporin A

(27) $R^1 = R^2 = Me$, H, $R^3 = CH{=}CH{-}Me$; $R^1 = Me$, $R^2 = H$, $R^3 = C{\equiv}C{-}Me$

(28) $R^1 = H$, $R^2 = Me$, $R^3 = OH$, OMe, OEt, OBzl *or* $OCH_2C_6H_4COPh$-4



(29) R = H or Me

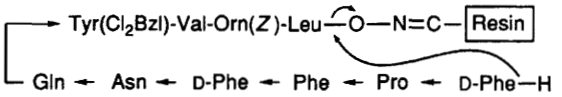
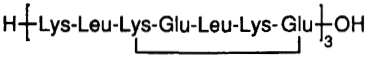


Figure 1



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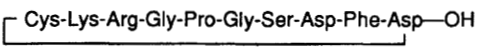

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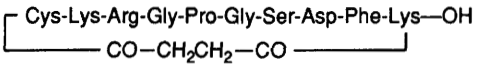


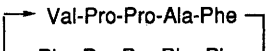
(32)

remained in a similar conformation but the orientation of the MeBmt analogue side chains differed significantly in $CDCl_3$. Systematic search and energy minimisation procedures have allowed the conclusions to be drawn that the bioactive conformations are close to those derived from X-ray and antibody binding studies already reported. Incorporation[32] of enantiomerically pure ε-oxygen functions into the MeBmt residue as depicted in (28) was made possible by synthesis of the amino acid residue in position 1 by stereoselective epoxidation with a peracid followed by a base-catalysed intramolecular rearrangement of an epoxy urethane. These ε-oxygen analogues retained very little of the activity of cyclosporin A, the best being the analogue (28) with $R^3 = OCH_2Ph$ which retained 20-25% activity. Nmr analysis confirmed a similar main ring conformation to the parent molecule but the side-chain conformation revealed a tendency for an intramolecular H-bond between the β-OH and the ε-oxygen of the same residue in position 1. Conformationally restricted γ-lactam analogues of cyclosporin A have also been prepared[33]. The γ-lactam structure (29) was inserted into the 3/4 position either with (R = Me) or without an N-methyl substituent (R = H). The immunosuppressive activity of the γ-lactams were only 4-17% that of the parent compound, so adding constraint did not seem to enhance potency. The coupling reagent BOP-Cl proved useful[34] in four out of the five steps in the quite difficult assembly of the 2-7 hexapeptide fragment which was then used to make D-Lys[8]-cyclosporin A. The authors recommend BOP-Cl as a first choice reagent for *N*-alkylated nucleophiles giving high yields and negligible racemisation. A new nmr assessment[35] of cyclosporin A (26) has been carried out to obtain more accurate distance measurements. Measurements in the slow-motion limit (negative nOe effects) were carried out in $CDCl_3$. Build-up rates at 600 MHz using 6 different mixing times at low temperatures (252.5K) were transformed into distances using two spin approximation. With the new distance restraints in the molecular dynamics simulations using the GROMOS package it was concluded that the new structures deduced resemble more closely the previously reported X-ray structure, where the MeBmt side-chain is folded over the backbone. Another report[36] on cyclosporin A illustrates the potential of heteronuclear double-half-filters as a technique for conformational studies when the cyclopeptide is bound to its presumed receptor, the protein cyclophilin. Three types of sub-spectra were obtained; (a) exclusively the intramolecular nOe cross peaks between protons of cyclosporin A, which enables structure determination of the receptor bond molecule to be made without interference from the receptor; (b) exclusively intramolecular nOe's between protons of cyclophilin, so the ligand does not feature in these values; and (c) intermolecular nOe's between protons of cyclophilin and cyclosporin A. Thiocyclosporin A is known to exist in two conformations in $CDCl_3$, and was taken[37] as a model for recording 3D hetereonuclear nmr spectra in natural abundance. The high resolution and relatively short measuring time was brought about by reduction of spectral width by multiplicity selection *via* heteronuclear

quantum coherence and a TOCSY transfer to all protons coupled to these groups. The effect of solvent environment on the conformation of cyclosporin A has been studied[38] using molecular dynamics. Simulations of the properties in H_2O and CCl_4 have been compared, and it seems that the backbone conformation is independent of solvent type. The conformation in 'simulated CCl_4' agreed with the model obtained from nmr studies in $CDCl_3$ while in H_2O the relaxation of atomic motion tended to be slower than in apolar solvents.

Cyclisation on solid supports is expanding as a technique for the preparation of cyclic peptides. Two examples of the use of the Kaiser oxime resin can be cited. For the synthesis[39] of tyrocidin A the cyclisation was based on Figure 1 and was achieved in 55% yield. A model tricyclic amphiphilic α-helical peptide (30) has also been synthesised[40] using the oxime resin methodology. Cd spectra revealed that the multicyclic compound (30) adopted a mostly disordered conformation in aqueous solution but a high α-helix content in 50% CF_3CH_2OH solution or when adsorbed on to siliconised quartz slides. Mimics of the site A loop structure in the hemagglutinin of the influenza virus have been chosen[41] to be a series of cyclic peptides derived from the region 139-147. Examples of the structural types synthesised are represented by (31) and (32), the cyclisations being carried out directly on the support prior to final cleavage off the resin support. For the cyclisations the diisopropylcarbodiimide/HOBt approach seemed faster than DCC/HOBt approach seemed faster than DCC/HOBt, while the BOP reagent although more efficient gave a less pure product. It was also reported that at high resin loading and faster reaction rates there was a considerable amount of polymerisation.

Detailed conformational studies[42] have been carried out on antamanide (33), using vicinal proton coupling constants and the ^{13}C-relaxation times. Coupling constants were obtained by a least-squares analysis of a 2D E.COSY (exclusive correlation spectroscopy) spectrum. The Pro^3 and Pro^8 residues have been found to be conformationally rigid, while Pro^2 and Pro^7 were mobile with a significant population of a second conformation. An X-ray crystallographic determination of (34) revealed[43] that it had two double bends linked together by a Gly-Val bridge. The analogue is based on the repeat pentapeptide unit of elastin, but with an L-Ala residue in position 3. The usual Pro^2-X^3 β-turn has not been maintained because of the inserted residue, but instead there is a type III Pro^2-X^3 β-turn followed by a type I Ala^3-Val^4 β-turn. A reinvestigation[44] of the phytotoxic metabolites produced by *Aspergillus niger*, using hplc has given rise to a further 4 peaks in the chromatogram of malformin A type compounds. Two of the peaks have been shown to be due to (35) and (36), on the basis of their mass spectra and nmr characteristics. Molecular modelling has suggested a requirement for a D,D,L,D,L configuration but this has not been checked. Compound (35) possessed optimum malformation activity in the corn root test at a concentration of 10^{-7} mol/L with (36) being considerably less active. Macrocycles with 27- and 36- membered rings



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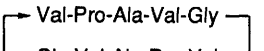

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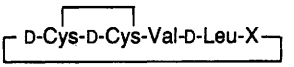
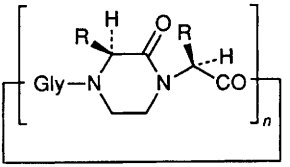


(35) X = Ile (36) X = Val



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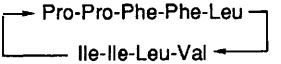

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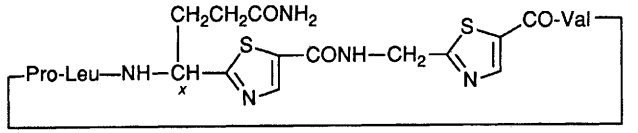
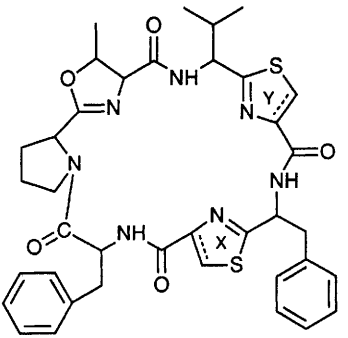
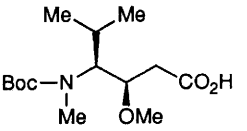


(39) *x* = L (40) *x* = D



(41) Y = thiazoline, X = thiazoline
(42) Y = thiazoline, X = thiazole



(43)

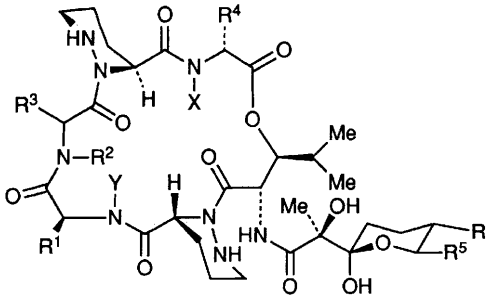
as in (37) where n = 3,4 have been prepared[45] and their interactions with 4-R′-$C_6H_4CH(Me)NH_2$.HBr studied by ^1H and ^{13}C-nmr in $CDCl_3$. Both macrocycles can distinguish between enantiomers of the amine hydrobromide, with the series where n = 4 showing superior enantioface-differentiating abilities than those where n = 3.

The antanamide analogue, cyclolinopeptide A(38) in its dihydrate form, has been analysed by X-ray diffraction[46]. The data show correspondence with the previously reported monohydrate with the Pro^1-Pro^2 bond *cis* and all others *trans*. Similarities with antamanide are seen in the two proline residues. ^1H and ^{13}C-Nmr data[47] on cyclolinopeptide (38) show that the two neighbouring Phe aromatic rings are oriented nearly perpendicular to each other, which is interpreted as strong limitation to free rotation of the aromatics, probably due to the steric hindrance of neighbouring aliphatic side chains.

2.8 Peptides containing Thiazole Type Rings - Dolastatin 3 (39) from the Indian Ocean sea hare *Dolabella auricularia* has been synthesised[48] in high yield (76%) from a pentafluorophenyl active ester linear precursor without high dilution conditions. Diethylphosphoryl cyanide was the coupling reagent of choice in the preparation of the precursor Boc-Leu-(Gln)Thz-(Gly)Thz-Val-Pro-OMe. The conformation of the molecule is characterised by reduced mobility probably due to the presence of the two linked thiazolyl carboxamide system and a *trans* Pro peptide bond. The diastereoisomer of dolastatin 3 (40) has also been synthesised[49] and cd/nmr studies carried out. The conformation of (40) was close to that of (39), yet the analogue (40) was inactive. The configuration at (Gln)Thz must influence interaction at receptors. The success of cytotoxic peptides from marine organisms as potential antineoplastic agents has sparked a great deal of interest in this field especially now that the cyclic peptide didemnin B is undergoing phase II clinical trials. Recently (1989) the isolation and characterisation of three new cyclic peptides from the ascidian *Lissoclinum patella* was reported, and identified as patellamide D, lissoclinamide 4 and 5. A report[50] has now followed this up with a further two lissoclinamides (41) and (42) from the same species. The most potent component was (41) which rivalled didermin B in its *in vitro* activity. Efficient syntheses of the hetero ring systems for incorporation into the lissoclinamides have been reported[51]. Simple condensation reactions between cysteine esters and N-protected imino ethers RNHCHR(OEt)=NH derived from chiral amino acids led to the formation of small peptides suitable for further elaboration. The methylated analogue (43) of the γ-amino-β-hydroxy acids present in the didemnins occurs naturally in dolastatin 10. The stereocontrolled synthesis[52] of (43) based on a chiral allylic ketone reduction with $NaBH_4$ has been reported.

2.9 Cyclodepsipeptides - The richness and diversity in nature's structures is very well exemplified this year again by the number of new cyclic depsipeptides identified. Details of the structural elucidation of the novel cyclodepsipeptide L156,602 isolated from *Streptomyces spp.* M.A6348 apparently have been submitted for publication but it was not retrieved as a publication during 1990. But three reports relating to the synthesis of L156,502 (44) have appeared. The antibiotic is closely related to azinothricin and A835586C. The cyclisation point used for ring closure[53] was the NH_2 of Gly and the carboxyl group of N-hydroxyalanine using phosphonic anhydride for activation. The depside link was formed by reaction of an acyl imidazole with an alcohol group. The construction of the tetrahydropyranylpropionic acid side chain in (44) as a methyl ester has also been reported[54]. Subtle modifications to the basic antibiotic structure (44) have also been carried out[55] to ascertain the function of the various units. The N-OH groups could be selectively mono- or bis-protected as benzyl carbonates which could be readily removed by catalytic hydrogenolysis. Both the (*R*)- and the (*S*)-piperazic acid secondary nitrogens resisted acylation but both could be reductively reduced under the conditions of Borch *et al* (J.Amer.Chem.Soc. 1971, **93**, 2897). Bis-dehydropiperazic acids could be characterised after addition of metachlorperbenzoic acid. Variapeptin (45) and citropeptin (46) produced by *Streptomyces variabilis* and *S.flavidovirens* have also been found [56] to be related to azinothricin and A83586C using 2D nmr.

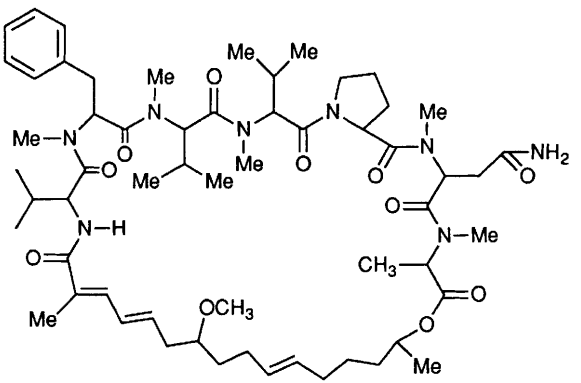
2D Nmr at 400MHz has assisted[57] greatly in the characterisation of the novel cytostatic (PS ED_{50} 0.022 μg/mL) cyclodepsipeptide dolastatin 14 found in the Indian Ocean sea hare *Dolabella auricularia.* The structure offered is shown in (47). In a continuing search of antineoplastic metabolites from marine invertebrates, extracts from the sponge *Pseudoaxinyssa* sp. from Papua New Guinea showed a good spectrum of activity[58]. Four components in the extracts designated geodiamolides C to F have been characterised as (48) - (51) based on nmr data. Coinciding with last year's report from the U.S. of a synthesis for geodiamolides A (52) and B (53), another total synthesis has been reported[59] with full account of the diastereo-controlled steps in the synthesis. The strategy centres around the coupling of the hydroxynonenoic acid derivative (54) to the tripeptide (55) using 1.05 eq of DCC/HOBt. Removal of the Bu^t ester with simultaneous partial desilylation were effected with TFA/ethanedithiol/CH_2Cl_2. The acid thus produced on treatment with trichlorobenzoylchloride in benzene followed by dimethylaminopyridine under reflux gave the 18-membered ring in 18% yield. Microviridin a novel tricyclic depsipeptide from the toxic cyanobacterium *Microcystis viridis* has been identified[60] as having structure (56). Again nmr proved a powerful technique in the elucidation of the toxin's structure which is believed to be the first of its kind from nature. Microviridin strongly inhibits Lys tyrosinase activity which is involved in forming melanin from tyrosine. A novel peptide lactone hormaomycin (57) produced by



(44) R = EtCH(Me)CH_2, R^1 = Me, R^2 = H, R^3 = H, R^4 = Me, R^5 = Me, X = Y = OH

(45) R = EtCH(Me)CH_2, R^1 = Me, R^2 = Me, R^3 = $PhCH_2$, R^4 = CH_2OH, R^5 = Me, X = H, Y = OH

(46) R = Me, R^1 = CH_2OMe, R^2 = Me, R^3 = CH_2CHMe_2, R^4 = MeCH(OH)–, R^5 = MeCH=C(Me)COCH(Me)–CH=C(Me)–, X = Y = H



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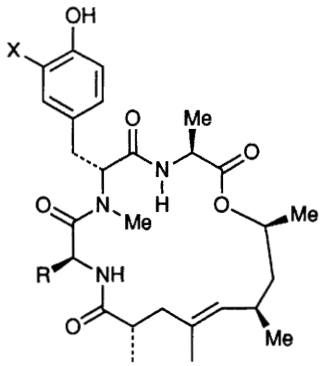
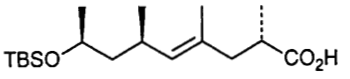


(48) X = Cl, R = Me
(49) X = I, R = H
(50) X = Br, R = H
(51) X = Cl, R = H
(52) X = I, R = Me
(53) X = Br, R = Me



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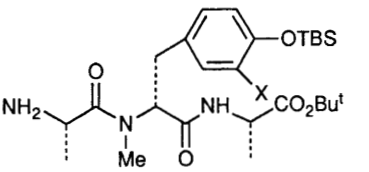

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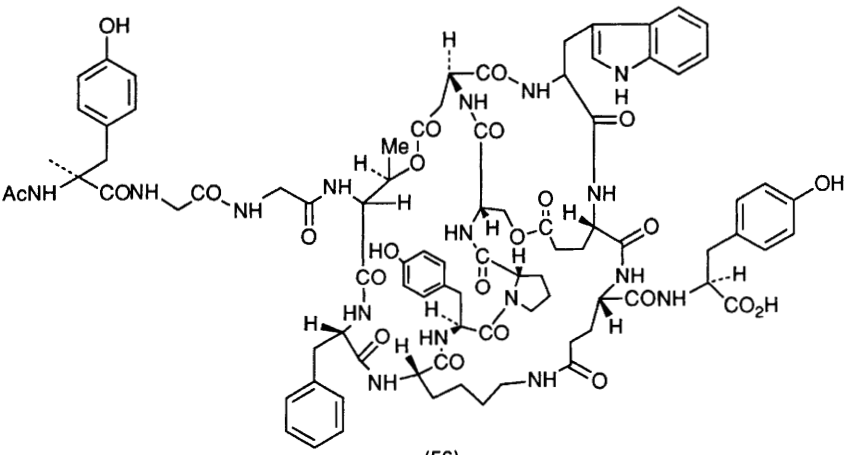
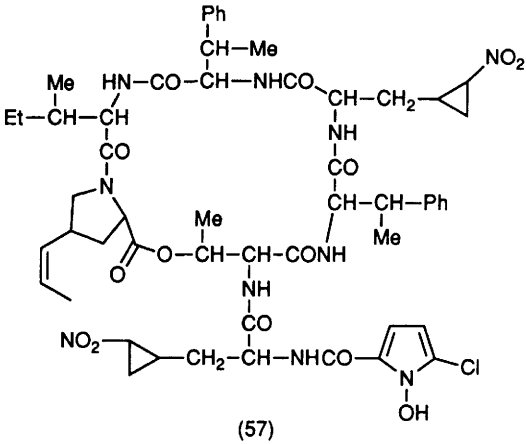


(56)

Streptomyces griseoflavus was identified[61] from its 2D nmr and mass spectra, with chirality being confirmed using a chirasil Val column to separate CF_3CO-derivatised residues. The peptide lactone was selectively active against some Gram positive bacteria. Promising data from clinical studies in transplantation on the immunosuppressive FK-506 has resulted in a further search for analogues. Two analogues of FK-506, sarcosine FK506 (58) and proline FK506 (59) have been synthesised by first of all degrading FK506 at positions x and y. The cleavage involved a multistep process with $Pb(OAc)_4$ and $LiAlH_4$ being key cleavage reagents in the pathway. Once obtained[62], analogues (58) and (59) showed a propensity for rearrangement to ring expanded compounds such as (60). Analogues (58) and (59) exist with their major amide bond rotamer oriented opposite to that of FK-506. Treatment of virginiamycin S_1 (61) with trifluoroacetic acid[63] cleaved the ring at the 4-5 residues, and when recyclised by BOP-Cl gave the 4-epimer [D-MePhe^4]-virginiamycin S. This analogue was resistant to acid hydrolysis in contrast to the native form. Conjugates of virginiamycin S based on the structures in (62) have been prepared[64] using the appropriate acylating agent on the hydroxyl amine derivative. A crystal structure[65] of vernamycin B_α (63) revealed that the 19-atom depsipeptide ring assumes a cup-like conformation folded around the 3-hydroxypicolinic acid residue to form a globular entity with a predominantly hydrophobic surface very similar to the conformation (61).

The structures of the syringomycins, phytotoxins produced by the phytopathogenic bacterium, *Pseudomonas syringae* pv *syringae* have been explored for two decades culminating in the recent structure determination of syringomycin E by Segre. A major syringomycin from sugar cane isolate has now been determined[66] by nmr and mass spectrometry to be (64) which is similar to E except that chlorothreonine and hydroxyaspartic acid residues are exchanged, and in (64) the ClThr is α-linked. The same authors have found[67] that *P. syringae* pv *syringae* SY12 isolated in Japan from lilac blights produced two novel phytotoxins which have been identified as syringostatins A (65) and B (66).

The didemnins A (67), B (68) and C (69) share a common macrocyclic structure and a full report has now appeared of their total synthesis[68] which has been successfully achieved by introducing the substituents onto the macrocycle as the last stage in each case. A disconnection between a leucine tetrapeptide (71) and the HIP statine unit (70) produced target molecules for synthesis, which were then coupled using isoprenylchloroformate to give a linear precursor which was cyclised at the NH_2 group of leucine using diphenylphosphoryl azide, once the Z-group had been hydrogenolysed. The side-chains of didemnins A, B and C were introduced onto the threonine NH_2 group using the BOP reagent. Conformational isomers of [Me-L-Leu^7]didemnin B have been investigated[69] by 2D nmr techniques and refined by molecular dynamics calculation using the GROMOS programme. Comparison of this analogue with the solution structure of didemnin B (68) showed that one



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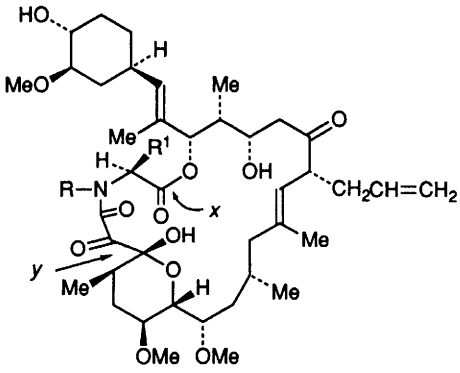
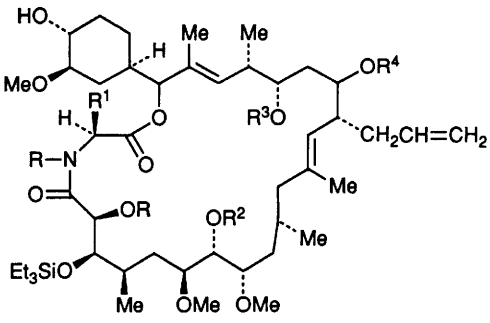


(58) R = Me, R^1 = H
(59) RR^1 = $(CH_2)_3$



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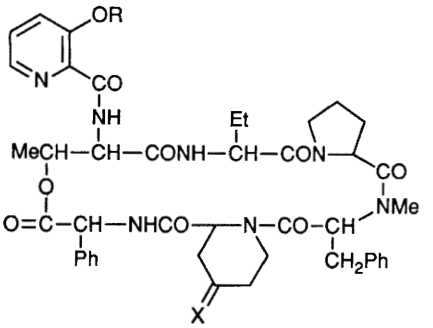
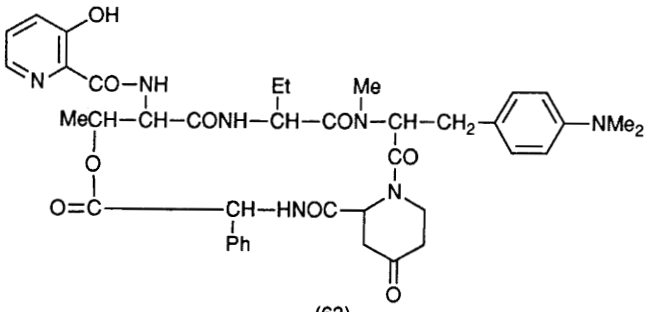
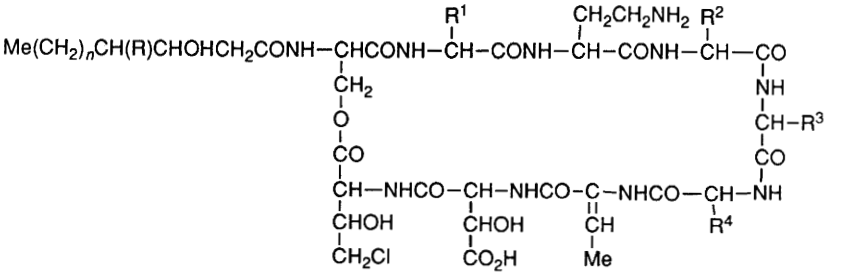


(61) R = H, X = O

(62) R = H, X = $NOCH_2CH_2NHR^1$ (R^1 = H, Ac, $COCF_3$, CO_2Me, CO_2Et, $CONMe_2$, $CO_2CH_2CCl_3$)



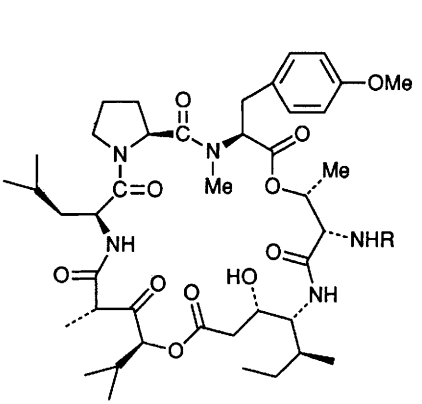
(63)



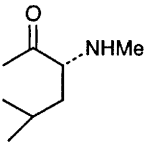
(64) *n* = 8, R = H, R^1 = CH_2OH, R^2 = $(CH_2)_2NH_2$, R^3 = $(CH_2)_3NHC(=NH)NH_2$, R^4 = CH_2Ph

(65) *n* = 9, R = H, R^1 = $(CH_2)_2NH_2$, R^2 = $(CH_2)_2OH$, R^3 = $(CH_2)_3NH_2$, R^4 = $CH(OH)CH_3$

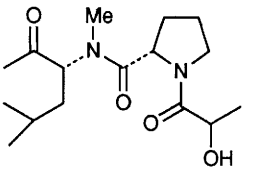
(66) As (65) but R = OH



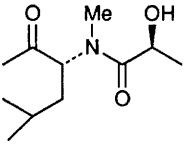
(67) R =

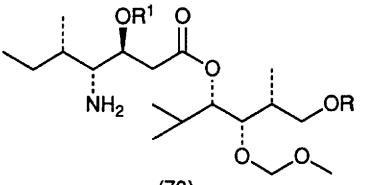


(68) R =



(69) R =

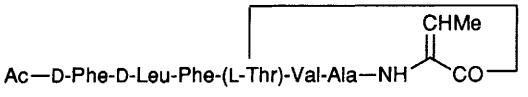




(70)

+ Z—Leu-Pro-MeTyr(Me)—Boc—Thr—OH

(71)



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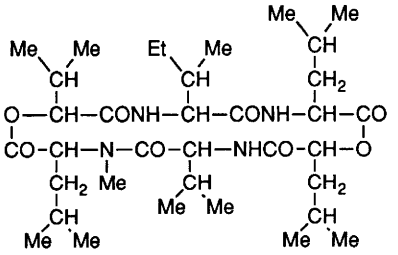

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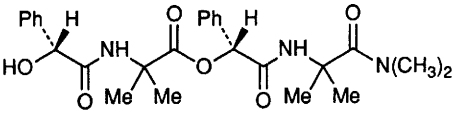


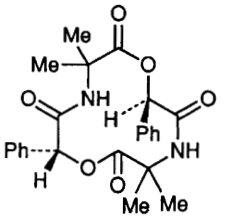
(74)

conformer (designated conformer A) was very similar to the B (68) conformation though the isostatine-hydroxyisovalerylpropionic acid region of the ring was slightly extended in conformer A. Another conformer (conformer B) exhibited a βVI-turn in the linear side-chain moiety. Protected *(2R,3S)*alloisoleucine and *(3S,4R,5S)*-isostatine derivatives have been synthesised[70] as part of a didemnin synthetic programme.

When the peptide lactone antibiotic TL-119 and/or A-3302-B was synthesised[71] according to structure (72) its properties did not concur with the natural form. A reassessment of the configurations of the amino acids has confirmed that the antibiotic contains a D-*allo*threonine instead of L-Thr. Four sporidesmolides have already been characterised before, but a fifth sporidesmolide V has now been found[72] in the cultures of *Pithomyces chortarum* and found to be of the same cyclic structure (73) as other members of the family but possesses different side chains. Direct amide cyclisations of linear precursors such as (74) to give 12-atom cyclodepsipeptides such as (75) have been carried out[73] using HCl in toluene at 100°C. The linear precursor (74) was synthesised *via* the azirine/oxazolone method for the synthesis of α,α-disubstituted compounds. The macrocyclic ionophore (76) was a typical structure of the compounds resulting from the acylation of H-Val-Val-OH with 2-chloroacetyl chloride followed by treatment with $CsCO_3$ in DMF[74].

2.10 Cyclic Peptides containing Other Non-Protein Ring Components - Full details have now emerged[75] for the implementation of an activated Ullmann condensation for the synthesis of the ACE inhibitor from *Micromonospora halophytica* ssp *exilisia* K.13 (77) and of OF4949-III (78, R = H, R^1 = Me) and OF4949-IV (78, R = H, R^1 = H). The methodology proceeded without amino acid racemisation and could be useful for selectively protected DOPA-derivatives. A thioether analogue (80) of K-13 has also been synthesised[76], and provided material for a nmr and modelling study. The diphenyl ether link in (79) was built in at the linear precursor stage using a coupling between the iodonium salt (81) and the phenoxide (82). The conversion of the linear precursor to the cyclic analogue (79) was made by activation of the Ala COOH group using diphenylphosphoryl azide. A new ACE inhibitor has been identified[78] as ancovenin (83), isolated from *Streptomyces* sp No.A647P-2. The cyclic peptide forms a tricyclic structure bridged by sulfide bonds based on 3 lanthionine residues. The Ugi four-component condensation reaction has been used[79] to synthesise the key intermediate (84), which was then processed to the 28-membered cyclopeptide alkaloid nummularine F.

The marine sponge of the genus *Theonella* has been shown to contain inhibitors of various proteinases particularly thrombin. The structures of two active compounds in *Theonella*, cyclotheonamide (A) (85) and (B) (86) have been elucidated[80]. Two analogues (87) and (88) of the biphenomycin antibiotics, having a



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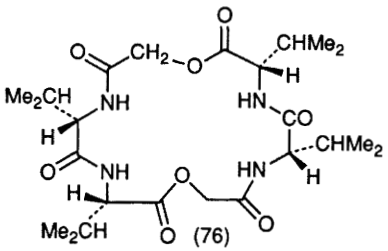
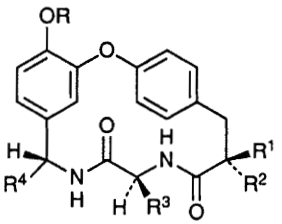
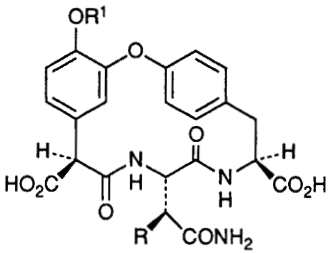


(76)



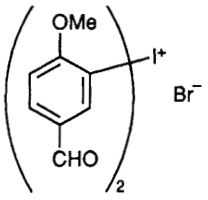


(78)

(77) R = H, R^1 = H, R^2 = NHAc, R^3 = 4OH-$PhCH_2$, R^4 = CO_2H

(79) R = Me, R^1 = NHBoc, R^2 = H, R^3 = Me, R^4 = H

(80) As (77) but S replacing aryl ether link



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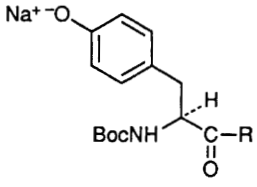

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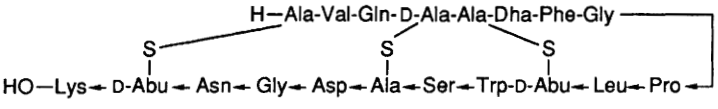

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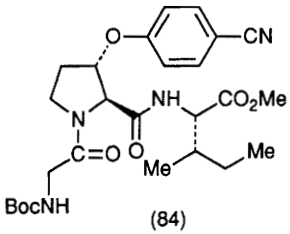

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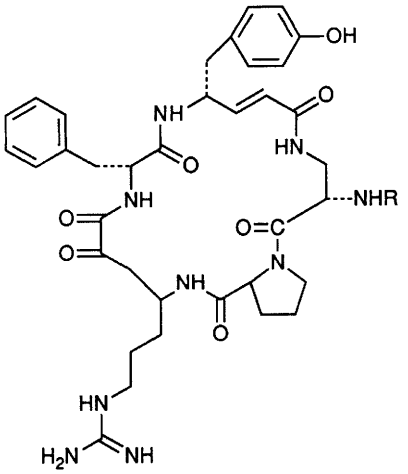
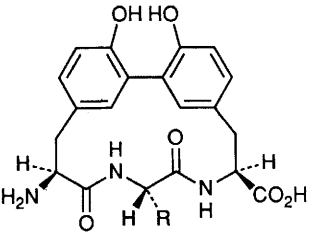
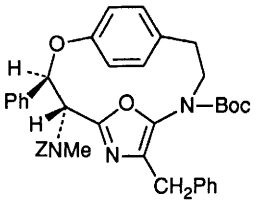


(85) R = CHO
(86) R = Ac



(87) R = Me
(88) R = $(CH_2)_3NH_2$



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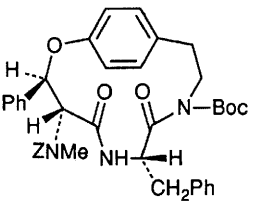

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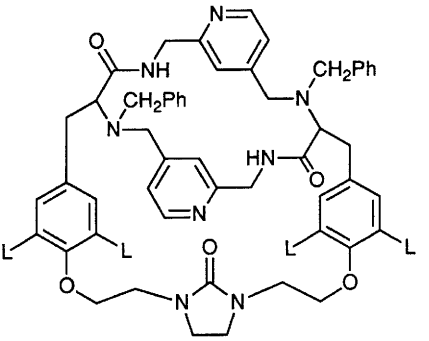


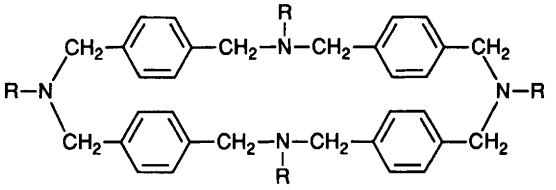
(91)

biphenyl link *ortho* to phenolic hydroxyls have been synthesised[81] from tyrosine derivatives using vanadium oxytrichloride. In an interesting use of heterocyclic rings as masking groups for dipeptide units, the oxazolophane (89) has been used as a model for the synthesis[82] of compounds such as (90) which contains the 14-membered ring typical of a number of cyclopeptide alkaloids. The macrotricyclic compound (91) has been synthesised[83] starting from L-Tyr, and has been shown to be enantioselective in its binding to a guest molecule N-Ac-L-Ala-amides. The cationic host molecules (92) and (93) have been shown[84] to strongly bind anionic and nonionic hydrophobic guest molecules such as 8-anilinonaphthalene-1-sulfonate, and N-phenyl-1-naphthylamine to form 1:1 inclusion complexes. ^{1}H Nmr applied to the host-guest complex indicated that the guest molecule was undoubtedly incorporated into the 3D cavity provided intramolecularly by the macrocyclic ring and the eight hydrocarbon chains. The total synthesis[85] has been reported of a conformationally flexible 18-membered cyclic pentapeptide (94) bearing a simple rigid mimic of the Tyr^5-Tyr^6 moiety in bouvardin/deoxybouvardin. The analogue (94) was made from the coupling of Boc-D-Ala-L-Ala-L-MeTyr-L-Ala-OH with an aromatic amino ester using DCC, and the resulting linear precursor after deprotection cyclised using $(PhO)_2P(O)N_3$. Acyl antibiotics cepafungins I, II and III (R = $CH_2CH_2CHMe_2$, Bu and $CHMe_2$, respectively) with the basic structure (95) have been elucidated[86] using degradation reactions and nmr evidence. The major component I and minor component III are new, but II is identical to glidobactin A which has recently been reported.

A protected peptide based on hen egg white lysozyme sequence 87-97 has been condensed[87] onto a cyclic peptide carrier (96) (R = H) using the BOP reagent to give (96). The final ring cyclisation stage between Gly and Lys was effected using the DPPA reagent. A comparative conformational analysis[88] has been carried out using high field nmr on two model molecules *cyclo*(3Me-*o*-aB-Gly$_4$-AA) and *cyclo*(2X-*m*aB-Gly$_4$-AA) where *o*aB and *m*aB represent *ortho*- and *meta*-aminobenzoic acids and AA was either Phe or Arg. The *meta* analogues had greater conformational mobility than the *ortho* analogues, which was also reflected in the former's greater reactivity towards enzymic hydrolysis.

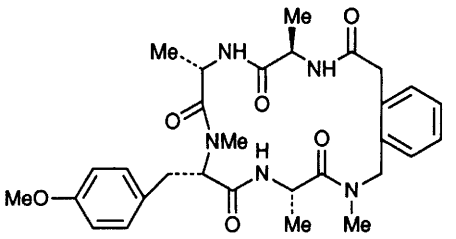
3. Modified Linear Peptides

This section sees the greatest change of format this year, in that common authorship between Chapters 3 and 4 has resulted in discussions of enzyme inhibitors, dehydropeptides α,α-dialkylamino acids, amide bond surrogates being covered solely in Chapter 3.

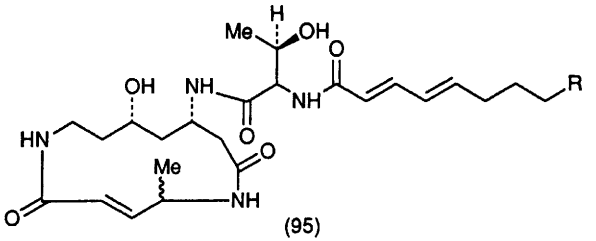


(92) R = $Me_3N^+CH_2CONHCH(CH_2CO-)CON[(CH_2)_{13}Me]_2Br^-$

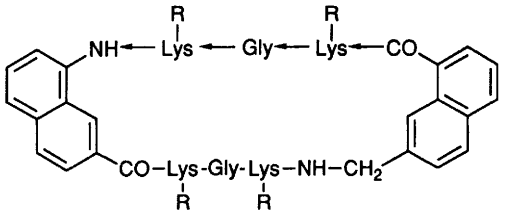
(93) R = $Me_3N^+CH_2CONHCH(CO-)CH_2CON[(CH_2)_{13}Me]_2Br^-$



(94)



(95)



(96) R = Ac—Asp-Ala-Aib-Aib-Thr-Ala-Ala-Aib-Asn-Ala-Aib-Lys-Lys-Leu-Gly-

3.1 Phosphonopeptides - The long term research of an Australian group of researchers has matured into a number of papers on *O*-phospho peptides this year. Boc-Tyr(PO_3Me_2)-OMaq where Maq = anthraquinon-2-ylmethyl has been prepared[89] in high yields by either a phosphorotriester or phosphite-triester phosphorylation of Boc-Tyr-OMaq. The former methodology involved generating a phenoxide ion which was then treated with di-methyl phosphorochloridate $(MeO)_2P(O)Cl$, while the latter technique utilised $(MeO)_2PNEt_2$ in the presence of 1H-tetrazole. *m*Chloroperoxybenzoic acid oxidation of the phosphite led to the protected phosphate. Several deprotecting reagents and conditions were used on the Me phosphate group including 'hard' acids such as CF_3SO_3, $(CH)_3SiBr$ or $CF_3SO_3Si(CH_3)_3$. The presence of thioanisole enhanced the rate of cleavage. In work related to the synthesis of peptides from casein, serine peptides have been phosphorylated using the two step phosphate triester approach[90]. Thus Ac-Ser-NHMe reacted successfully with $(EtO)_2PCl$/pyridine or $(EtO)_2PNEt_2$/1H-tetrazole followed by iodine/water to give Ac-Ser(PO_3Et_2)-NHMe in high yield. However use of $(PhO)_2POCl$/pyridine or $(EtO)_2PCl$/pyridine failed to give the phosphorylated derivative. A ^{31}P nmr investigation did show that phosphorylation took place but it was followed by rapid dephosphorylation. The two step phosphite triester approach above also proved successful in the synthesis of Boc-Glu(Bu^t)-Ser[$PO_3(CH_2Ph)_2$]-Leu-OBu^t which after hydrogenation and acidolytic treatment gave H-Glu-Ser(PO_3H_2)-Leu-OH. Good yields[91] in the phosphorylation step using $(PhCH_2O)_2PNEt_2$/1H-tetrazole were seen for the series Ac-[Ser(PO_3H_2)]$_n$NHMe where n = 1-3. The advantages of using a side-chain isostere of -Ser-PO_3H_2 for biological examination has been studied through the use of its methylene isostere 2-amino-4-phosphonobutanoic acid NH_2-CH($CH_2CH_2PO_3H_2$)-COOH (Abu PO_3H_2). An improved seven step procedure for the synthesis of Boc-Abu(PO_3Me_2)OH has now been reported[92]. Researchers working on a long term perspective should note[93] that Ac-Ser[$PO_3(CH_2Ph)_2$]-NHMe gave H-Ser-NHMe after 12 months at 20°C, which amounts to an O to N phosphorus shift followed by acid hydrolysis. Ac-Ser(PO_3H_2N)HMe gave the same product over a period of 4 years!

Three alternative approaches to the use of solid phase techniques in the synthesis of phosphopeptides have been reported. One approach[94] involved synthesising the non-phosphorylated peptide on the resin using a Wang linker and arranging for all side-chains to be protected except for Ser (or Thr). The hydroxyl side-chains of these amino acids were then phosphorylated on the resin using N,N-diisopropyl bis(4-chlorobenzyl)phosphoramidite/1H-tetrazole followed by t-butylhydroperoxide as part of an automated protocol. Another protocol used[95] was to couple the initial amino-acid on to the p-alkoxybenzyl resin, then subsequent residues (including acid sensitive groups such as the phospho-derivatised Ser or Tyr) were added as *N*-allyloxycarbonyl(Alloc) derivatives which were deprotected at each

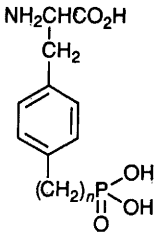
step by hydrostannolytic cleavage. It was only as a last step in cleavage off the resin was CF_3COOH used. Alloc-Ser-OH was derivatised using di-*t*-butyl *N,N*-diethylphosphoramidite in the presence of tetrazole, followed by oxidation *in situ*. Boc-phosphotyrosine derivatives have been synthesised[96] and incorporated into slightly modified conventional solid phase protocol. An enzymatic synthesis of *O*-phosphorylated tyrosine has been carried out[97] by enzymatic transfer of adenosine monophosphate moiety onto the Tyr phenolic group using *E. coli* glutamine synthase adenylyltransferase. The phosphotyrosine is produced from this derivative, either by another enzyme micrococcal nuclease or by sodum *m*-periodate. Peptides phosphorylated in this way included Tyr^5-bradykinin, Leu-enkephalin, angiotensin II and Val^5 angiotensin II in yields ranging from 3 to 40%. Interest in 4-phosphono and 4-phosphonomethyl DL-Phe lies in their potential[98] use as mimics which could cause interference with the metabolism of *O*-phosphotyrosine. Compound (97, n = 0) was synthesised from 4-bromo-DL-Phe while (97, n = 1) was obtained from methyl *p*-toluate. Neither compound showed cytostatic activity but a diethyl derivative of (97, n = 1) inhibited cell growth in the range 250 μg - 1 mg ml^{-1}.

First steps in the development of acylphosphonic acids and oxyiminophosphonic moieties as novel bioactive agents have been achieved[99] by the synthesis of compounds such as (98), made by reacting Z-Pro-Cl with $(MeO)_3P$ and phthaloyl derivatives (99).

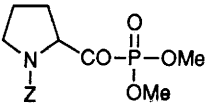
4.0 Conjugate Peptides

4.1 Glycopeptide Antibiotics - The structure of a novel glycopeptide antibiotic UK-68,597 has been elucidated[100] as (100) using FAB/MS and nmr techniques. It is a similar structure to ristocetin and teicoplanin, but unusual in its high degree of chlorination, its aromatic sulfate ester and an α-keto group in place of N-terminal amine. In UK-72,051, a novel antibiotic structurally related to vancomycin (101) and isolated[101] from a streptomycete fermentation, the sugar unit and the position of aromatic chlorine differ from vancomycin itself as seen in (102). Again nmr and mass spectrometry featured as key techniques in the elucidation.

The formation of diaryl ethers is a key stage in any attempt to synthesise the vancomycin group of antibiotics. The traditional Ullmann approach to diaryl ethers appears to be too vigorous in its high temperatures so other alternatives are being developed. Last year's Report included a synthesis based on an oxidative coupling using $Tl(NO_3)_3$. The originators of this technique have now reported[102] their approach using the steps outlined in Scheme 1. Organomanganese chemistry has also been brought to bear[103] on the diarylether synthesis as outlined in Scheme 2 for the part synthesis of a deoxy analogue of ristomycinic acid.



(97) n = 0 or 1



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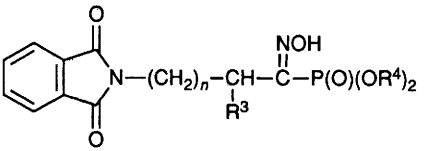
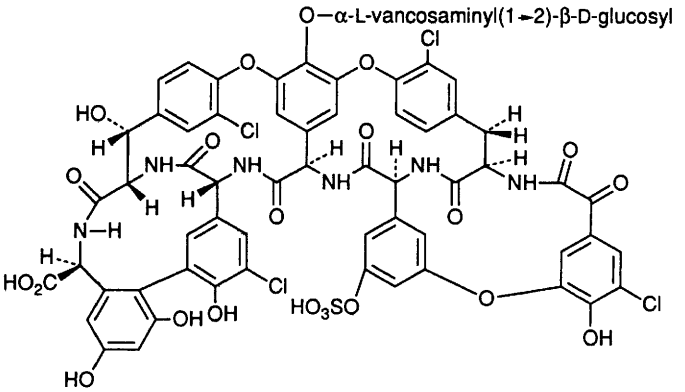


(99) R^3 = Me, CH_2Ph, H, R^4 = Me, $CHMe_2$, Et, n = 0, 1, 2



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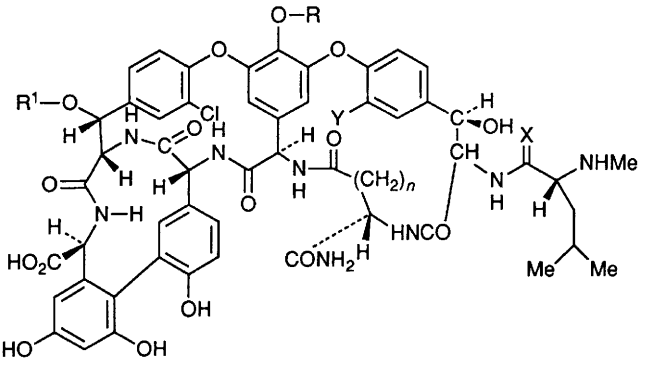
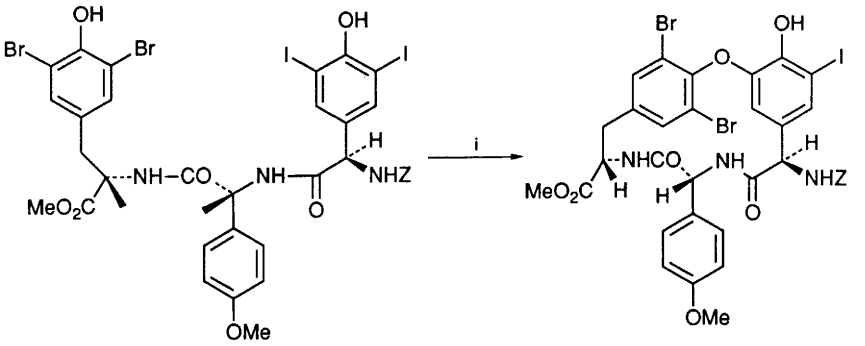


(101) R = α-L-Vancosaminyl(1→2)-β-D-glucosyl
R^1 = H, Y = Cl, X = O, *n* = 1
(102) R = 4-*epi*-Vancosaminyl(1→2)-glucosyl
R^1 = 4-*epi*-Vancosaminyl, Y = H, X = H_2, *n* = 0



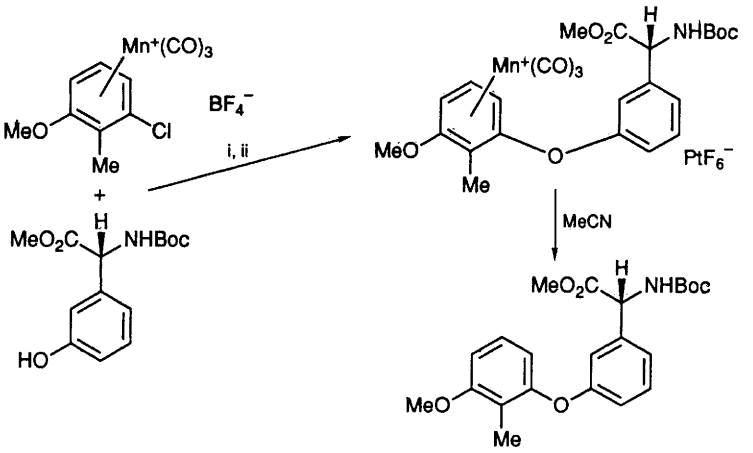
Reagent: i, $Tl(NO_3)_3$

Scheme 1

4.2. Other Glycopeptides - Glycopeptides with T_N and T antigen structures (103) and (104) representing the *N*-terminal tripeptide of asialoglycophorin with blood group M specificity, have been synthesised[104] using Fmoc and Pyoc (2-pyridylethoxycarbonyl) for *N*-terminal protection. The *O*-glycosyl linkages were stable to the morpholine conditions used to remove the N-terminal protection. On coupling to bovine serum albumin *via* carbodiimide procedures an average of 20 or more T_N and T antigen glycopeptides per mole of protein was achieved. Again using the Fmoc-protection protocol it has been shown[105] that solid phase peptide synthesis can tolerate use of unprotected mono- and di-saccharide units. Scheme 3 summarises the stages in a T cell epitope peptide synthesis of this kind. Multivalent T_N antigen cluster-Lys-Lys conjugates such as (105) have also been synthesised[106] by coupling (GalNAc $\alpha 1 \rightarrow O$-Ser) to Lys-Lys. Continuous flow solid phase technology has allowed[107] the incorporation of Fmoc pentafluorophenyl ester derivative (106) into a nonapeptide analogue of antifreeze glycopeptides without loss of glycoside unit.

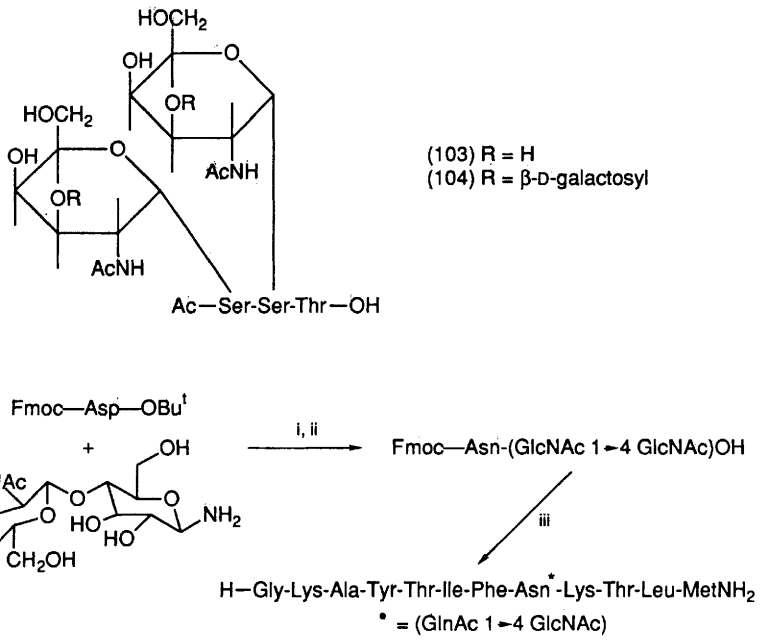
The PAL linker (107) has shown compatibility with Fmoc protection for the synthesis of morphiceptin analogue (108). The hydroxyproline residues were introduced as the Fmoc acetylated sugar derivatives. There was no damage to the glycosidic bond during the cleavage conditions which also involved deprotection of the sugar unit on the resin. Branched glycopeptide (109) has been prepared[109] by coupling the 6-aminohexyl 6-O-[bis(2,2,2-trichloroethoxy)phosphinyl] α-D-manno-pyranoside with Ac-Tyr-Asp(-Ala-OH)-Ala-OH. Four tuftsin analogues, H-Thr(R)-Lys-Pro-Arg-OH where R can represent α- or β-glucopyranosyl, or α- or β-D-galactopyranosyl have been synthesised[110]. Fmoc Thr (αGlc)-OH and Fmoc Thr(αGal)-OH were introduced without OH protection of the sugars using DCC/HOBt which were reacted with H-Lys(Z)-Pro-Arg(NO_2)-OBzl.

In the synthesis of proline containing *O*-glycopeptides the threonine derivative (110) can be extended[111] either at the *N*- or *C*-termini. For extension at the *C*-terminus the allyloxycarbonyl group (Aloc) was used for *N*-protection, while for *N*-terminal extension the Bu^t ester was preferred. Glycosyl amino acids based on 2-acetamido-2-deoxy-D-galactose derivatives of Ser or Thr have been coupled[112] on *p*-alkoxybenzylpolystyrene resin using Fmoc protection and DCC/HOBt activation. The coupling[113] of glycosyl amines to aspartic acid to form (111) has been the key to the formation of a number of asparagine-linked glycopeptides. The BOP and HBTU reagents proved best in the sugar/amino acid linking which resulted in the formation of (111) which included R = Ac, R^1 = Val-PheNH_2, Pro-Phe-NH_2, Gly-Phe-NH_2; R = Ac-Tyr, R^1 = Leu-Thr-Ser-NH_2. Sialo-glycopeptides have been produced[114] in a 'one-pot' procedure utilising the enzymes galactosyl-transferase and α 2,6-sialyl transferase. Again the Aloc-protecting group proved successful in the synthesis as typified by (112).



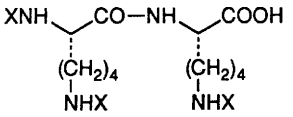
Reagents: i, NaH; ii, NH_4PtF_6

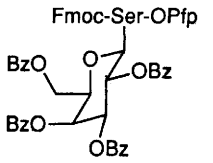
Scheme 2



Reagents: i, Pfp-OH/DCC; ii, TFA; iii, automated solid phase

Scheme 3





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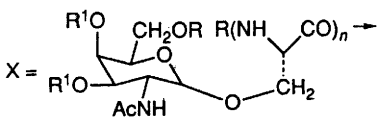

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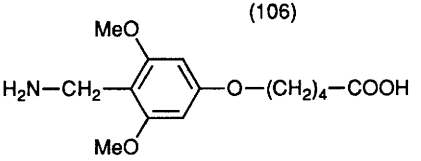

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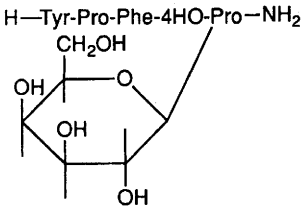

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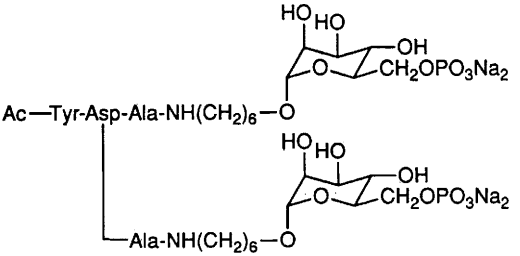
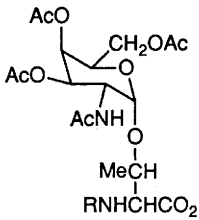
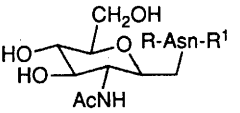


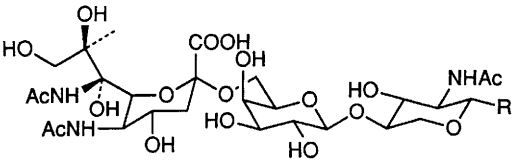
(109)





(111)

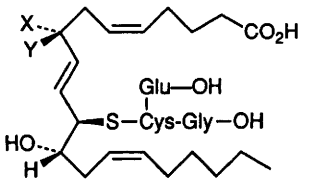
(110) R = H, R^1 = Bu^t or R = Aloc, R^1 = H



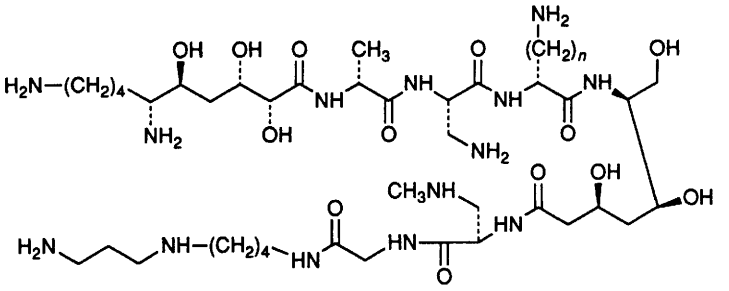
(112) R = Aloc—Phe-Asn-Ser-Thr-Ile—OH

or R = H—Gly-Gly-Asn-Gly-Gly—OH

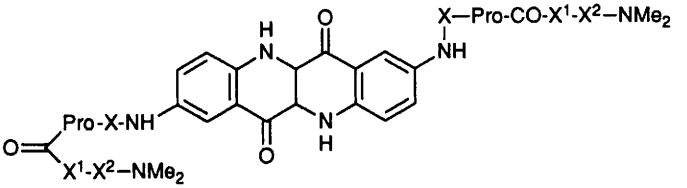
4.3 Non-Carbohydrate Peptide Conjugates - Interesting potential neuromodulator molecules have emerged[115] after the absolute configuration of 8(*R*)- and 8(*S*)-hepoxilin A_3 has been proven. The 11-(*R*) glutathione thiol conjugates (113) and (114) were synthesised, and it could be shown that they are also present in homogenates of rat brain hippocamus and that they cause membrane hyperpolarisation and changes in postsynaptic potential. The original structure of galantin I isolated in 1981 from *Bacillus pulvifaciens* has had to be subjected to one or two changes over the decade. However, a synthesis[116] of the proposed structure has contributed to another modification with the correct structures now believed to be (115) and (116) representing a mixture of D-Orn and D-Lys analogues. In an effort to determine the role of H-bonding in stabilising secondary structure, the conjugate (117) has been synthesised[117] and found to be a model for β-sheet formations. Novel morphinan peptides based on ethenisomorphinan and enkephalin residues (118) have been synthesised[118] by coupling the morphinan residue *via* the acid chloride and demethylating the product with HBr/HOAc to give (118) with R = PheOEt, D-PheOEt, Gly-PheOEt. It has been reported[119] that newly synthesised lipopentapeptides with (*R*)-glycerol moieties showed higher mitogenic activities than those with the (*S*)-configuration. Transient protection of serine residues by *O*-p-(methylsulfinyl)benzyl derivatives has provided[120] the opportunity of selective sulfation of tyrosine with SO_3/DMF to give cholecystokinin CCK-12 as a model test.



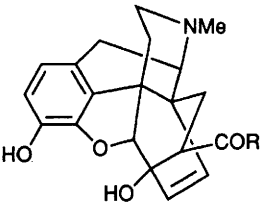
(113) X = OH, Y = H
(114) X = H, Y = OH



(115) n = 3 (116) n = 4



(117) X = Gly, D-Ala; X^1 = Gly, Ala, D-Ala, $NHMe_2CO$
X^2 = Phe, Ala, D-Phe



(118)

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5
β-Lactam Antibiotic Chemistry

BY C.H. FRYDRYCH

1. INTRODUCTION

The organic chemist's fascination with the chemistry and reactivity of the highly strained β-lactam ring remains undiminished. This year has seen a slight increase in the number of publications appropriate to this review. A larger than usual number of papers dealing with structure-activity relationships in cephalosporins is noted, many of these are listed in the Appendix.

The section headings which follow are identical to those used in Volume 22.

A smaller number of reviews have been published this year. Of those published, three are concerned with various aspects of biosynthesis. An article has appeared reviewing the enzymatic synthesis of β-lactams.[1] The various enzymes involved in biosynthetic pathways are the subject of another publication.[2] A review of the elegant studies involving Isopenicillin N Synthase (IPNS) highlights the considerable range of viable substrates, the products from their interaction with IPNS, and the mechanistic information gleaned from the structure of the products.[3] A further review of the synthesis of intermediates for 1β-methyl carbapenems has appeared.[4] The preparation of monobactams by intramolecular cyclisation is the subject of a review.[5]

2. NEW NATURAL PRODUCTS

No reports of new β-lactam containing natural products have been found in the 1990 literature.

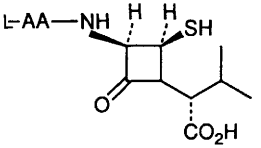
3. BIOSYNTHESIS

Three reviews covering various aspects of β-lactam antibiotic biosynthesis were noted in the Introduction (*vide supra*). A published account of a lecture given at a conference in Israel provides a useful overview of work on the biosynthesis of penicillins and cephalosporins.[6] A report has appeared detailing the purification of the enzyme δ-(L-aminoadipyl)-L-cysteinyl-D-valine (ACV) synthetase from *Cephalosporium acremonium* to electrophoretic homogeneity.[7]

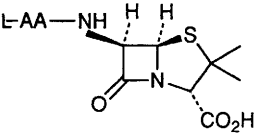
The successful preparation and characterisation of seco-Isopenicillin N, (**1**) a compound which is too unstable to allow isolation from solution, has been reported.[8] Incubation of (**1**) with the enzyme IPNS did not result in its conversion to Isopenicillin N. The biosynthesis of Isopenicillin N (**2**) from ACV was not affected by co-incubation with (**1**). Incubation of the allenyl derivative of valine (**3**) with IPNS provided 3-acetopenam (**4**) *via* a "hydroxylative" process[9] in disagreement with an earlier hypothesis that such substrates would be processed by a "desaturative" pathway. In contrast the propargylglycine-containing tripeptide (**5a**) provided the α-acetylenic penicillin (**6a**) in good yield while incubation of the cyanoglycine tripeptide (**5b**) resulted in only 10% conversion to give a 1:1 mixture of α- and β-cyano penicillins (**6b, 6c**).[9a] A full account of studies on the incubation of tripeptides having unsaturated amino acids in the C-terminal position has appeared.[10] The precise stereochemical requirements for their conversion to bicyclic β-lactams was studied. A number of tripeptides modified in the side-chain were used to probe the specificity of the enzyme IPNS. While (**7a**), having a rigid transoid structure is converted with >90% efficiency, the cisoid (**7b**) undergoes only 12% conversion.[11] This and other results are seen as strong evidence for a fixed distance between carboxy and cyclisation binding sites and that this is best matched by a 6-carbon side-chain in a linear transoid configuration.

The acylation of 6-aminopenicillanic acid (**6-APA**) by the enzyme acylCoA:6APA acyltransferase using various glutathione S-acyl derivatives as acyl donors provided penicillins G, V and K. All reactions were enhanced by the addition of CoA.[12] In a separate study using the same enzyme various acyl-CoA derivatives were tested as substrates. The results revealed a number of stereochemical requirements for successful incorporation into penicillins. Most notable are the importance of free rotation around the side-chain C(2)-C(3) bond and the overall volume of the substrate.[13]

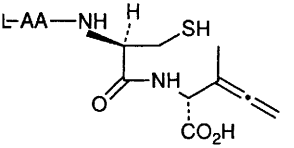
Full accounts of two different syntheses[14,15] of proclavaminic acid (**8**), a monocyclic β-lactam precursor of clavulanic acid, have appeared. One of these allows the absolute stereochemistry to be assigned as (2S,3R). This isomer was an efficient substrate for the enzyme clavaminic acid synthase providing clavaminic acid (**9a**). In a separate publication the purification and characterisation of the enzyme clavaminate synthase from *Streptomyces clavuligerus* is described. In this study all four isomers of proclavaminic acid (**8**) were prepared as two racemic pairs. The stereochemistry of the natural substrate was deduced by kinetic measurements.[16]



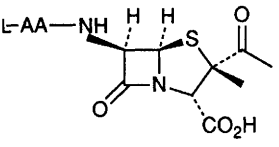
(1) L-AA = L-α-aminoadipyl



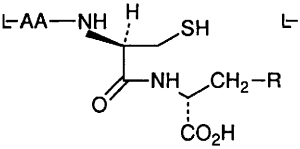
(2)



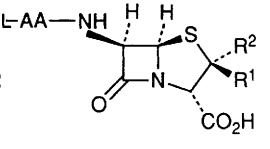
(3)



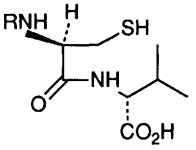
(4)

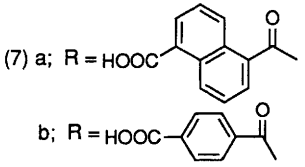


(5) a; R = C≡CH
b; R = C≡N

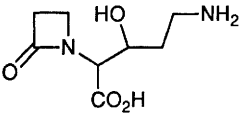


(6) a; R^1 = H, R^2 = C≡CH
b; R^1 = H, R^2 = C≡N
c; R^1 = C≡N, R^2 = H

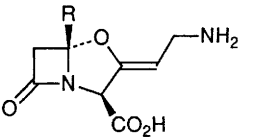




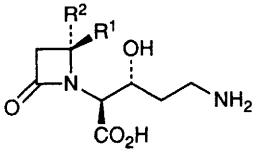
(7) a; R = HOOC
b; R = HOOC



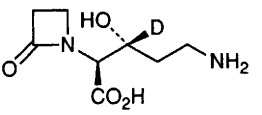
(8)



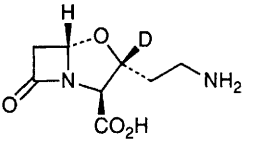
(9) a; R = H
b; R = D



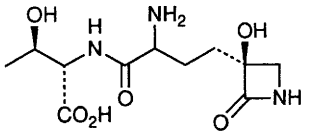
(10) a; R^1 = H, R^2 = D
b; R^1 = D, R^2 = H



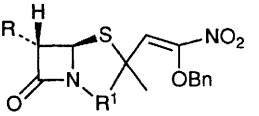
(11)



(12)



(13)



(14) a; R = $SiMe_3$, R^1 = $SiMe_2Bu^t$
b; R = R^1 = H
c; R = $OSiPh_2Bu^t$, R^1 = $SiMe_2$Thexyl
d; R = OH, R^1 = H

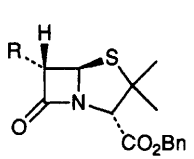
Incubation of the two stereospecifically mono-deuterated proclavaminates (**10a,b**) with the enzyme clavaminate synthase gave rise to clavaminic acid (**9a**) and the 4-deuterated derivative (**9b**) respectively.[17] In both cases ring closure occurs with retention of configuration at C(4) of the β-lactam. Racemic proclavaminic acid labelled with deuterium at the 3'-position (**11**) gave two products when incubated with clavaminate synthase. In addition to clavaminic acid, the operation of a primary isotope effect resulted in the accumulation of a hitherto unknown intermediate, namely monodeuterio dihydroclavaminate (**12**).[18] This result suggests that ring closure precedes desaturation in the formation of clavaminic acid.

A further study on the biosynthesis of Tabtoxin (Wildfire Toxin) (**13**) by *Pseudomonas syringae pv tabaci* has shown that the two ^{13}C atoms of [2,3-$^{13}C_2$] pyruvic acid are incorporated intact into the β-lactam unit of (**13**). The result suggests that the biosynthesis proceeds in part along the lysine pathway.[19]

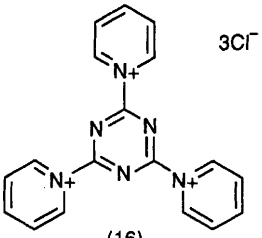
4. PENICILLINS AND CEPHALOSPORINS

This year has seen the publication of only one paper on the total synthesis of these bicyclic β-lactams. Following a route previously described for benzyl penicillanate (see Volume 22), the monocyclic precursor (**14a**) was deprotected to give (**14b**). Cyclisation and ozonolysis to (**15a**) was followed by oxidation and hydrogenation to give penicillanic acid sulphone. In a similar manner (**14c**) was deprotected to give (**14d**) which was cyclised and ozonolysed to give (**15b**). Formation of the mesylate and displacement by azide with inversion at C(6) was followed by reduction to give 6-aminopenicillanic acid (6-APA).[20]

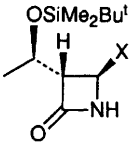
A new esterification catalyst (**16**), formed by the reaction of three molar equivalents of pyridine with cyanuric chloride, allows the preparation of esters using alcohols under mild conditions.[21] An improved synthesis of the monocyclic penem precursor (**17a**) from dibromo-penicillanic acid sulphone has appeared.[22] Full details have now appeared on the reaction of 6-diazopenicillanates (**18**) with furan to give substituted 6-methylene penicillanates (**19**). The investigation was extended to include substituted furans, revealing a strong steric effect with furan 2-substituents and the detrimental effects of electron-withdrawing groups at furan C(2) and C(3). Addition of (**18**) to benzofuran results in ring expansion of the latter to give the novel 6-spiro-penicillanate (**20**).[23] Metal halogen exchange of the 6-(iodoallenyl)penam (**21a**) with an alkyl-



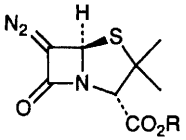
(15) a; R = H
b; R = HO



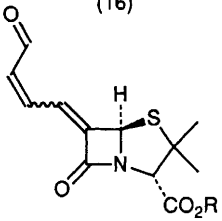
(16)



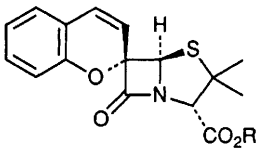
(17) a; X = SO_2Me
b; X = OAc



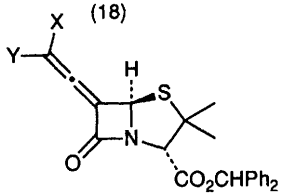
(18)



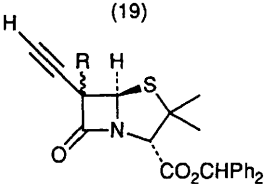
(19)



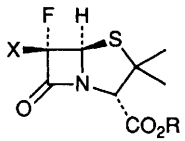
(20)



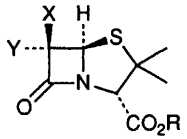
(21) a; X = I, Y = H
b; X = Mg, Zn, Y = H
c; X = CO_2Et, Y = I
d; X = CO_2Et, Y = H



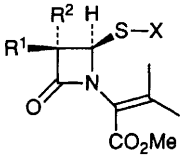
(22) a; R = Li
b; R = COR^1



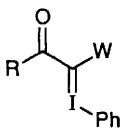
(23) a; X = Cl
b; X = Br



(24) a; X = F, Y = H
b; X = H, Y = OH
c; X = H, Y = F



(25) a; HgPh
b; $CHCl_2$



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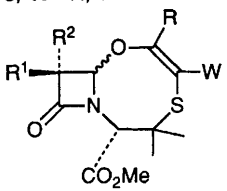

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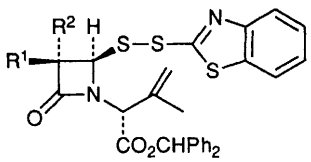


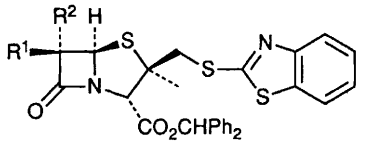
(28)

lithium gives rise to a species which behaves as a 6-lithio penam (**22a**) upon protonation. Treatment of (**21a**) with zinc/copper or a Grignard reagent results in an intermediate which reacts as allene (**21b**) when protonated. Attempted acylation gave rise to a 6-acetyleno-6-acyl penam (**22b**) in all cases. The desired alkoxycarbonylallenes were eventually prepared by reduction of the corresponding iodoallene (**21c**) by treatment with a Grignard reagent followed by an acid quench to give (**21d**).[24] Reaction of a 6-diazopenicillanate (**18**) with a N-halosuccinimide and tetrabutylammonium bifluoride gives the 6α-fluoro-6β-halopenicillanates (**23a,b**). Reduction with the hindered trineophyl tin hydride gives the 6β-fluoropenicillanate (**24a**).[25] Preparation of the 6α-fluoropenicillanate (**24c**) was achieved by treatment of the 6α-hydroxy derivative (**24b**) with diethylaminosulphur trifluoride (DAST).

A detailed study of the 1,2-cleavage of penicillins using non-nucleophilic bases and thiophilic heavy metals provides the best conditions for the conversion of numerous 6-substituted penicillanates to 1,2-secopenicillanates.[26] An alternative method for 1,2-cleavage involves reaction of penicillanates with Seyferth reagent $PhHgCCl_3$ in the presence of sodium iodide. The product is the mercury mercaptide (**25a**) in contrast to the reaction observed in the presence of triethylamine which gives 4-dihalomethylthio azetidinones (**25b**) *via* a carbene reaction.[27] The same authors report a ring expansion reaction of penams upon treatment with iodonium ylides (**26**) providing the unusual (4,8) bicyclic product (**27**).[28] An acid catalysed thermal rearrangement of azetidinyl benzothiazolyl disulphides (**28**) provides a direct route to 2β-benzothiazolylthiomethyl penams (**29**).[29]

A method for the direct introduction of a 6α-formamido group in penicillins (and at 7α- in cephalosporins) involves preparation of the Schiff base (**30a**). Oxidation with lead oxide and reaction with bis(trimethylsilyl)formamide provides (**30b**) from which the free 7β-amino derivative can be generated by the action of Girards reagent T.[30]

Moving on to cephalosporin chemistry, a synthesis of 3-hydroxy (**33a**) and 3-alkoxy (**33b**) cephems from the penicillin-derived (**31**) is reported. Oxidative double bond cleavage of (**31**) with ruthenium chloride and periodic acid gives enol (**32a**) which can be alkylated to (**32b**). Cyclisation of (**32a,b**) using a bimetal redox system involving tin metal provides (**33a,b**).[31] The 7-unsubstituted and 7α-phenylacetamido 3-cyano cephems (**34a**) and (**34b**) have been prepared from a protected 3-hydroxymethyl cephem (**35**). Both were less active than the 7α-(1-hydroxyethyl) deriva-



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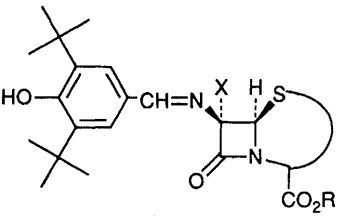
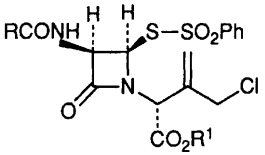


(30) a; X = H
b; X = NHCHO



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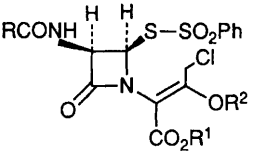
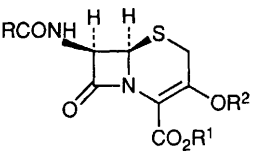
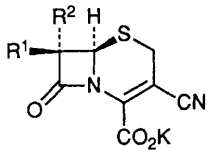
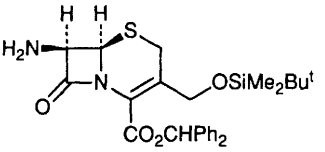


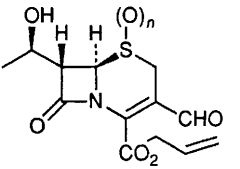
(32) a; R^2 = H
b; R^2 = alkyl



(33) a; R^2 = H
b; R^2 = alkyl



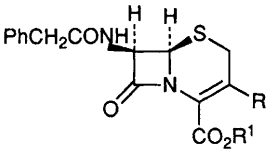


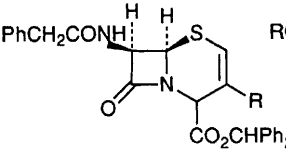


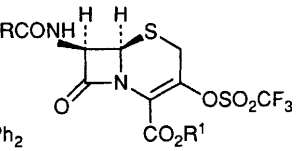
(34) a; R^1 = R^2 = H
b; R^1 = H, R^2 = $PhCH_2CONH$
c; R^1 = H, R^2 = $CH_3CH(OH)$

(35)

(36) n = 1, 2



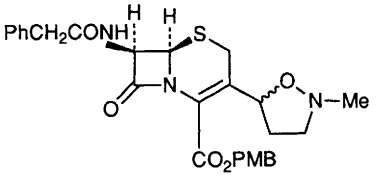




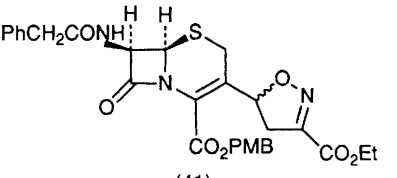
(37) a; R = CHO
b: R = CH_2I
c; R = $CH(OMe)_2$

(38) a; R = CHO
b; R = $C(Cl)=CH_2$
c; R = $COCH_3$

(39)



(40) PMB = *p*-methoxybenzyl

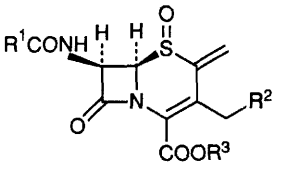


(41)

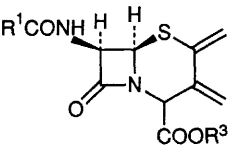
tive (**34c**) (see Volume 22) as β-lactamase inhibitors.[32] The same authors report the prepration of (*R*)- and (*S*)-sulphoxides and the sulphone of 3-formyl-7α-(1-hydroxyethyl)cephem (**36**) as allyl esters. The (*R*)-sulphoxide and sulphone esters were potent β-lactamase inhibitors.[33] A synthesis of the Δ3-isomer of 3-formyl cephems (**37a**) is reported by oxygen or air oxidation of 3-iodomethyl cephems (**37b**) using rhodium trichloride/aluminium. Vanadyl acetylacetonate and vanadyl sulphate were also effective under these conditions.[34] Different authors report the formation of the dimethyl acetal of (**37a**), that is (**37c**), upon reaction of 3-chloromethyl cephems with methanol under an atmosphere of dry air.[35] The Δ2 3-formyl cephem (**38a**) has been used to prepare the 3-(α-chlorovinyl) derivative (**38b**). Reaction of (**38a**) with methylmagnesium iodide followed by oxidation gave 3-acetyl compound (**38c**). Reaction with triphenylphosphine in carbon tetrachloride gave (**38b**).[36] The C(3)-triflate (**39**) continues to be exploited for the synthesis of a variety of directly substituted derivatives. The carbon-carbon bond forming reactions used recently for carbacephalosporins (see Volume 22) are also effective with (**39**). Treatment of (**39**) with vinyl stannanes and palladium acetate in the absence of phosphines or halide sources gives a range of 3-(substituted vinyl) cephems.[37] Full details have now appeared of similar reactions which use palladium catalysts containing tri(2-furyl)phosphine (see also Volume 21).[38] The same authors report the addition-elimination reactions of (**39**) with a variety of organocuprates which provides access to a range of 3-alkyl-, 3-aryl- and 3-alkenyl cephems.[39]

A full account has now appeared detailing the reactions of 3-vinylcephems with diazomethane and diphenyldiazomethane.[40] 1,3-Dipolar cycloadditions of 3-vinylcephems with a nitrone provided a 2.7/1 mixture of 3-(2-methylisoxazolidin-5-yl) cephems (**40**) which could be quaternised by reaction with methyl iodide. A similar reaction with a nitrile oxide provided a 1/1 mixture of 3-(3-ethoxycarbonyl-2-isoxazolin-5-yl) cephems (**41**).[41]

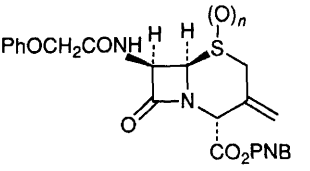
The 2-methylenecephem sulphoxide (**42**), available from cephem sulphoxides *via* a Mannich reaction (see also Volume 22), undergoes reductive elimination with zinc-copper amalgam to give the 2,3-dimethylenecepham (**43**).[42] Cycloadditions of 3-methylenecepham (**44a**) and the corresponding sulphoxide (**44b**) with diazomethane give rise to the 3-spiropyrazolinocepham (**45a**) and sulphoxide (**45b**) respectively. Attempted elimination of nitrogen from (**45a**) resulted in decomposition. The sulphoxide (**45b**) underwent elimination in refluxing dimethylformamide to give the expected 3-spirocyclopropane derivative (**46b**) together with the 3-vinyl-



(42)

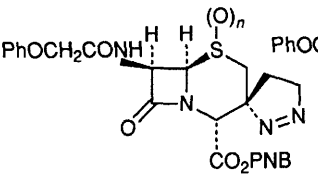


(43)

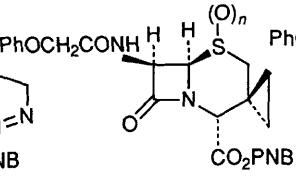


PNB = *p*-nitrobenzyl

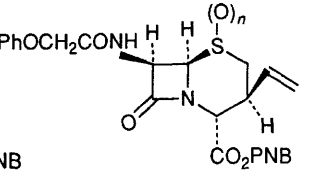
(44) a; *n* = 0
b; *n* = 1



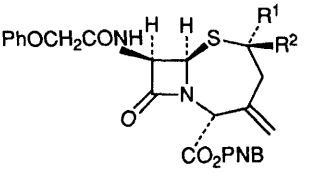
(45) a; *n* = 0
b; *n* = 1



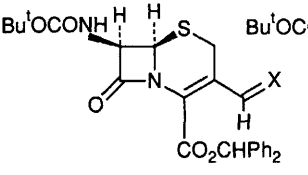
(46) a; *n* = 0
b; *n* = 1



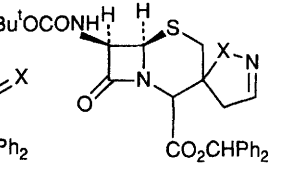
(47) a; *n* = 0
b; *n* = 1



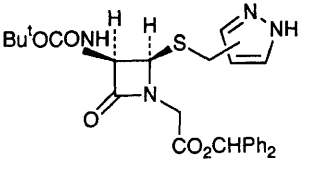
(48)



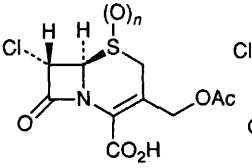
(49) a; X = O
b; X = NNH_2
X = NOH



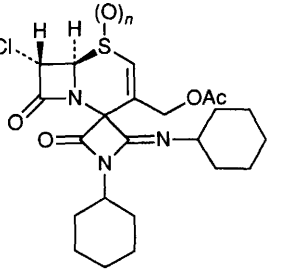
(50) a; X = NH
b; X = O



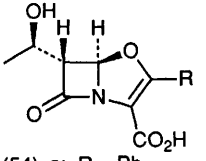
(51)



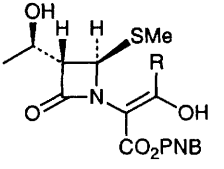
(52) a; *n* = 0
b; *n* = 1, β-isomer
c; *n* = 1, α-isomer
d; *n* = 2



(53) a; *n* = 0
b; *n* = 1, β-isomer
c; *n* = 1, α-isomer
d; *n* = 2



(54) a; R = Ph
b; R = Me
c; R = Pr^i



(55)

cepham (**47b**). The corresponding cephams (**46a**) and (**47a**) were obtained by sulphoxide reduction.[43] The reaction of 2-methylene cephems like (**44a**) with diazoacetates and diazomalonate resulted in ring expansion to give 4-methylenehomocephams (**48**). The rearrangement is proposed as occurring by carbenoid addition to the sulphur atom followed by a [2,3] sigmatropic rearrangement.[44]

Reaction of the cephem aldehyde (**49a**) with hydrazine and hydroxylamine provided the 3-spirocephams (**50a**) and (**50b**) respectively presumably *via* intramolecular Michael addition of the hydrazine (**49b**) and oxime (**49c**). In the case of methylhydrazine, the intermediate spirocepham was not isolated, reaction went directly to azetidinone (**51**) resulting from opening of the six-membered ring. A minor by-product from the esterification of cephem sulphone (**52d**) using alcohols and dicyclohexylcarbodiimide (DCC) was shown to have the unusual 4-spiroazetidinyl-Δ2-cephem structure (**53d**). In the absence of alcohol and with two equivalents of DCC (**53d**) could be isolated in good yield. A similar, but slower reaction, was observed for the cephem sulphoxides (**52b**) and (**52c**) providing (**53b**) and (**53c**) in the latter case as a mixture of C(4)-isomers. The cephem sulphide (**52a**) did not react under these conditions but gave (**53a**) in poor yield when triethylamine was added. A plausible mechanism involves the formation of a ketene by dehydration *via* an intermediate acylisourea. Reaction of the ketene with a second molecule of DCC would then provide the observed products. The reactivity of the various cephems correlates with the acidity of the C(2)-protons (sulphone>sulphoxide>sulphide), one of which must be removed to allow ketene formation. Other 7-substituted cephem sulphones undergo similar reactions with DCC.[46] Full details have now appeared concerning the ring contraction of 2-diazocephem sulphoxides to carbapenems by photorearrangement.[47]

5. CLAVULANIC ACID AND OXAPENAMS

The commercially available chiral 4-acetoxyazetidinone (**17b**) has been used in a synthesis of oxapenems (**54a-c**). The route involves preparation of the enols (**55**). Chlorination and ring closure provided the oxapenem esters which were deprotected to give (**54a-c**). The instability of oxapenems was highlighted by measurement of half-lives of (**54a-c**) as 24, 43 and 200 minutes respectively.[48]

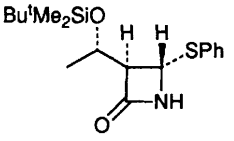
6. PENEMS

An unusual synthesis of 5,6-*cis* penems begins with the cycloaddition of a vinyl sulphide with chlorosulphonyl isocyanate (CSI) to give azetidinone (**56**) as the major isomer obtained. Elaboration by established routes then provides the (5S) penem derivative (**57**). Irradiation of (**57**) with Pyrex-filtered UV light in ethyl acetate resulted in partial isomerisation to the (5R) penem (**58a**) which was in turn photo-labile providing thiazole (**59**). The (5R) isomer was separated and deprotected to give (**58b**) which proved to have lower antibacterial activity than the more usual 5,6-*trans* penems.[49] 4-Dihalomethylazetidinones like (**25b**) have been converted to 2-unsubstituted penems (**60a,b**) by ozonolysis, cyclisation with trimethyl phosphite, and subsequent base treatment.[50] A synthesis of penems from azetidinone disulphide (**61a**) involves reaction with phosphoranes to give ylids (**61b**). Ozonolysis to an oxalimide and Wittig cyclisation provided 2-substituted penems (**62a,b**).[51] A novel method of introducing substituents at C(2) of penems, analogous to that used for carbapenems, involves the enol triflate (**63a**). This was obtained by reaction of the corresponding thiolactone with triflic anhydride. The addition-elimination reaction with substituted thiols provided C(2)-thiosubstituted penems (**63b**) while reaction with cuprates gave C(2)-alkyl derivatives (**63c,d**).[52] Oxidation of penems (**64a**) with m-chloroperbenzoic acid (m-CPBA) in dichloromethane gave a mixture of sulphoxide isomers (**64b**) and (**64c**) in a 4:1 ratio. The isomer ratio was insensitive to protection of the C(8)-hydroxyl group and was explained as resulting from the directing influence of the β-lactam carbonyl group. Evidence for this was provided by changing the solvent to ethyl acetate which can itself interact with m-CPBA. The selectivity was reduced to a 3:2 ratio of (**64b**) and (**64c**).[53] An overview of the synthesis and antibacterial properties of a large number of 2-(oxygen substituted) penems has appeared.[54] Two publications describe the synthesis and β-lactamase inhibitory activities of a series of 6-(substituted methylene)penems (see also Volume 22).[55,56]

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.

Cyclisation of suitably substituted pyrrolidines, available from (L)-glutamic acid in six steps, allowed conversion of the separable isomers of (**65**) to intermediates (**66a**) and (**66b**) for PS-5 and epi-PS5 respectively (for a similar carbapenem synthesis see Volume 22).[57] A total



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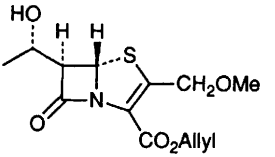

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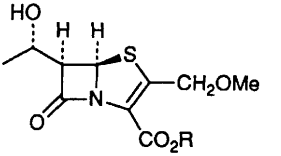
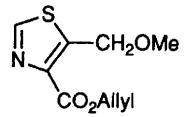


(58) a; R = Allyl
b; R = H



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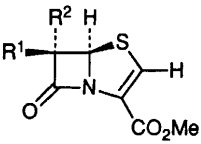
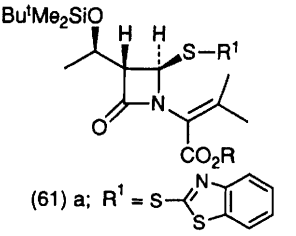
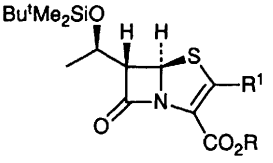


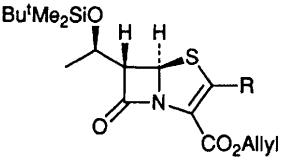
(60) a; $R^1 = R^2 = H$
b; $R^1 = H$, R^2 = MeCH(OH)



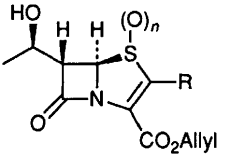
(61) a; R^1 = S-(benzothiazolyl)
b; $R^1 = C(=PPh_3)-X$



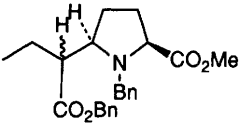
(62) a; $R^1 = CO_2Et$
b; $R^1 = CONH_2$



(63) a; $R = OSO_2CF_3$
b; R = SR^1
c; R = Me
d; $R = CH_2OMOM$
MOM = CH_3OCH_2



(64) a; *n* = 0
b; *n* = 1, β-isomer
c; *n* = 1, α-isomer



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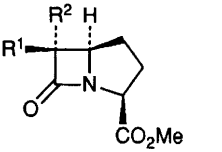
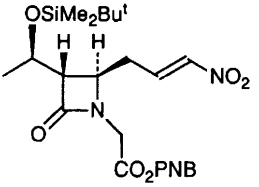
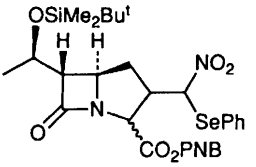


(66) a; R^1 = H, R^2 = Et
b; R^1 = Et, R^2 = H



(67)



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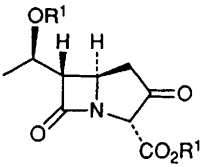
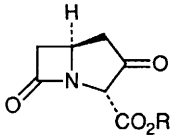


(69) a; $R^1 = SiMe_2Bu^t$
b; R^1 = H

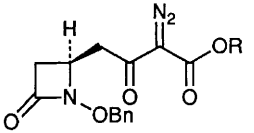
synthesis of (+)-thienamycin used a Michael addition of the anion of (**67**) and quenching with phenylselenenyl chloride to give (**68**). Oxidative elimination to the nitroolefin and ozonolysis provided the bicyclic ketone (**69a**). This material was then progressed *via* chemistry devised by the Merck group for the unprotected derivative (**69b**). Considerable difficulty was experienced in removing the t-butyldimethylsilyl group at the penultimate stage of the syntheis.[58] An alternative synthesis of 6-unsubstituted derivatives of (**69b**) namely (**70a,b**) involves rhodium-catalysed cyclisation of (**71**) without the need to remove the N-benzyloxy function. Benzaldehyde is formed in the reaction, which is proposed as occurring *via* intermediate (**72**).[59] Cyclisation of the β-hydroxyamide (**73**) under modified Mitsunobu conditions provided a β-lactam intermediate (**74**) which was progressed to a carbapenem *via* the known Horner-Emmons cyclisation route.[60]

An efficient preparation of 2-aryl substituted carbapenems from the enol triflate (**75a**) involves the Pd(0) catalysed cross coupling of the temporarily protected (**75b**) with aryl stannanes to give (**75c**).[61] Two publications have appeared reporting the synthesis of a range of 5,6-*cis* and 5,6-*trans* carbapenems by modification of the olivanic acids MM 22382 and MM 22383 respectively.[62,63] Full details have now appeared on the procedures for C(2)-functionalisation of 2-unsubstituted carbapenems by various addition-elimination procedures.[64,65] A synthesis of Melillo's lactone has appeared in which the cyclisation of a chiral acyl-nitroso derivative with cyclopentadiene is the key step.[66] The extension of an already published route to Melillo's lactone allows incorporation of a further methyl group for the synthesis of 1β-methylcarbapenems.[67] The cycloaddition of a chiral nitrone with α-chloroacrylonitrile allows preparation of isoxazolidinone (**76**) which is proposed as a potential carbapenem intermediate.[68]

Following last years plethora of carbacephalosporin papers, only two publications discussing their synthesis have appeared during 1990. The previously reported chiral azetidinone (**77**) (see Volume 22) has been transformed to the carboxylic acid (**78a**). Esterification with phenol or thiophenol gave (**78b**) or (**78c**) respectively. The Dieckmann cyclisation of the diesters occurred exclusively with the desired regiochemistry to give the 3-hydroxycarbacephem (**79a**).[69] A synthesis employing a similar cyclisation strategy began with a four component condensation of the β-amino acid (**80**) with formaldehyde and allyl isocyanide providing azetidinone (**81**). Further transformations gave the ester-amide (**82**) which could be cyclised with base to give a 7-unsubstituted carbacephem (**79b**).[70]



(70) a; R = Me
b; R = CH_2Ph



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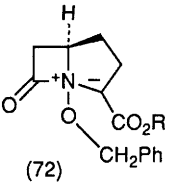

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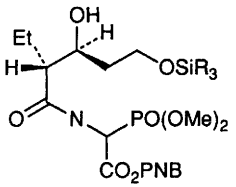

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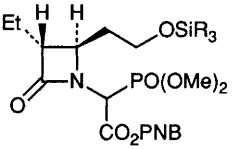

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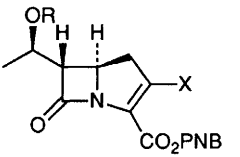
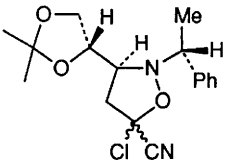
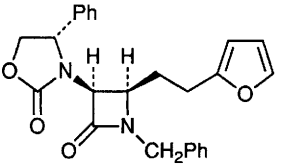


(75) a; R = H, X = OSO_2CF_3
b; R = $SiMe_3$, X = OSO_2CF_3
c; R = H, X = Ar



(76)



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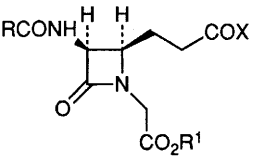
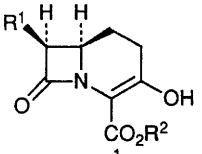
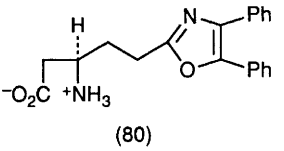


(78) a; X = OH
b; X = OPh
X = SPh



(79) a; R^1 = RCONH
b; R^1 = H



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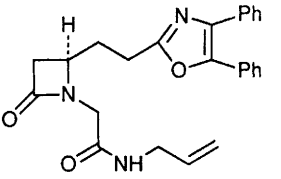

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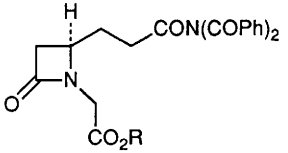

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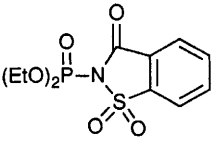

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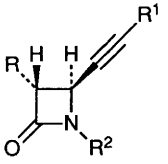

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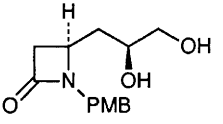

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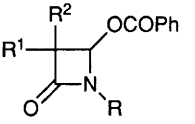


(86)

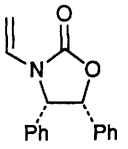
8. AZETIDINONES

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 actually isolated. A study of phosphorus reagents for the cyclisation of β-amino acids revealed that ethyl and phenyl dichlorophosphates as well as phenylphosphinic dichloride were all effective.[71] A Saccharin derived phosphonate (**83**) also gave good yields of azetidinones from β-amino acids.[72] A further example of copper (I) triflate/calcium carbonate mediated cyclisation of β-aminothiolesters (see also Volume 22) provided the 4-alkynyl azetidinones (**84**).[73] Ring closure of a carbohydrate-derived β-amino acid with 2-chloro-1-methylpyridinium iodide provided the carbapenem precursor (**85**).[74] Full details, together with a lengthy mechanistic discussion, have appeared concerning the reactions of lithium ester enolates with 2-arylamino-2-methoxy-1-phenylethanones (synthetic equivalents of benzoyl imines). Included are the syntheses of a number of 4-benzoyl azetidinones (86).[75] Palladium (II)-assisted carboacylation of the optically active ene carbamate (**87**) allowed preparation of a β-amino acid which was converted to the thienamycin intermediate (**88**).[76] The addition of lithium chloride to the condensation of the lithium dianion of 3-hydroxybutanoates with N-acylamines gives higher stereoselectivity in the formation of β-amino esters (see also Volume 22).[77] The use of N-(alkylamino)benzotriazoles (**89**) in place of imines, in the condensation with lithium ester enolates gives β-amino esters, some of which were cyclised to β-lactams.[78]

3,4 bond-forming reactions.- The base-induced intramolecular alkylation of the bromoacetamide (**90**) provided the 4-cyanoazetidinone (**91a**). Removal of the N-protecting group was followed by decyanation with sodium in liquid ammonia/tetrahydrofuran. The resulting azetidinone alcohol (**91b**), a carbapenem intermediate, was obtained as a 2:1 mixture of C(4)-isomers with the unnatural isomer predominating.[79] The cyclisation of the α,β-epoxy amides (**92a-c**) provided the *trans* β-lactams (**93a-c**), together with the unusual bicyclic hemiacetals (**94a-c**). Acylation of *trans* derivatives (**93a-c**) was followed by Baeyer Villager oxidation to give the corresponding 4-acyloxyazetidinones (**95a-c**), useful penem precursors. Acylation of the



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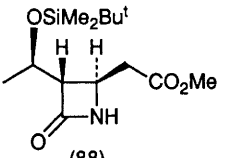
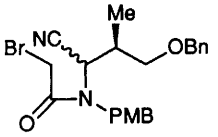


(88)

(89)



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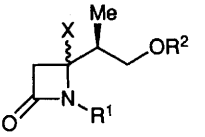
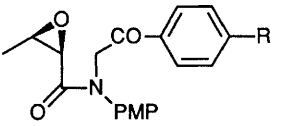
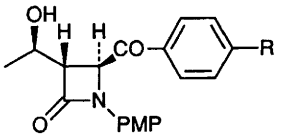


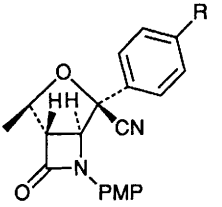
(91) a; X = CN, R^1 = PMB, R^2 = Bn
b; X = R^1 = R^2 = H



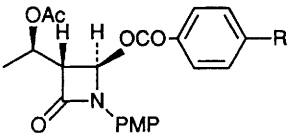
(92) a; R = H
b; R = Cl
c; R = Me



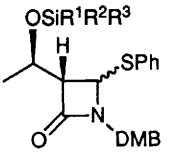
PMP = *p*-methoxyphenyl
(93) a; R = H
b; R = Cl
c; R = Me



(94) a; R = H
b; R = Cl
c; R = Me

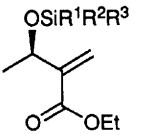


(95) a; R = H
b; R = Cl
c; R = Me



DMB = 2, 4-Dimethoxybenzyl

(96)



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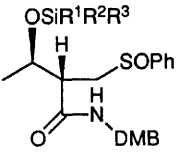

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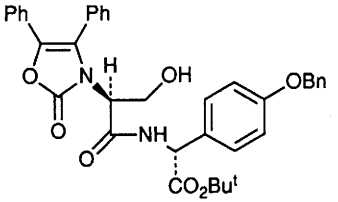

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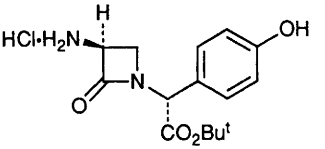
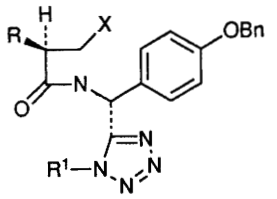


(100)

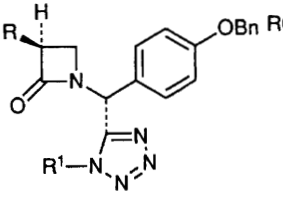
hemiacetals (**94a-c**) resulted in ring-opening to give cis β-lactams corresponding to (**93a-c**) which were resistant to Baeyer-Villager oxidation.[80]

1,4 bond-forming reactions.- An extension of the silicon-induced Pummerer methodology for azetidinone formation (see also Volume 22), has provided the carbapenem precursor (**96**). The Michael addition of thiophenol to the acrylate (**97**) occurred with increasing diastereoselectivity as the bulk of the silyl protecting group increased; amidation and S-oxidation then gave (**98**), cyclisation in the presence of a ketene silyl acetal provided (**96**).[81] The reported total syntheses of Nocardicins A-G relies upon a ring closure of the β-hydroxy amide (**99**) under Mitsunobu conditions to give the key intermediate (**100**) after catalytic hydrogenation.[82] In a similar approach, the tetrazole derivative (**101a**) was cyclised by phase transfer alkylation to give (**102a**) while the phthalimido-substituted β-hydroxy amide (**101b**) underwent ring closure by the Mitsunobu procedure to give (**102b**) which was transformed to the tetrazole analogue of the Nocardicin nucleus (**100**).[83] The same authors have also reported the synthesis of the phosphinic acid analogue of Nocardicins (**103**) following a similar synthetic strategy.[84] The intramolecular alkylation-cyclisation of (**104**) to give β-lactams (**105**) was performed in aqueous base in the absence of either organic solvents or phase-transfer catalysts.[85] The facile ring closure of β-substituted hydroxamates to N-oxyazetidinones continues to be exploited. The allylic alcohol (**106**) underwent Mitsunobu ring closure to give the 4-(1-methylvinyl)azetidinone (**107a**). Ozonolysis then gave the 4-acetyl derivative (**107b**) which underwent Baeyer Villager oxidation to 4-acetoxy-1-oxyazetidinones (**108**). Attempted displacement of the acetoxy group resulted in β-lactam ring opening.[86] The S-phenylalanine-derived isonitrile (**109a**) underwent stereospecific rearrangement to the nitrile (**109b**) by flash pyrolysis. Elaboration to the hydroxamate (**110**) was followed by ring closure, removal of the benzyl group and reduction to the azetidinone (**111**).[87] Ring opening of the cyclic sulphite in hydroxamate derivative (**112**) with lithium azide provided the α-azido-β-hydroxy hydroxamate (**113**) which was cyclised under Mitsunobu conditions to the single 3-azido β-lactam (**114**).[88]

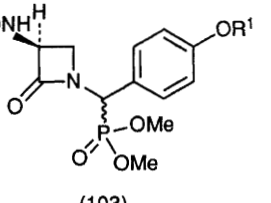
Cyclisation of the amino acid hydrazide (**115**) provided the N-phthalimido azetidinone (**116a**) which could be deprotected to the N-amino azetidinone (**116b**). N-Sulphonation then gave the novel heteroatom-activated β-lactam (**116c**).[89] The photochemical synthesis of N-amino β-lactams by ring contraction of pyrazolidin-3-ones has been improved by the use of a photo-labile



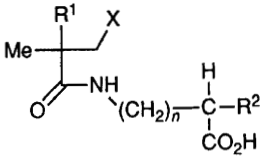
(101) a; R = H, X = Br
b; R = Ft, X = OH



(102) a; R = H
b; R = Ft



(103)



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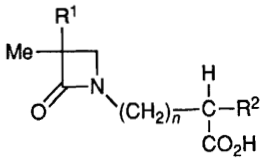

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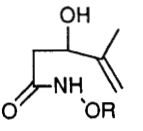

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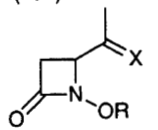
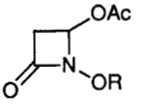


(107) a; X = CH_2
b; X = O



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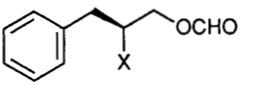
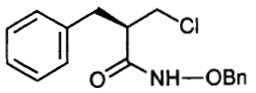


(109) a; X = NC
b; X = CN



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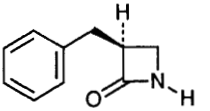

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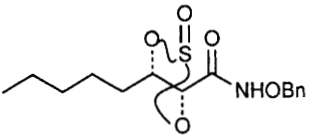

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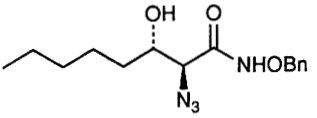

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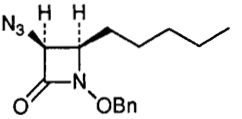

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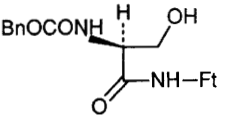

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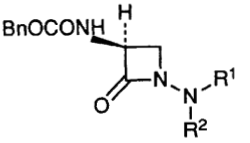

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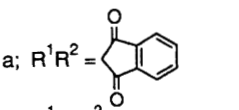
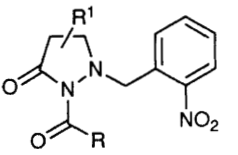


a; R^1R^2 =
b; $R^1 = R^2 = H$
c; $R^1 = H$, $R^2 = SO_3^-M^+$



(117)

protecting group. Condensation of hydrazine hydrate with α,β-unsaturated carboxylic acids and esters gave, after protection of N(1) and acylation of N(2), the 1-(o-nitrobenzyl)-2-acylpyrazolidin-3-ones (**117**). Tandem photochemical reactions, firstly with pyrex-filtered light (λ>300nm) to remove the o-nitrobenzyl group and then with lower wavelength (254nm) light to effect the ring contraction, resulted in formation of the N-(acylamino) β-lactams (**118a**). Deacylation then gave the N-amino derivatives (**118b**). The reaction is thought to occur *via* the bicyclo [2.1.0] intermediate (**119**).[90]

Reactions in which two bonds are formed

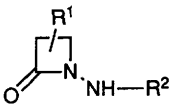
This sub-section includes formal [3+1] and [2+2] additions which may be concerted or stepwise under the conditions used.

[3+1] additions

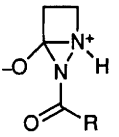
1-2 and 2-3 bond formation.- The ring expansion of aziridines upon reaction with lithium iodide and nickel tetracarbonyl results in insertion of the CO unit into the N-C bond of the least substituted carbon adjacent to nitrogen. The reaction works well with simple alkyl substituents (**120**, R^1,R^2 = Alkyl), less well with electron withdrawing substituents, and not at all for aryl substituents.[91]

[2+2] additions

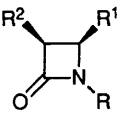
1-2 and 3-4 bond formation.- As in previous volumes detailed mention of ring formation of this type will only be made where new chemical features are apparent. A number of further examples of this type are listed in the Appendix. Full details have now appeared on the reactions of α,β-unsaturated acid chlorides with imines to give 3-vinyl β-lactams (**121**). Further transformations provided intermediates for the synthesis of various carbapenem antibiotics.[92] A study of [2+2] additions included the preparation of the silicon derivatives (**122**) by reaction of an α-iminoester with a suitable silicon-containing ketene.[93] Further examples of the cycloadditions of N-vinyl imines with ketenes have appeared. The procedure for removal of the vinyl group to give N-unsubstituted β-lactams has been simplifed.[94] Variation of the [2+2] addition which incorporate a chiral substituent in order to induce diastereoselectivity continue to appear. The use of a chiral aldehyde, derived from mandelic acid, gave a chiral imine which underwent diastereoselective



(118) a; R^2 = COR
b; R^2 = H



(119)



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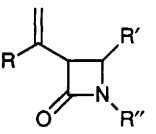
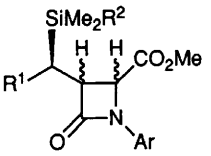
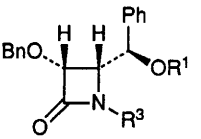


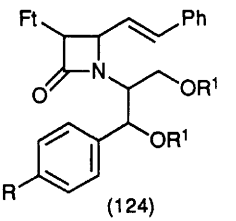
(121)



(122)



(123)



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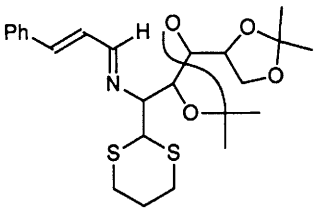

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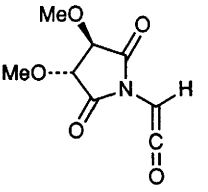
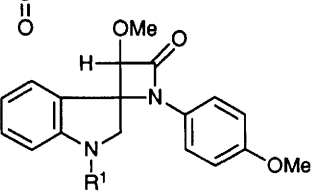


(126)



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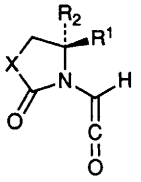
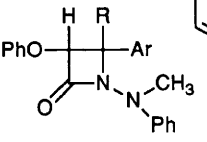
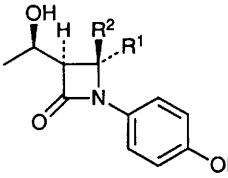


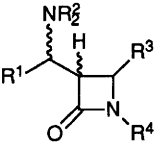


(128)

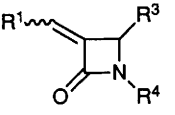
(127) a; X = CH_2, R^1 = CH_2OMOM, R^2 = H
b; X = O, R^1 = H, R^2 = CH_2OMOM



(130) a; R^1 = CH=CHPh, R^2 = H
b; R^1 = H, R^2 = CH=CHPh



(131)

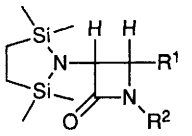


(132)

[2+2] addition with benzyloxyketene to give azetidinones (**123**). β-Lactam ring-opening and further transformation gave β-amino-α,γ-dihydroxyesters useful for further synthesis.[95] The use of a chiral 2-amino-1,3-propanediol as the amine precursor of an imine resulted in diastereoselective [2+2] addition with phthalimidoketene. The ratio of (3*S*,4*R*) and (3*R*,4*S*) isomers of the product (**124**) was dependant upon the bulk of the protecting group R^1.[96] Similar compounds were prepared using the chiral imine (**125**). The reaction with phthalimidoketene gave a single (3*S*,4*R*) isomer while methoxyketene addition gave rise to a mixture.[97] Chiral ketenes derived from (L)-tartaric acid (**126**), (S)-glutamic acid (**127a**), and (S)-serine (**127b**) have been used to prepare 3-amino β-lactams. Mixtures of *cis* and *trans* isomers were obtained with the *cis* compounds predominating.[98] N,N-Disubstituted ketone hydrazones undergo [2+2] addition with phenoxyketene to give N-amino azetidinones (**128**).[99] Spiro indolinone β-lactams (**129**) were obtained from [2+2] additions of an imine derived from indoline-2,3-dione with methoxyketene.[100]

Moving now to the ester enolate plus imine variant, an organocopper derivative of a β-silylenolate gives compounds similar to (**122**).[101] The effect of additives on the reaction of the lithio dianion of ethyl 3-hydroxybutyrate with a cinnamaldehyde imine has been studied. Addition of t-butylmagnesium chloride gives rise to the (1*RS*,3*RS*,4*SR*) 3,4-*trans* isomers (**130a**) while triethylborane provided the (1*RS*,3*RS*,4*RS*) 3,4-*cis* isomers (**130b**).[102] The reaction of the lithium enolates of 3-(N,N-dialkylamino) esters with imines gives 3-aminoalkyl β-lactams (**131**). Deamination then provides α-alkylidene derivatives (**132**). The ratio of *cis* and *trans* β-lactams (**131**) formed depends upon the nature of R^1 and the method used to generate the enolate.[103] The lithium enolates of esters derived from chiral alcohols react with imines with high enantioselectivity. The E/Z-geometry of the chiral ester-enolate is responsible for the *cis/trans* stereochemistry of the β-lactam (**133**) formed. A comparison of five different chiral auxiliaries was reported.[104] Similar chiral azetidinones were obtained from the reaction of an achiral enolate with a chiral amine-derived imine. The best chemical and optical yields of 3,4-*trans* β-lactams (**134**) were obtained using the zinc enolate.[105] The boron enolate of a thioester was used to prepare the azetidinone (**135**), a precursor for the carbapenem (+)-PS-5.[106]

1-4 and 2-3 bond formation.- The reaction of allylsilanes with chlorosulphonyl isocyanate (CSI) gives the corresponding azetidinones (**136a,b**). A similar reaction with (allenylmethyl) silanes provides 3-alkylidene derivatives (**137a,b**).[107]



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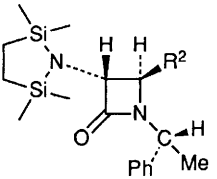

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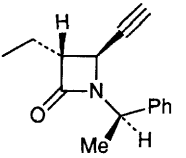

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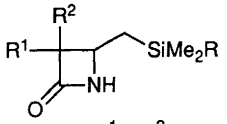
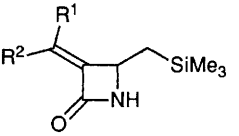
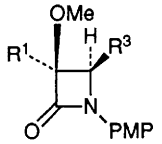


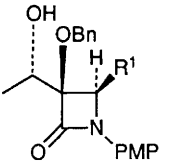
(136) a; $R = R^1 = R^2 = Me$
b; $R^1 = TMS$, $R^2 = H$, $R = Ph$



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



(138) a; $R^1 = Me$
b; $R^1 = Et$
c; $R^1 =$ 



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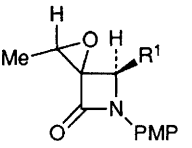

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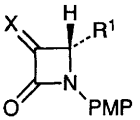
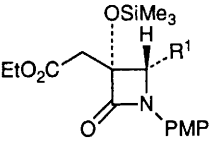
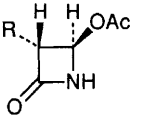


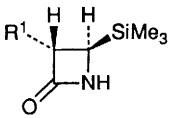
(141) a; X = O
b; X = $CHCO_2Et$ (*E*-isomer)



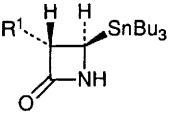
(142)



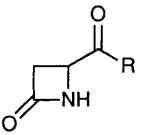
(143) a; R = H
b; R = MeCH(OH)



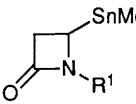
(144) a; $R^1 = H$
b; $R^1 = MeCH(OH)$



(145) a; $R^1 = H$
b; $R^1 = MeCH(OH)$



(146)



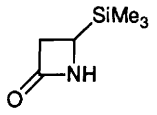
(147) a; $R^1 = SiMe_2Bu^t$
b; $R^1 = H$

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 3-methoxyazetidinones reacts with alkyl halides to give (**138a,b**) and with acetaldehyde to give (**138c**). The incoming group enters *trans* to the C(4)-substituent. The 3-(1-hydroxyethyl) derivative (**138c**) was obtained as a mixture of side-chain isomers. Methods were described for the preparation of either single isomer by an oxidation-reduction sequence.[108] The same authors used similar chemistry to prepare the 3α-(1-hydroxyethyl)-3β-benzyloxy azetidinone (**139**). Conversion of one oxygen function to a leaving group and displacement by the other allowed the preparation of both possible 3-spiroepoxy azetidinones (**140**).[109] The Reformatsky reaction of azetidine-2,3-diones (**141a**) with ethyl bromoacetate in the presence of chlorotrimethylsilane provided 3,3-disubstituted azetidinones (**142**). Desilylation, mesylate formation and base-induced elimination provided the 3-alkylidene azetidinones (**141b**) exclusively as E-isomers.[110]

Moving to chemistry at C(4), reaction of 4-acetoxyazetidinones (**143a,b**) with a silyl cuprate gave the 4-(trimethylsilyl) azetidinones (**144a,b**). Similar reaction with a tributylstannyl cuprate provided 4-(tributylstannyl) azetidinones (**145a,b**). Attempts to replace the trimethylsilyl group with an electrophile were unsuccessful. The 4-(tributylstannyl) derivative (**145a**) could be used as a C(4)-anion equivalent, undergoing Stille palladium catalysed coupling reactions with acyl chlorides to give 4-acyl azetidinones (**146**).[111] This chemistry contrasts with the more usual use of 4-acetoxy and other 4-substituted azetidinones as C(4)-cation equivalents. Other authors have also reported the preparation of 4-(trialkylstannyl) azetidinones like (**145a**) by reaction of 4-chloro-, 4-phenylthio- and 4-phenylsulphonyl azetidinones with stannyl lithiums or stannyl cuprates. 4-(Trimethylstannyl) derivative (**147a**) undergoes transmetallation to give a C(4)-lithio anion (a homoenolate) which can be methylated. The N-unsubstituted analogue (**147b**) undergoes transmetallation to give the N(1), C(4) dianion which reacts with chlorotrimethylsilane to give (**148**).[112]

A publication on the oxidative decarboxylation of α-(acylamino) acids includes the conversion of the azetidinone-4-carboxylic acid (**149**) to 4-benzoyloxyazetidinones (**150a,b**) by reaction with the appropriate peracidic in the presence of dicyclohexylcarbodiimide.[113] The copper-catalysed acyloxylation of 4-unsubstituted azetidinones with t-butyl perbenzoate or peracetate gives the corresponding 4-(acyloxy) β-lactams (**151a,b**). 3-Monosubstituted azetidinones gave pre-



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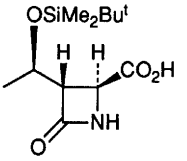
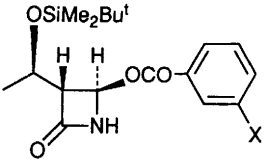
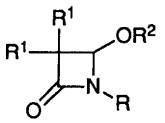


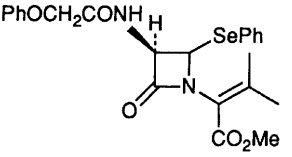
(149)



(150) a; X = H
b; X = Cl



(151) a; R^2 = Ac
b; R^2 = Bz



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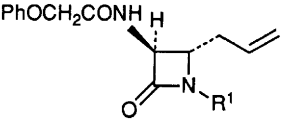
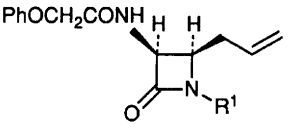
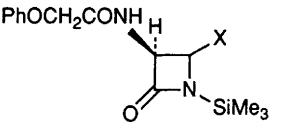


(153) a; R^1 = $C(CO_2Me)CMe_2$
b; R^1 = H



(154) a; R^1 = $C(CO_2Me)CMe_2$
b; R^1 = H



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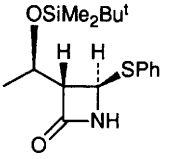

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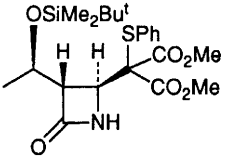

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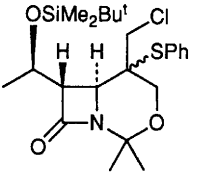

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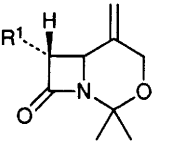
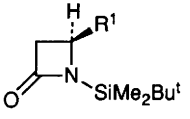
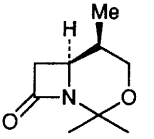


(159) a; R^1 = Me
b; R^1 = H



(160) a; R^1 = CO_2Bn
b; R^1 = $C(Me)=CH_2$
c; R^1 = $C(CH_2OH)=CH_2$
d; R^1 =



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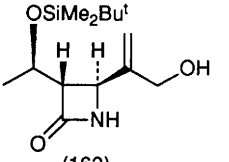

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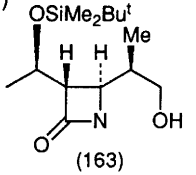


(163)

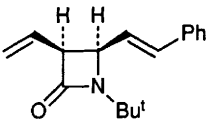
dominantly 3,4-*trans* products.[114] A paper detailing the ruthenium-catalysed oxidation of amides and lactams with peroxides provides 3 examples of the introduction of a 4-acetoxy group onto 4-unsubstituted β-lactams using peracetic acid.[115]

Radical allylation of the 4-(phenylselenenyl) azetidinone (**152**) with allyltributylstannane in the presence of azoisobutyronitrile (AIBN) gave a 2/1 mixture of *trans* and *cis* 4-allyl derivatives (**153a**) and (**154a**). A similar reaction of various 4-substituted N-trimethylsilyl azetidinones (**155**; X = SPh, SO_2Ph, SePh) gave mixtures of 4-allyl analogues (**153b**) and (**154b**).[116] Reaction of the 4-phenylthio β-lactam (**156**) with dimethyldiazomalonate in the presence of rhodium acetate gave the formal insertion product (**157**). Reduction of the diester to a diol was followed by conversion to a monochloro derivative. Acetonide formation gave the intermediate (**158**) which underwent elimination of phenylsulphenyl chloride to give olefin (**159a**), a known intermediate for 1β-methylcarbapenems.[117] The related olefin (**159b**) has been prepared by reaction of 4-(benzyloxycarbonyl) azetidinone (**160a**) with two equivalents of methylmagnesium iodide and elimination *via* a mesylate to give (**160b**). Selenium dioxide allylic oxidation then provided (**160c**). Removal of the silyl group and acetonide formation then gave (**159b**). A direct synthesis of a 1β-methylcarbapenem precursor (**161**) was achieved by hydroboration of (**160b**) to (**160d**) followed by acetonide formation.[118] The related allylic alcohol (**162**) has been converted to the 1β-methylcarbapenem precursor (**163**) by asymmetric hydrogenation using a chiral ruthenium-BINAP catalyst; only 0.1% of the corresponding 1α-methyl derivative was formed.[119]

The β-lactam-containing 1,5-diene (**164**) reacts with sulphur dichloride to give the bicyclic derivative (**165a**) which undergoes facile hydrolysis to (**165b**). Oxidation and double elimination then provided the diene (**166**). The α-methylene β-lactam (**167**) reacts with sulphur dichloride providing a mixture of the two (4,5)-bicyclic systems (**168**) and (**169**).[120]

Moving on to the chemistry of azetidinone nitrogen substituents, the regioselective halogenation of β- or γ-lactams (**170**) with N-bromosuccinimide provides the exocyclic bromides (**171**).[121] An alternative to the use of excess ceric ammonium nitrate for removal of the p-methoxyphenyl group from β-lactam nitrogen involves reaction with ammonium persulphate under silver nitrate catalysis. Yields quoted were 57-62%.[122]

Finally, a procedure for the synthesis and optical resolution of β-lactams from oxoamides using host-guest chemistry has been extended. In addition an optical resolution of 4-acetoxy-



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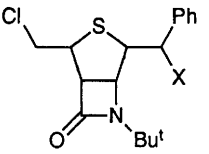
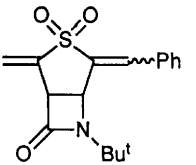


(165) a; X = Cl
b; X = OH



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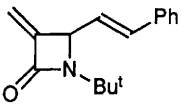

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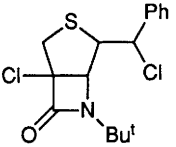

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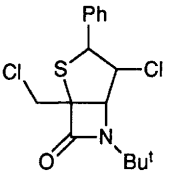

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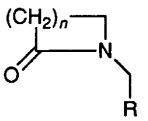
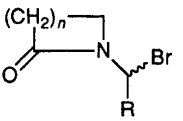
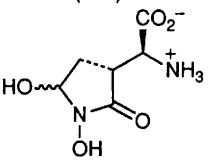


(170)
n = 1, 2; R = CN, CO_2Me



(171)
n = 1, 2; R = CN, CO_2Me



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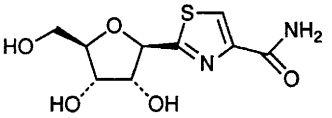

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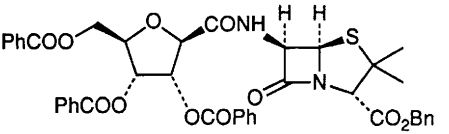

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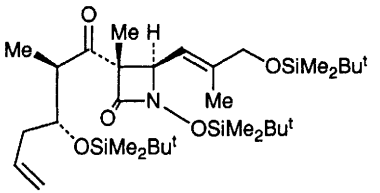

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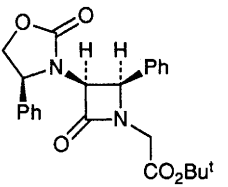

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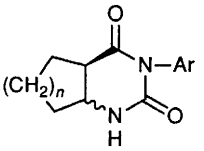
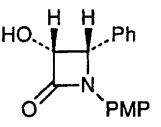


(177) n = 1,2



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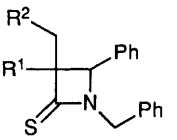


(179)

azetidinone was achieved by complexation with a chiral host.[123]

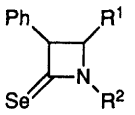
Further uses of azetidinones

Two publications have appeared detailing the synthesis of a novel α-amino acid dealanylalahopcin (**172**) from 3-allylazetidinone-4-carboxylic esters.[124,125] The antitumour, antiviral compound tiazofurin (**173**) has been synthesised from penicillanate (**174**).[126] An improved β-lactam-based synthesis of a fragment of the antitumour antibiotic lankacidin includes C(3)-acylation to give (**175**).[127] The azetidinone (**176**) has been used as a phenylalanylglycinate equivalent in the synthesis of α-alkyl-α-aminoacids.[128] An asymmetric synthesis of α,β-diamino acids and alcohols involves the stereoselective alkylation and aldol reaction of enantiomerically pure 3-amino-4-styryl β-lactams.[129] Bicyclic 2,4-pyrimidinediones (**177**) have been prepared by ring expansion of suitably N-substituted bicyclic β-lactams.[130] Reduction of an azetidine-2,3-dione with sodium borohydride provided a 3,4-*cis* 3-hydroxy azetidinone (**178**) further elaboration and β-lactam ring opening provided a β-amino-α-hydroxy ester corresponding to the side-chain of the anticancer compound taxol. A different approach provided the side-chain of the enzyme inhibitor bestatin.[131]

9. MAJOR STRUCTURAL VARIANTS

As usual, systems retaining a β-lactam ring (or at least a four-membered ring) will be dealt with first. The order will be monocycles, four-six fused systems (cephem analogues) and finally other systems. Irradiation of N,N-dibenzyl-α,β-unsaturated thioamides gives β-thiolactams (**179**) in a reaction involving γ-hydrogen abstraction by the alkene unit.[132] The reaction of lithium phenylalkyneselenolate with alkylideneamines at low temperature gives β-selenolactams (**180**). The reaction may be explained as occurring *via* a selenoketene anion canonical form in [2+2] cycloaddition with the imine component.[133] Diaziridines undergo metal-catalysed carbonylation/ring-expansion to give 1,3-diazetidinones (**181**). Palladium (0) catalysis works for C-mono-substituted diaziridines while stoichiometric quantities of cobalt carbonyl are required for C-disubstituted diaziridines.[134]

Moving to cephem analogues, reaction of the proline-based chiral chromium carbene complex (**182**) with an appropriate cyclic imino ether give the 7-substituted oxacepham (**183**).[135]



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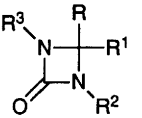

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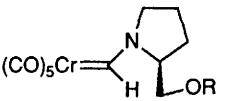

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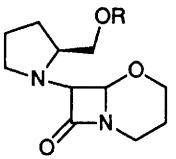

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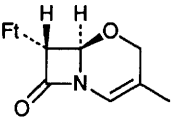

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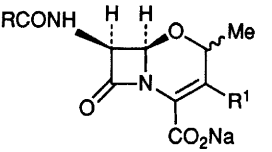

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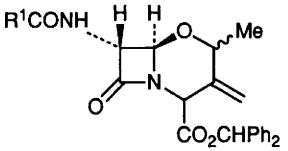

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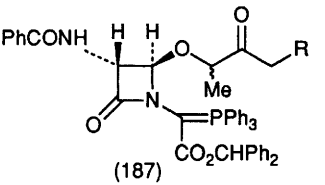

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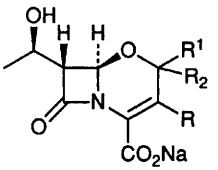
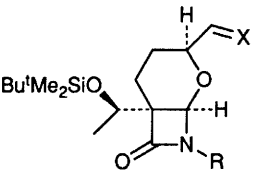
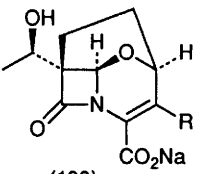


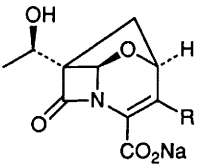
(188)
a; $R^1 = R^2 = H$
b; $R^1, R^2 = O$



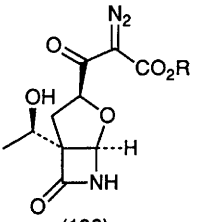
(189)
a; X = CH_2, R = H
b; X = O, R = $C(PPh_3)CO_2R^1$



(190)
a; R = H
b; R = SCH_2CH_2NHAc



(191) a; R = H
b; R = Ome



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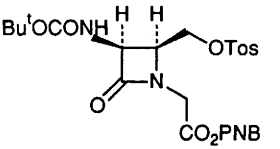
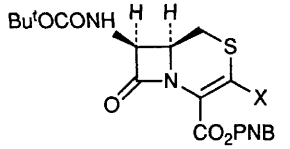


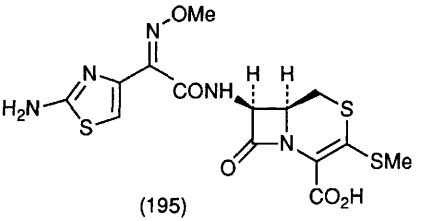
(193)

A simple oxacephem, lacking the C(4)-carboxylic acid, has been prepared by a Peterson-type intramolecular alkenylation of an N-bis(trimethylsilyl)methyl azetidinone under fluoride ion catalysis, providing (**184**).[136] A series of 2-methyl oxacephems (**185**) has been prepared using the exo-methylene oxacepham (**186**) derived in turn from a 3-hydroxy oxacephem by an indirect Wittig approach. Other compounds in the series were obtained by ring closure of the appropriate phosphorane-ketones (**187**).[137] Similar methodology has been used to prepare oxacephems having a thienamycin-type side-chain at C(7) (**188a**). The use of an α-ketoester gave 2-oxo oxacephems (**188b**) which were biologically inactive.[138] The 4,6-bicyclic system (**189a**), prepared from the much-used 4-acetoxyazetidinone derivative (**17b**), was converted to aldehyde-phosphorane (**189b**). Intramolecular Wittig cyclisation provided the ethano-bridged oxacephem (**190a**). An addition-elimination sequence provided the N-acetylcysteaminyl derivative (**190b**). Unsubstituted (**190a**) was virtually inactive while (**190b**) showed weak antibacterial activity.[139] Similar methodology provided the methano-bridged structure (**191a**). An intramolecular carbene insertion strategy involving (**192**) gave the 3-methoxy compound (**191b**).[140]

The reaction of the anion of azetidinone (**193**) with CS_2 or CSO gave the isocephems (**194a**) and (**194b**) respectively. These were trapped with diazomethane to give the corresponding methyl derivatives (**194c**) and (**194d**). Manipulation of the C(7)-amine in (**194c**) provided (**195**). Similar chemistry on (**194d**) resulted in ring-opening to a monocyclic azetidinone.[141] Reaction of the (azetidin-4-yl)methanethiol (**196**) with a suitably functionalised α,β-epoxyester provided the isocepham (**197**) in a single step. Elimination of water with P_2I_4 in pyridine gave isocepehem (**198a**). Removal of the phthalimido group gave amines (**198b**) or (**198c**) depending on the exact conditions used.[142] The azetidinone (**199**) has been used in the synthesis of isocephems and isooxacephems. Base-induced ring closure of (**199**) provided (**200a**) while conversion to a bis-mesylate and treatment with H_2S-triethylamine gave isocephem (**200b**).[143] Reaction of (azetidin-4-yl)methanol (**201a**) with carbonyl diimidazole gave the reactive intermediate (**201b**). Anion formation resulted in ring closure to give lactones (**202a,b**). Reaction with diazomethane provided the corresponding 3-methoxy isooxacephem.[144] The azetidinone disulphide (**203a**) reacts with aniline or ethylamine to give enamines (**203b,c**); subsequent cyclisation with silver acetate gives the 2-azacephem (**204a**). Similar chemistry provided the 7-acylamino analogue (**204b**). In an alternative procedure, reaction of disulphide (**203a**) with ethylamine and silver acetate gives a



(194) a; X = SH
b; X = OH
c; X =SMe
d; X = OMe



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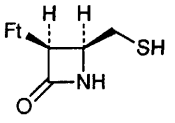

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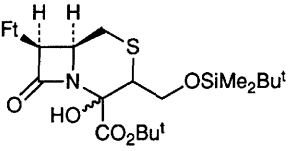

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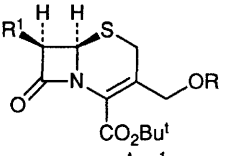
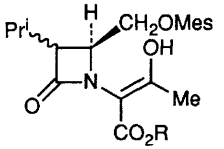
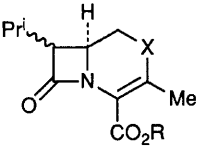


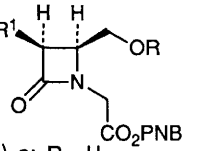
(198) a; R = $SiMe_2Bu^t$, R^1 = Ft
b; R = H, R^1 = NH_2
c; R = $SiMe_2Bu^t$, R^1 = NH_2



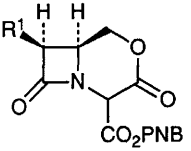
(199)



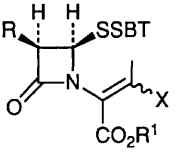
(200) a; X = O
b; X = S



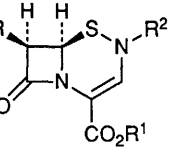
(201) a; R = H
b; R = CO—Im



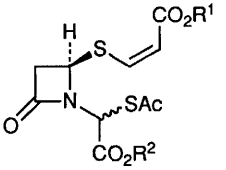
(202) a; R^1 = H
b; R^1 = $PhCH_2CONH$



(203) a; X = OMes
b; X = NHPh
c; X = NHEt



(204) a; R = H
b; R = $PhCH_2CONH$
R^2 = Et,Ph



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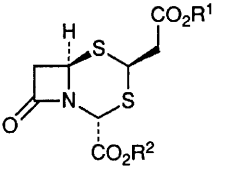
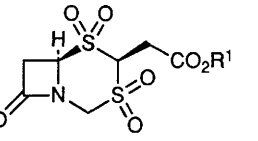


(206) a; R^2 = Bu^t
b; R^2 = H

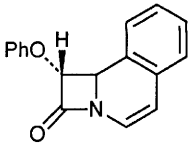


(207)

sulphenamide. Cyclisation with DABCO gives 2-azacephem (**204a**) presumably *via* an allene intermediate.[145] The azetidinone thioacetate (**205**) undergoes intramolecular addition to give 3-thiacepham (**206a**). Selective deprotection of the C(4)-ester provided acid (**206b**). Oxidation of (**206a**) gave the corresponding bis-sulphone possessing two highly acidic hydrogens, at C(2) and C(4). Deprotection of the bis-sulphone gave the corresponding acid which undergoes rapid decarboxylation to (**207**).[146] A further example of Peterson-type olefination of a N-bis(trimethylsilyl)methyl azetidinone provided tricyclic benzocarbacephem (**208**).[147]

Moving on to other ring systems, the N-amino azetidinone (**209**), prepared by cycloaddition of chloroketene with a hydrazone (see also Section 8), undergoes thermal cyclisation to the 2-oxo-3-azapenam (**210**).[148] Deprotection of the known azetidinodiazepines (**211**) and their tricyclic photoisomers (**212a**) has been achieved using methyllithium at low temperature providing (**213**) and (**212b**) respectively. The β-lactam ring proved to be remarkably resistant to these conditions.[149] Addition of the 4-(aminomethyl) azetidinone (**214a**) to the vinyl vicinal tricarbonyl ester (**215**) provided the tricyclic 2-azadethiapenam (**216a**). In a similar manner the homologous 4-(aminoethyl) derivative (**214b**) gave 3-azadethiacepham (**216b**).[150]

Moving on now to non-β-lactam analogues, pyroglutamic acid has been used to prepare (**217a**) and (**217b**), γ-lactam analogues of 1-hydroxy and 1-acetoxy carbapenems. The former was obtained as a stable free acid, while the latter decomposed rapidly in aqueous solution. Attempted oxidation of (**217a**) to a 1-oxo derivative provided instead the bicyclic pyrrole (**218**) which was also unstable as a free acid.[151] There have been modifications of lactivicin involving the preparation of various acylamino derivatives and ester prodrugs. One point of particular interest was the synthesis of the 4α-methoxy analogue (**219**).[152] Other authors have reported the preparation of cycloserine derivatives (**220a,b**).[153] A synthesis of the chiral pyrazolidin-3-one (**221a**) which avoids racemisation under basic conditions involves the Mitsunobu ring-closure of the N-trifluoroacetamide (**222**).[154] An alternative method for formation of the second ring in the pyrazolidinone antibacterials involves a Wadsworth-Horner-Emmons reaction of an oxoamide (**221b**).[155] A synthesis of a γ-lactam analogue of penems has appeared.[156] Four publications detail the design and synthesis of various oxaziridines[157,158] and epoxides[159,160] as topological analogues of β-lactam antibiotics.



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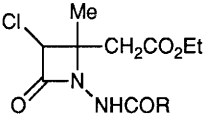

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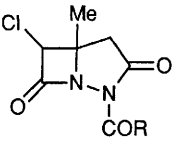

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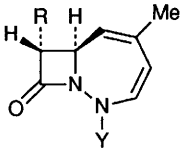
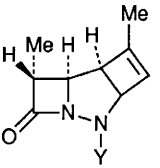
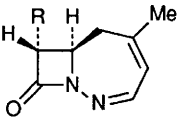


(211) Y = CO_2Et, COPh
R = H, Me



(212) a; Y = CO_2Et, COPh
b; Y = H



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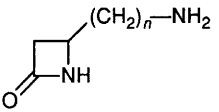
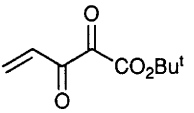
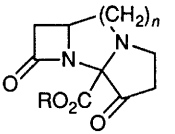


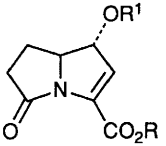
(214) a; *n* = 1
b; *n* = 2



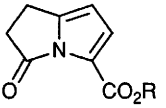
(215)



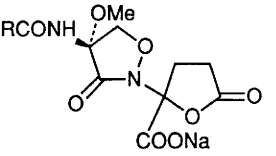
(216) a; *n* = 1
b; *n* = 2



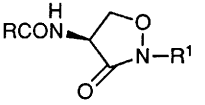
(217) a; R^1 = H
b; R^1 = Ac



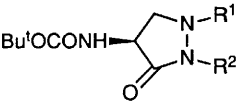
(218)



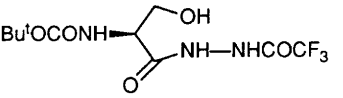
(219)



(220) a; R^1 = $CH(OEt)CO_2K$
b; R^1 = $C(CO_2NH_4)$=$CH{\sim}CO_2NH_4$

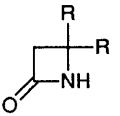


(221) a; R^1 = R^2 = H
b; R^1 = $CH_2CHXP(O)(OR)_2$
R^2 = $COCO_2$Allyl

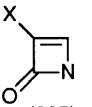


(222)

(223)



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

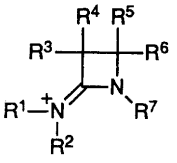


(225)

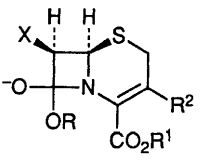
10. MECHANISTIC STUDIES, MODE OF ACTION AND DEGRADATION

As is usual, this section will encompass general mechanistic studies, interactions of β-lactams with enzymes, molecular graphics and mechanisms and products of degradation of β-lactams. The use of a flow reactor has allowed the observation and characterisation of the reactive intermediate 1-azetin-4-one (**223**) in solution. Compound (**223**) was generated by reaction of polymer-bound 4-substituted azetidinones with nucleophiles. The UV spectrum of (**223**) was recorded and its lifetime determined as ≤2 seconds.[161] A comparison of a number of pairs of cephems and carbacephems revealed broadly similar antibacterial activities. The β-lactam carbonyl group of carbacephems absorbed at a lower infra-red frequency than the corresponding cephem. The most striking difference was in chemical stability; at pH 10-11 in water carbacephems hydrolysed 8-32 times more slowly.[162] A report details a new interaction model for β-lactam antibiotics with their target enzyme. An optimised geometry was obtained using CNDO/II energy-gradient methods for the complex of a model β-lactam and the serine residue of an enzyme site flanked by groups capable of hydrogen bonding.[163]

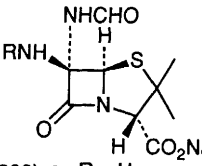
Moving now to degradation and hydrolysis of β-lactams, mercury photosensitised decomposition of azetidin-2-one (**224a**) and its 4,4-dimethyl analogue (**224b**) gave carbon monoxide and an olefin as major products in each case. Ammonia was obtained from (**224a**) while 2,2-dimethylaziridine was observed from (**224b**).[164] A study of the hydrolysis of several monocyclic β-lactams, and of a penam and penicillins revealed that all reacted with rate-determining addition of hydroxide ion. The increased reactivity of penicilins was attributed to both their bicyclic structure and the presence of an acylamino side-chain.[165,166] An examination of the reaction of N-phenyl and N-benzyl, 4-chloro and 4-(methylthio) β-lactams with sodium methoxide/methanol reveals that ring-opening is the first and rate-controlling reaction and that elimination of the C(4)-substituent occurs afterwards.[167] The same authors report a further study of nearly fifty azetidinones varying in reactivity by 10^9. All attempts to produce a putative intermediate azetinone (**225**), resulting from initial expulsion of the C(4)-substituent were without success.[168] The study of the reaction of 4-(aryloxy) azetidinones with aqueous alkali has been extended to their 4-(arylthio) analogues. The same El_CB_R mechanism is proposed, resulting in 3-hydroxyacrylamide and thiophenoxide ions.[169] Two publications have appeared discussing the hydrolysis of azetidinyl amidinium salts (**226**). The reaction gives a mixture of β-lactam, by



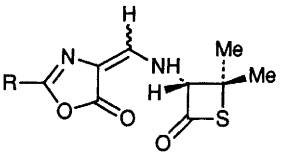
(226)



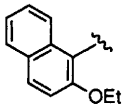
(227)



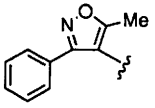
(228) a; R = H
b; R = CCl_3CH_2OCO

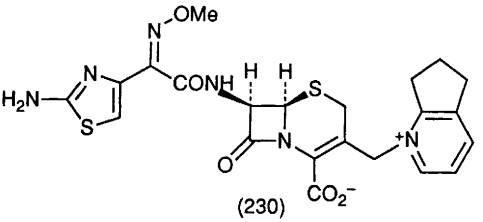


(229) a; R =

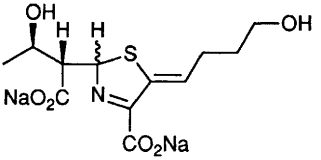


b; R =





(230)



(231)

exocyclic C-N fission and β-amino amide by endocyclic C-N rupture. Exocyclic fission is usually the major process, providing good yields of β-lactam products. The kinetics of the reaction indicate the presence of a neutral tetrahedral intermediate, there are two changes in rate dependence on hydroxide ion with increasing base concentration.[170,171] A similar study of the alcoholysis of cephalosporins reveals general acid-catalysed inhibition proposed to result from trapping of the anionic tetrahedral intermediate (**227**) by a proton to give a less reactive neutral species. At low pH this process is dominant with protonation of (**227**) occurring faster than ring-opening.[172] A full account has now appeared detailing the aqueous degradation of 6α-formamido penicillins by C(5)-C(6) and N(4)-C(7) cleavage. Under anhydrous conditions the 6β-amino-6α-formamido derivatives (**228a,b**) undergo base-catalysed methanolysis involving N(4)-C(7) cleavage to give the corresponding penicilloates.[173] The aqueous degradation of sodium nafcillin and sodium oxacillin are reported as yielding the novel thietan-2-one degradation products (**229a**) and (**229b**) respectively.[174] A comprehensive study of the degradation of cefpirome (**230**) in aqueous solution revealed syn-anti oxime isomerisation with light and Δ2-formation and C(7)-epimerisation with base. N(5)-C(8)/C(6)-C(7) cleavage and loss of the C(3)-substituent resulted from treatment with acid.[175] Metabolism of the 2-tetrahydrofuryl penem SUN 5555 in rats provided two major metabolites after oral dosing. The same compounds were produced by hydrolysis with 1.2 equivalents of aqueous sodium hydroxide. The products were identified as the two possible C(5) (penem numbering) isomers of the doubly ring-opened compound (**231**).[176] Further studies on the hydrolysis and aminolysis of clavulanic acid (see Volume 22) have concentrated on the role played by metal ions in chelating to both amino alcohol and clavulanic acid, bringing the two reactants into proximity and simultaneously reducing the pK_a of the nucleophile.[177]

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
Tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives of Ampicillin	178
Tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives of Amoxycillin	179
2β-(Substituted-methyl)penicillins	180
6α-Formamido penicillins	181
Synthesis of penicillanic acid sulphoxides	182
Chemistry of 6β-thioamidopenam-1β-sulphoxides	183
Prodrugs of Sulbactam	184
3-Aminoxy-(*E*)-2-methoxyiminopropionyl penicillins and cephalosporins	185
Benzimidazolyl derivatives of penicillins and cephalosporins	186
Carbomethoxyphenoxy (α,α-dialkyl)acetyl penicillins and cephalosporins	187
Orally active cephalosporin esters	188, 189, 190, 191
Cephalosporin sulphone esters as Human Leukocyte Elastase inhibitors	192, 193, 194, 195
5-Furyltetrazole acetic acid acylations of 7-ACA	196
7-(4,7-Disubstituted coumarin-3-acetamido)cephalosporins	197
Alkylene bisthioureido derivatives of Cefadroxil	198
7-(1,7-Disubstituted-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxamido cephalosporins	199
7-(6-Substituted-2-quinolone-3-acetamido)cephalosporins	200
A mild method for introducing 7α-alkoxy substituents in cephalosporins	201
Improved preparation of N-salicylidene-7-aminodesacetoxy-cephalosporanic acid	202
7-[1-(2-Aminothiazol-4-yl)-1-cyclopropanecarboxyamido]cephalosporins	203
1,5-Dihydroxy-4-pyridone-2-carbonyl containing cephalosporins	204, 205, 206
1-Carboxyethoxyimino substituted aminothiazole cephalosporins	207

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6
Metal Complexes of Amino Acids and Peptides

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

1 Introduction

This chapter deals with the synthesis, structures and reactions of metal-amino acid and metal-peptide complexes and covers material published in 1990. A number of reviews on metal complexes of amino acids and peptides have appeared. In one of these the biological importance of alkali and alkaline earth cations with emphasis on magnesium deficiency and therapy in human and vetinary medicine is summarised.[1] The choice of compounds which lead to efficient absorption of this ion without side effects is of crucial importance. Popular magnesium formulations contain the amino acids L-aspartic acid, L-glutamic acid and L-pyroglutamic acid. The metal binding roles of these acids, their dissociation and metal complexation equilibria in aqeous solution, their effects on magnesium bioavailability and the solid state structures of a range of crystalline complexes are reviewed. The complexes include Ca(L-Asp).nH_2O (n = 2,4), Ca(L-Glu).3H_2O, Sr(L-Glu).6H_2O, Ba(L-Asp).6H_2O, Ca(L-GluH)Cl.H_2O and Ca(L-Glu)$_2$, Mn(L-Asp).3H_2O, Zn(L-pGlu)$_2$.2H_2O, Zn(L-AspH)Cl, Li$_2$(L-Asp).2H_2O, Na(L-GluH).H_2O, K(L-AspH).2H_2O and K(L-GluH).H_2O.

Other reviews cover the solution chemistry of metal peptide complexes,[2] metallothioneins and phytochelatins, heavy metal binding proteins from plants,[3] the formation, turnover, structure and compartmentalization of phytochelatins,[4] occurrence, synthesis and function of heavy metal binding proteins/peptides,[5] casein phosphonopeptides in calcium solubilisation and in physiological function,[6] calcium carbonate and phosphate-peptide interactions,[7] molecular recognition using metal complexes and studies of the interaction between metal complexes and amino acids, peptides, sugars and DNA,[8] macrocyclic polyamines for selective binding of alkali and alkaline earth cations and biological zwitterions such as amino acids,[9] the effect of amino acid side chain on protein absorption and retention by hydrophilic gels into which metal chelates are incorporated,[10] synthesis and biomimetic properties of transition metal thiolates including peptide thiolates,[11] binary and ternary palladium(II)/peptide/nucleoside or nucleotide complexes as models for metal ion mediated DNA-protein interactions and platinum(II)/DNA/protein cross links caused by the antitumour drug Cisplatin,[12] and the use of copper(II) chloride together with 1-hydroxybenzotriazole for racemisation-free and efficient peptide synthesis by the carbodiimide method.[13]

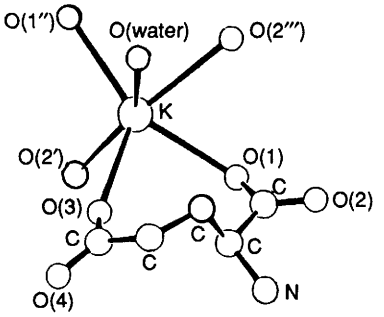
2 Amino Acid Complexes

2.1 Synthesis and Structures.- The crystal and molecular structures of amino acid complexes containing a wide range of metal ions have been reported. While the majority of structures reported deal with complexes of Cu(II) also included and described herein are complexes of K(I), Cr(III), Co(III), Cu(II), Mo(VI), Rh(III), Ir(III), Ru(II), Pd(II) and Pt(II). In addition the X-ray diffraction technique has been used to determine structures in solution of Zn(II)-amino acid complexes.

The crystal and molecular structure of the glutamate complex K(L-GluH).H_2O has been determined.[14] In this complex **(1)** the metal ion lies in a distorted trigonal-prismatic environment of six oxygen atoms from four different amino acids one of which is bidentate forming an 8-membered chelate ring and a water molecule. Bridging by the α-carboxylate oxygen atoms **(2)** produces a larger polymer in which the layers are cross linked by hydrogen bonding involving the $^+NH_3$ and γ-COO^- groups.

The synthesis of bis(imidazole)(amino acid-N,N-diacetato)chromium (III) complexes $Cr(Im)_2[N(CHRCOO^-)(CH_2COO^-)_2]$ capable of association with proteins in a geometry specific fashion has been described and the crystal and molecular structures of two of these i.e. the L-leucine, R = -CH_2CHMe_2, and L-phenylalanine, R = CH_2Ph, derivatives $Cr(Im)_2$(Leu-N,N-diac) **(3)** and $Cr(Im)_2$(Phe-N,N-diac) **(4)** have been determined.[15] In both cases only one isomer was isolated this having the amino acid side chain, R, on a ring in the equatorial plane. The Cr-N (amino acid) and Cr-O bond distances lie within characteristic limits while the Cr-N (imidazole) bond trans to the amino acid nitrogen is slightly shorter than that trans to carboxylate. Hydrogen bonding and aromatic/aromatic interactions within the crystal lattice have been examined as models for interactions which may occur between the complexes and proteins. The rates and stereochemical course of aquation reactions of the parent $Cr(NTA)(H_2O)_2$, R = H, complex have also been studied. A series of μ-carboxylato-μ-hydroxochromium(III) complexes some of which include amino acid ligands have been synthesised and studied by ^2H n.m.r. and electronic spectroscopy.[16] These complexes are $[Cr(en)_2OH(HAA)Cr(en)_2]^{5+}$, $[Cr(en)_2OH(CH_3COO)Cr(en)_2]^{4+}$ and $[Cr(NTA)OH(CH_3COO)CrNTA]^{2-}$ where en = 1,2-diaminoethane and HAA = Gly, Ala, Ser or Thre. The crystal structure of the NTA complex has been reported.

The synthesis and stereoselectivity of the complexes *fac*-Cr(L- or D-Ala)$_x$(L-Leu)$_{x-3}$ where x = 1 or 2 and the application of the synthetic method to the resolution of D-L-alanine are described.[17] Several binary chromium(III)-amino acid complexes and ternary chromium(III)-nucleotide (5'-AMP or 5'-CMP)-amino acid (L-Ser, L-Met or Gly) complexes have been synthesised and characterised by elemental and thermogravimetric analysis and by



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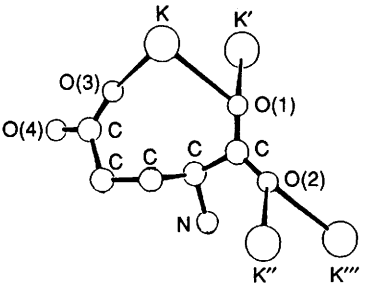

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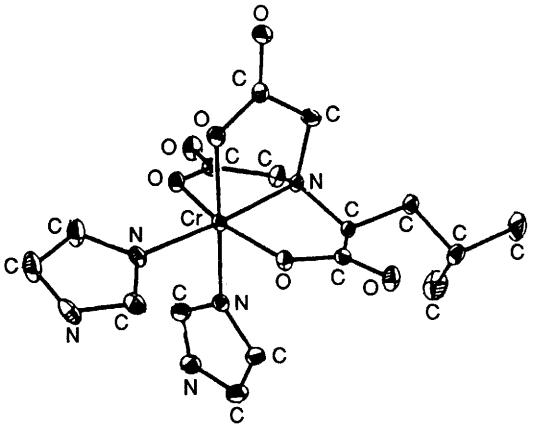

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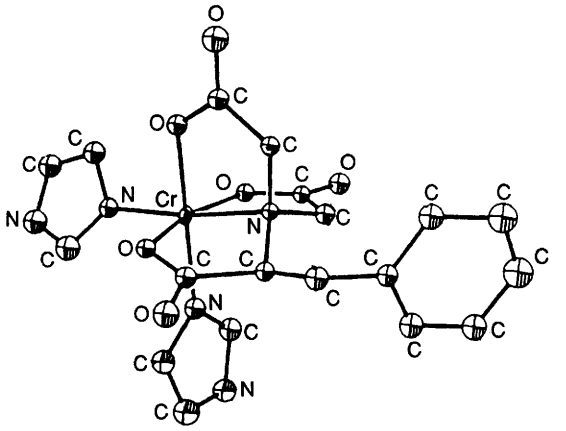

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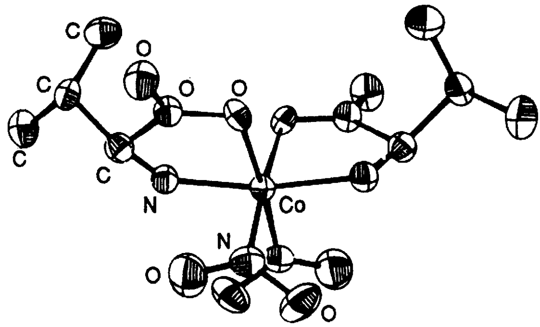
spectroscopic methods (i.r., electronic and e.s.r.).[18] The complexes are $Cr(L\text{-}Met)(L\text{-}MetH)Cl_2.3H_2O$, $Cr(L\text{-}Met)(5'\text{-}AMP).5^1/_2\ H_2O$, $Cr_2(L\text{-}Met)(5'\text{-}CMP)_2OH.10H_2O$, $Cr_2(L\text{-}SerH)_2(L\text{-}Ser)_4Cl_2.6H_2O$, $Cr(L\text{-}Ser)_2(H_2O)_2Cl.H_2O$, $Cr_2(L\text{-}Ser)(5'\text{-}AMP)_2Cl.16H_2O$, $Cr_2(L\text{-}Ser)_2(5'\text{-}CMP)OH(Cl).7H_2O$, $Cr(GlyH)_2(Gly)Cl_2.3H_2O$, $Cr_2(Gly)_2(5'\text{-}AMP)(OH)_2.6H_2O$ and $Cr_2(Gly)_2(5'\text{-}CMP)(OH)_2.7H_2O$. In these complexes the amino acids act as bidentate ligands and the nucleotides are bonded to the metal via the phosphate groups. The solid state reaction between powdered mixtures of glycine and $[Cr(NH_3)_6](NO_3)_3$ at 130-180°C produce the glassy purple complex $[Cr(NH_3)_3(OOCCH_2NH_3)_3](NO_3)_3$ which on dissolution in water gives *fac* -$Cr(Gly)_3$.[19] The fully deuterated form of this complex has also been prepared.

The reaction of V_2O_5 with hydrogen peroxide and glycine at pH 3-4 gives the peroxovanadium(V) complex $[VO(O_2)_2GlyH]^-$ which contains bidentate peroxo ligands and which was isolated as an NH_4^+ and a K^+ salt.[20] At pH 2 the reaction product is $V_2O_2(O_2)_3(GlyH)_2(H_2O)$ which also contains one μ-peroxo ligand. In both complexes glycine is present as a monodentate carboxylato-bonded zwitterion.

The crystal structure of the complex Δ-cis(NO_2), trans(NH_2) - Ag[Co(S-Val)$_2$(NO_2)$_2$], **(5)**, has been determined.[21] The fact that the S-Val ligand forms a strained envelope chelate ring conformation with one side chain methyl group axially oriented close to the metal confirms the idea that such axial positioning of the side chain induces a large contribution to the optical activity of the complex. The crystal structure of the complexes $[Co(tren)Memal\text{-}H]Cl_2.H_2O.^1/_2$ EtOH and $[Co(tren)Memal]Cl.3H_2O$ as models for the binding of glutamate in biological systems have been determined.[22] A strong H-bond involving the uncoordinated carboxylate oxygen allows methylmalonate (Memal) to act as a uninegative, bidentate ligand in the former case. The ethylenebiguanide (EBG, **6**) complex $[Co(Met)EBG]^{2+}$ has been synthesised and spectroscopic evidence points to the existence therein of bidentate N,O bonded methionine with non-involvement of the SH in coordination.[23] Several cobalt(III) ammine complexes with chiral amino acid ligands such as L-Asp and L-Asn (also L-malate) were synthesised and their c.d. spectra analysed.[24]

The crystal and molecular structures of a number of amino acid and N-protected complexes of copper(II) are described. The ternary complexes $Cu(bipy)(Tos\text{-}DL\text{-}Asn)_2\ H_2O.2H_2O$ **(7)** and $Cu(bipy)Tos\text{-}DL\text{-}Asn.H_2O$ **(8)** containing bipyridine and N-tosyl amino acid ligands have tetrahedrally distorted square pyramidal geometries.[25] In the first of these complexes each Tos-Asn anion acts as a monodentate carboxylate ligand giving a CuN_2O_3 chromophore while in the second it is tridentate through the sulphonamidic nitrogen, the carboxylate oxygen and the side chain amide oxygen giving a CuN_3O_2 chromophore. In a related solution study of complexes having tosylated asparagine and glutamine ligands using pH-metric and polarographic techniques the following species were detected; $Cu(bipy)(HL)_2$, $Cu(bipy)L$, $Cu(bipy)L_2^{2-}$, $Cu(bipy)HL^+$ and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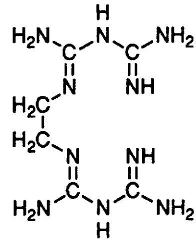
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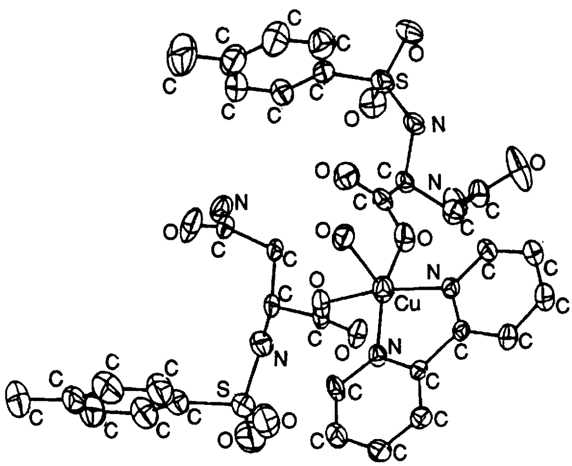
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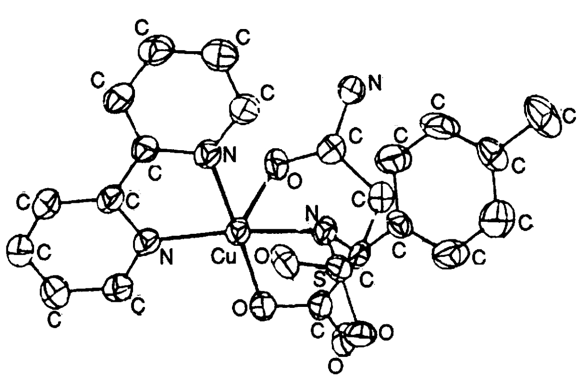
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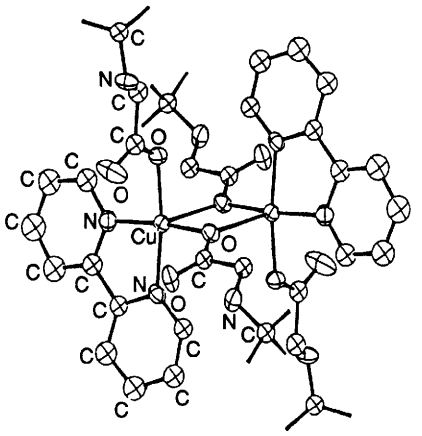
$Cu_2(bipy)_2L_2OH^-$, in which HL^- represents a monodentate carboxylato bonded ligand and L^{2-} is bidentate involving the additional deprotonated sulphonamide nitrogen site.[26] In the ternary complexes deprotonation of the sulphonamide group occurs at lower pH (~5) than in the corresponding binary systems (~7).

The N-tritylglycine complex $Cu_2(\mu\text{-Trt-Gly})_2$ $(bipy)_2$ (Trt-Gly) **(9)** has been obtained from $Cu(Trt\text{-}Gly)_2.3H_2O$ and bipy in methanol and its crystal structure determined.[27] Each of the units in this dimer consists of a square pyramidal CuN_2O_3 chromophore having a bipyridine and a monodentate O-bonded Trt-Gly-O ligand. The metal atoms in the dimer are bridged by two oxygen atoms one from each of the μ-Trt-Gly ligands. The crystal and molecular structure of $Cu(NCS)[N,N\text{-}(2\text{-}OHCH_2CH_2)_2Gly].H_2O$ **(10)** has been determined.[28] In this complex the metal is in a tetragonal bipyramidal ligand environment having OH, COO^- and N donor groups from the amino acid ligand as well as an N atom of the NCS^- ligand in the square plane with the second OH group and the S of the NCS^- apically coordinated.

The amino acid DL-α-amino-β-methylaminopropionic acid (AMAPA, **11**) is a chronic neurotoxin which has been found to induce upper and lower motor neuron dysfunction and Parkinsonian features in experimental animals.[29] This compound had previously been shown to form unusually stable metal complexes with copper(II) and zinc(II) and also gives a stable α-carbamate structurally similar to the excitotoxin N-methyl-D-aspartate. These observations may be relevant in explaining the neurotoxicity of the amino acid. The crystal structure of $Cu(AMAPA)_2(ClO_4)_2$ **(12)** has been determined.[29] In this complex the metal lies at a centre of symmetry with one L and one D amino acid forming a trans CuN_2O_2 square planar arrangement having bond distances, Cu-N 1.976 Å, Cu-O 1.942 Å and two axial perchlorate ligands, Cu-O 2.54 Å.

As part of a study on the role of lattice symmetry in superexchange interactions in copper(II) - amino acid complexes the crystal structure of the 2-aminobutyrate complex trans-$Cu(L\text{-}Abu)_2$ was determined and compared with that of the racemic amino acid complex.[30] This complex **(13)** consists of square planar trans CuN_2O_2 chromophores arranged in two dimensional sheets parallel to (001). Pairs of carboxylate oxygens from neighbouring molecules in the sheet complete elongated octahedral coordination around the metal. Large differences observed in e.s.r. line widths between $Cu(D,L\text{-}Abu)_2$ and $Cu(L\text{-}Abu)_2$ results from a modification in the exchange network due to a lowering in symmetry. Single crystals of the complexes $Cu(L\text{-}Phe)_2$, $Cu(L\text{-}Met)_2$ and $Cu(L\text{-}Leu)_2$ have been examined by e.s.r. spectroscopy for magnetostructural correlations in order to assess the effectiveness of carboxylate bridges and H-bonds as pathways for superexchange.[31]



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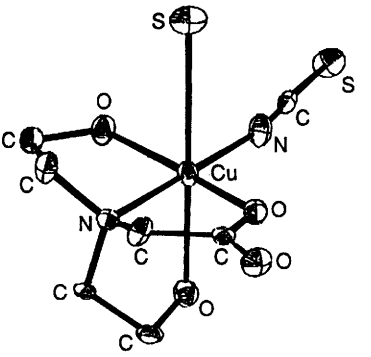

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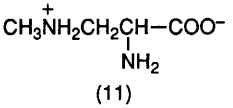

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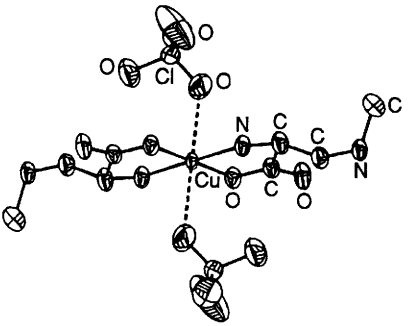

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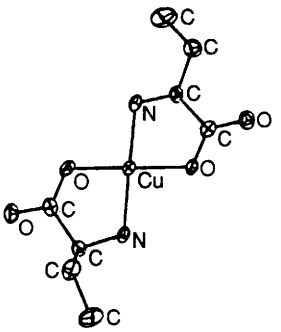

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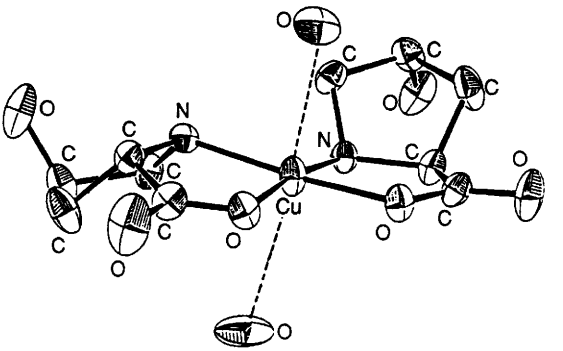

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The crystal structures of the complexes *cis*-Cu(L-Pro-4OH)$_2$.4H$_2$O (**14**) and *trans*-Cu(D-aPro-4OH)$_2$.2^1/$_2$H$_2$O (**15**) have been determined.[32] In both complexes the amino acid ligands are in a square planar arrangement around the metal and coordinated via the pyrrolidine nitrogens and carboxylate oxygens with cis configurations of the N,N O,O pairs in the former complex and trans in the latter. Weak Cu-O interactions complete distorted octahedral coordination around the metal.

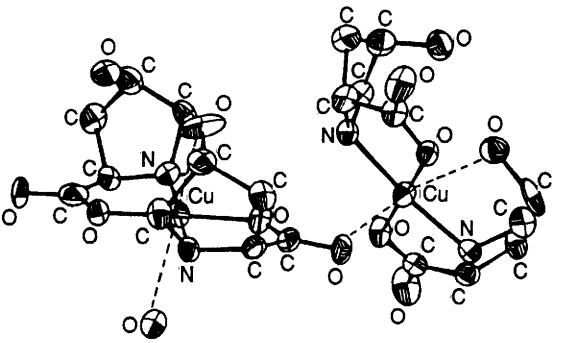
A number of ternary copper(II) complexes contaiing 'mono-condensed' Schiff bases derived from 1,3-diacetylbenzene and Phe-OMe (**16**), Tyr-OEt (**17**), Histamine (**18**), His-OMe (**19**) and 2-(2-aminoethyl)pyridine (**20**) with the same amino acids/amines as secondary ligands have been synthesised and characterised by molar conductance, magnetic and spectroscopic methods.[33] In the Phe and Tyr complexes (**21**) the Schiff base and coligands act as N,O-bidentate donors while in the other complexes (**22**) they act as 2N bidentate ligands.

Copper(II) and nickel(II) complexes of 2-aminooxyacids $^+NH_3OCH(R)COO^-$ where R = H, Me, Pr, i-Bu, Bzl and cobalt(II) complexes of some of their esters have been synthesised.[34] The acids behave as bidentate N,O anionic ligands giving neutral complexes with Cu(II) and Ni(II). The Co(II) ester complexes have pseudotetrahedral structures in which the ester ligands are monodentate and N-bonded to the metal.

The liquid X-ray diffraction method has been used to determine the structures of the 1:1, 2:1 and 3:1 complexes of alanine with Zn(II) in aqueous solution.[35] In contrast to the corresponding glycinato complexes which have regular octahedral structures with Zn-O and Zn-N bond lengths of 210 ± 2 pm the alaninato complexes have shorter Zn-O bonds of 202/203 pm in $[Zn(Ala)(H_2O)_4]^+$, $Zn(Ala)_2(H_2O)_2$ and $[Zn(Ala)_3]^-$ but Zn-N bonds of 213/214 pm. The difference has surprisingly been attributed to the inductive effect of the methyl substituent.

The reaction of MoO_2Cl_2 with methionine in water or methanol gave the octamolybdate $Mo_8O_{20}(OH)_4$(Met-O)$_4$ as a tetrahydrate or octamethanolate.[36] Neutralisation of a methanolic solution of MoO_2Cl_2 and methionine with morpholine produced the salt (H-Mor)$_4$[$Mo_8O_{24}(OH)_2$(Met-O)$_2$] the crystal structure of which is reported. The structure consists of eight centrosymmetrically condensed edge sharing octahedra in which the molybdenum atoms are octahedrally coordinated. The amino acid ligands are O-bonded and occupy terminal sites of the Mo_8O_{26} core.

The reaction of $RuCl_2(PPh_3)_3$ with the amino acids Gly, L-Ala and L-Val in methanol produced the complexes $Ru(AA)_2(PPh_3)_2$.[37] A crystal structure determination of the L-Ala complex which crystallises as the Δ diastereomer shows that the carboxylate oxygen of one amino acid ligand and the nitrogen of the second lie trans to the cis positioned triphenylphosphines.



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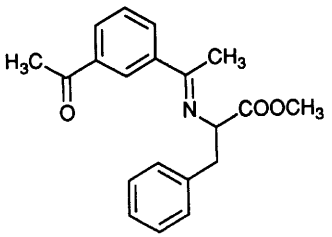

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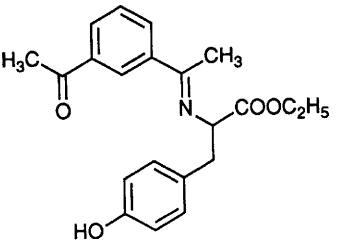

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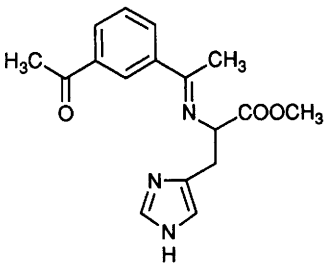

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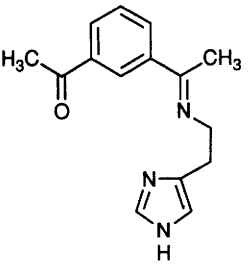

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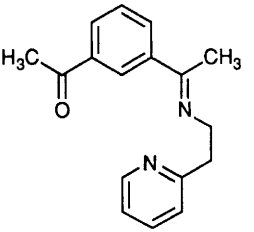

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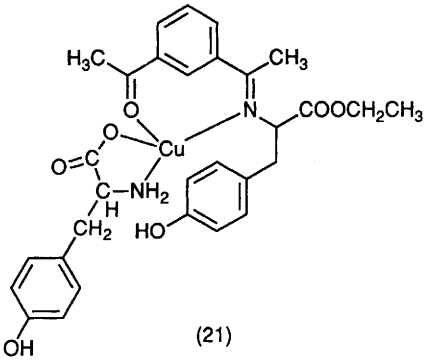

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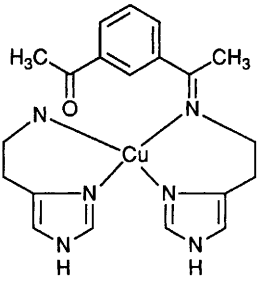

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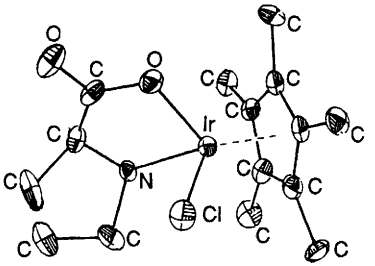

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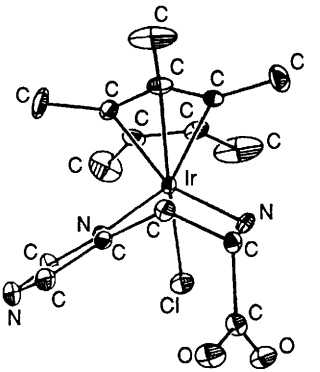

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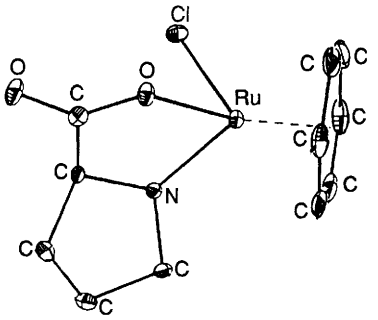

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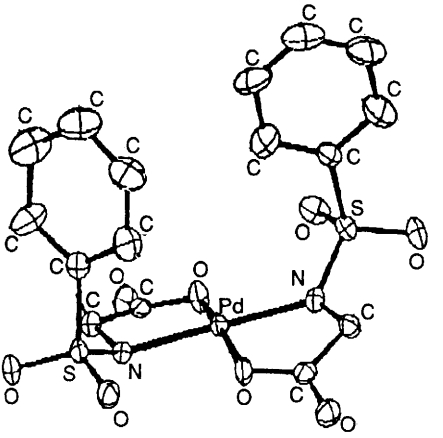


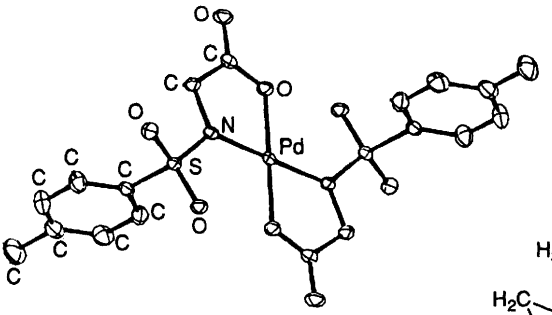
(26)

^{31}P and ^{1}H n.m.r. spectroscopy confirmed a similar structure for the L-Val complex and also that both Λ and Δ forms of this and the L-Ala complex exist in methanol solutions. The reaction of $RuCl_2(PPh_3)_3$ with Gly or L-Ala but not L-Val in acetone gives the Schiff base complexes $Ru[Me_2C{=}NCH(R)COO]_2(PPh_3)_2$ the crystal structures of which show that the phosphine ligands are trans to one another in the Gly complex but are trans to amine nitrogens in the L-Ala complex.

A number of Rh(III), Ir(III), Ir(I) and Ru(II) complexes containing amino acid or amino acid ester ligands have been prepared and the crystal structures of three of these determined by X-ray diffraction.[38] The compounds synthesised were $Me_5CpMCl(AA)$ where M = Rh or Ir and AA = Gly, L-Val, L-Phe, L-PhGly, L-Trp, L-Pro, L-His, L-Asp; Me_5CpM(L-Asp) where M = Rh or Ir; $[Me_5CpIr(Cl)$L-His$]Cl$; $(\eta^6\text{-}C_6H_6)RuCl(AA)$ where AA = L-Pro, L-Phe, L-4-NO_2Phe, L-Dopa, D-PhGly; $[(\eta^6\text{-}C_6H_6)Ru$(L-His$)]Cl$; (Cod)Ir(AA) where AA = L-Ala, L-Phe, L-Val, L-Leu, L-PhGly and Cod = η^4-1,5-cyclooctadiene and (Cod)Ir(Cl)AAOR where AAOR = GlyOEt, L-AlaOMe or L-ValOMe. The structures of $Me_5CpIr(Cl)$L-Pro **(23)**, $Me_5CpIr(Cl)$L-His **(24)** and of the ruthenium(II) complexes $(\eta^6\text{-}C_6H_6)Ru(Cl)$L-Pro **(25)**, $(\eta^6\text{-}C_6H_6)Ru(Cl)$L-Ala and $(\eta^6\text{-}C_6H_6)Ru$(L-AlaOMe$)Cl_2$ have been determined.[38,39] Reaction of the L-Ala complex with 9-ethylguanine gives $[(\eta^6\text{-}C_6H_6)Ru$(L-Ala)9-Etgua$]Cl_2$ while the L-AlaOMe complex gives $(\eta^6\text{-}C_6H_6)Ru$(9-Etgua$)Cl_2$.

The crystal and molecular structures of the N-benzenesulfonylglycinato (Bs-Gly) and the N-4-tolylsulfonylglycinato (Tos-Gly) complexes $Na_2[Pd(Bs\text{-}Gly)_2].H_2O$ **(26)** and $Na_2[Pd(Tos\text{-}Gly)_2]$ **(27)** have been determined.[40,41] In both cases the ligands are coordinated through the carboxylate oxygen and deprotonated nitrogen atoms giving square planar complexes with trans configurations. Intramolecular contacts of Pd with S,O and aromatic C atoms and intramolecular stacking interactions involving phenyl rings are reported for the Bs-Gly complex the solution behaviour of which has been studied by ^{1}H n.m.r. The interaction of Bs-Gly, Ts-Gly and Dn-Gly where Dn is dansyl with Pd(II) in aqueous solution was investigated by polargraphic and pH-metric methods and N,O chelated 1:1 and 2:1 complexes were identified in the pH range 4-11.5.[42] The reactions of cis-$Pd(Guo)_2Cl_2$ where Guo is guanosine with the sodium salts of Gly, Ala, Val, Ile, Pro and Phe in methanol solution have been investigated and the N,O chelated amino acid complexes cis-$[Pd(Guo)_2AA]Cl$ were isolated.[43] Hydrophobic interligand interactions in these complexes were investigated.

A number of papers describing purine, pyrimidine and nucleoside complexes of palladium(II) and platinum(II) have been published. Eight complexes of the type [Pd(D,L-ethionine)L(Cl)]Cl.nH_2O where L is adenine, adenosine, guanine, guanosine, hypoxanthine, inosine, cytosine and cytidine were synthesised and characterised.[44] In all of these complexes which contain N,S coordinated ethionine the purines and their nucleosides are coordinated through N7, **(28)** while the pyrimidines and their nucleosides are coordinated through N3, **(29)**.The reactions of the complexes *cis*-$[Pt(NH_3)_2AA]NO_3$ where HAA = Gly, L-Ala or



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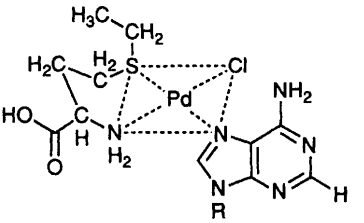

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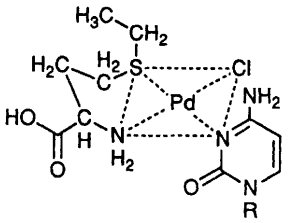

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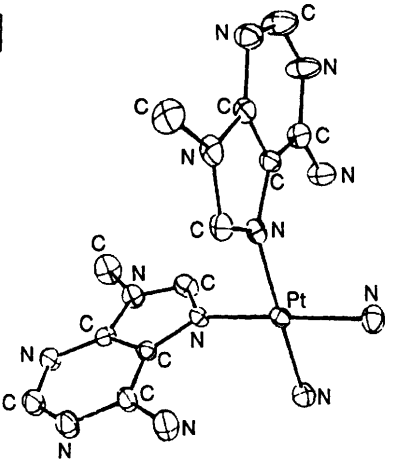

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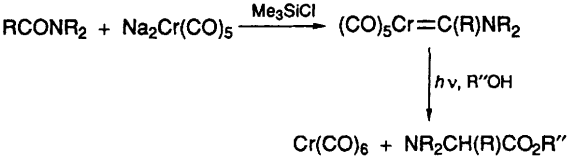


Scheme 1

2-aminobutyric acid with 9-methylguanine, 9-MeGH, and 9-methyladenine, 9-MeA, resulted in the formation of *cis*-$[Pt(NH_3)_2(9\text{-MeGH})_2](NO_3)_2$ in neutral solution and *cis*-$[Pt(NH_3)_2(9\text{-MeA})_2](NO_3)_2$ in strong acid.[45] The crystal structure of the latter complex was determined and this shows that the 9-MeA molecules are both coordinated through N7, (**30**). The crystal structures of the complexes *trans*-$[Pt(MeNH_2)_2(l\text{-MeCyt})Cl]Cl.H_2O$ and *trans*-$[Pt(MeNH_2)_2(l\text{-MeCyt})Gly]NO_3.2H_2O$ containing 1-methylcytosine ligands have also been reported.[46] The ^1H n.m.r. spectra of the glycinato complex in the pH range 0.4 - 13.5 are indicative of two acid-base equilibria with pK_a values 2.5 and 12.5.

$$[Pt(MeNH_2)_2(l\text{-MeCyt})(H\text{-Gly})]^{2+} \overset{-2H^+}{\rightleftharpoons} Pt(MeNH_2)_2(l\text{-MeCyt}^-)Gly$$

The cysteine ester complexes $[MeHgSCH_2CH(NH_3^+)CO_2R]Cl$, R = n-C_4H_9, n-$C_{16}H_{33}$ have been synthesised and their partition coefficients between n-octanol and water measured and compared with those of the cysteine complex.[47] Esters of 2,3-dimercaptosuccinic acid give polymers with Hg(II) and with methyl mercury give the bimetallic complexes $MeHgS(RO_2C)CHCH(CO_2R)SHgMe$ where R = C_2H_5, n-C_4H_9, n-$C_{16}H_{33}$.

The diamagnetic peroxo complexes of UO_2^{2+} i.e. $UO_2(O_2)$ phen, $UO_2(O_2)$bipy, $UO_2(O_2)$en, $UO_2(O_2)H_4$edta and $UO_2(O_2)$Gly have been synthesised.[48] In these complexes both the peroxo and coligands (except Gly) are bidentate while in $UO_2(O_2)$Gly, the amino acid is monodentate and O-coordinated. This complex oxidises triphenylphosphine to the oxide, cyclohexene and styrene to 1,2-diols and SO_2 to sulphate.

2.2 Reactions.- The reaction of tertiary amides with $Na_2Cr(CO)_5$ and Me_3SiCl gives aminocarbene complexes which on photolysis in alcohol solvent produces amino acid esters in good yields, Scheme 1.[49]

The kinetics of formation of Cr(D,L-Trp)$_3$ from $[Cr(H_2O)_6]^{3+}$ and the amino acid has been investigated by visible spectroscopy over the pH range 2.75-3.75.[50] A mechanism involving equilibrium formation of an outer sphere complex prior to anation is proposed.

The electrochemical preparation of manganese(III) solutions is described and the kinetics of oxidation of L-histidine by Mn(III) in H_2SO_4 solutions have been investigated spectrophotometrically.[51] The reaction is first order in Mn(III) and L-histidine concentrations and is retarded by Mn(II) and H^+. The effects of ionic strength, solvent and certain complexing agents on the rate were also investigated.

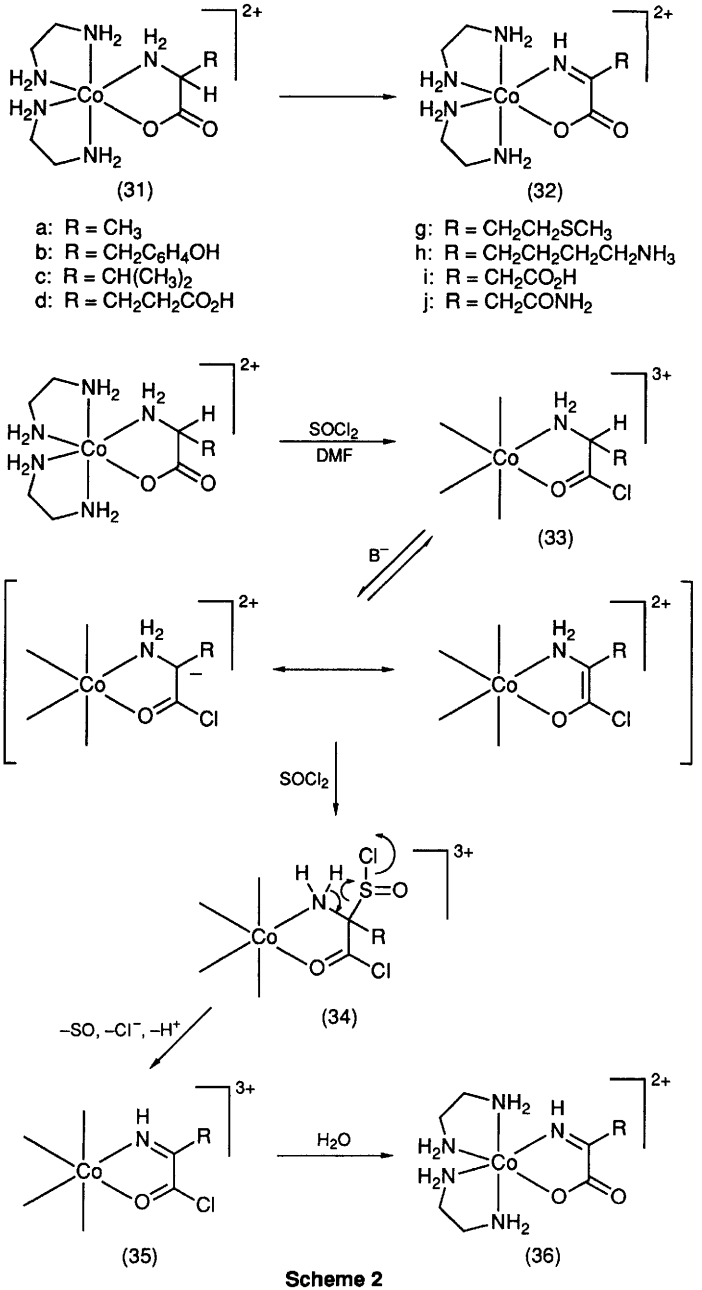
Paramagnetic relaxation rates and shifts of the CH_2 protons have been used to measure rates of complex formation between Fe^{2+}, Co^{2+} and the ligands ethylenediamine, glycinate and malonate in aqueous solution.[52,53] Kinetic evidence for the formation of the species $[Fe(Gly)]^{2+}$, $[Fe(Hen)]^{3+}$, $[Fe(Hmal)]^{+}$, $[Co(Hen)]^{3+}$, $[Co(Hmal)]^{+}$ and $[Co(mal)_3]^{4-}$ has been reported and a carboxyl displacement mechanism is suggested for the reaction of both $M(Gly)_3^-$ species with free glycinate. The kinetics of oxidation of Lys, Arg and His by alkaline $[Fe(CN)_6]^{3-}$ have been studied at T=318-338 K.[54] The reaction follows second order kinetics and proceeds via rate determining formation of an α-imino acid which undergoes hydrolysis to the corresponding α-keto acid.

The kinetics of aquation of $Co(Gly)^{-}$ formed from $Co(Gly)_3$ with hydrated electrons has been investigated by pulse radiolysis.[55] The observed rate constants are given by the expression $k_{obs} = k^o + k^H[H^+]$. For $Co(Gly)_3^-$ $k^o = 4.2x10^3\ s^{-1}$, $k^H = 2.7x10^7\ M^{-1}s^{-1}$ while for $Co(Gly)_2$ $k^o = 3.5x10^2\ s^{-1}$, $k^H = 8.1x10^5\ M^{-1}s^{-1}$ and for $Co(Gly)^+$ $k^o = 49\ s^{-1}$, $k^H = 2.1x10^4\ M^{-1}s^{-1}$.

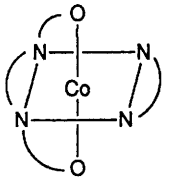
The reaction of thionyl chloride with various amino acid/cobalt(III) complexes **(31)** gives the related imino acidato complexes **(32)** by oxidation, Scheme 2.[56] The mechanism of this reaction involves initial formation of an acid chloride **(33)** followed by reversible deprotonation at the α-carbon and formation of an α-sulfinyl chloride **(34)** by reaction with $SOCl_2$. Loss of SO and HCl gives the chelated α-imino acid chloride **(35)** which is converted to the product **(36)** by hydrolysis. In this reaction the role of the metal ion is crucial. It acts as an N-protecting group, it moderates the reactivity of the carboxylate group and increases the acidity of the α-CH group facilitating formation of the carbanion which is a necessary step in the reaction. This reaction appears to be quite general for chelated amino acids which do not contain highly reactive side chains.

The cobalt(III) promoted hydrolysis of coordinated glycylanilides bearing internal carboxylate and phosphonate substituents is described.[57] While the internal carboxylate group contributes nothing to the rate of hydrolysis the internal phosphonate group is effective. This may be due partly to stereoelectronic effects although the main difference appears to result from the increased basicity of the phosphonate group. It is therefore proposed that phosphate or phosphonate groups are better models than carboxylate for the abnormally basic carboxylates found in enzymes such as carboxypeptidase A.

Isomerisation of the complexes $[Co(edda)en]^+$ and Co(edda)Gly were studied in basic solution. For these complexes two, **(37)** and **(38)**, and three **(39)-(41)**, geometric isomers respectively are possible.[58] For the isomerisation of Λ(R)-β-$[Co(edda)en]^+$ to the Λ(S,S)-α-isomer an optical purity of 45% was observed. In the case of Co(edda)Gly the following optical purities were observed:


(31)
(32)
a: R = CH3
b: R = CH2C6H4OH
c: R = CH(CH3)2
d: R = CH2CH2CO2H
g: R = CH2CH2SCH3
h: R = CH2CH2CH2CH2NH3
i: R = CH2CO2H
j: R = CH2CONH2
SOCl2
DMF
(33)
B−
SOCl2
(34)
−SO, −Cl−, −H+
H2O
(35)
(36)

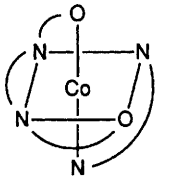
Scheme 2



(37)

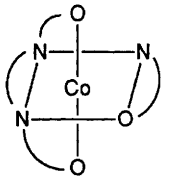
α

$[Co(edda)(en)]^+$



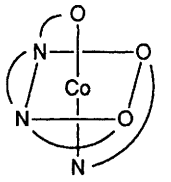
(38)

β



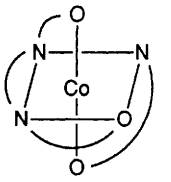
(39)

α–*mer* (O)



(40)

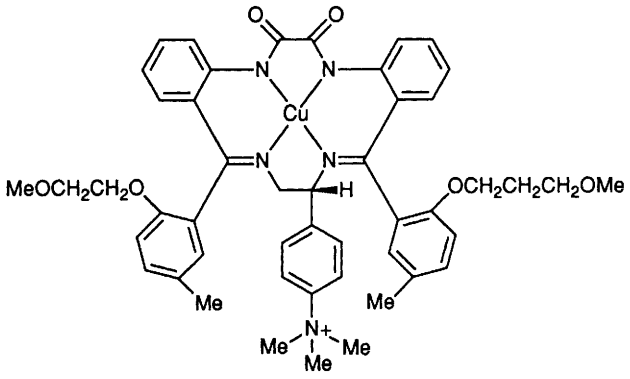
β–*fac* (O)



(41)

β–*mer* (O)

$[Co(edda)(gly)]$



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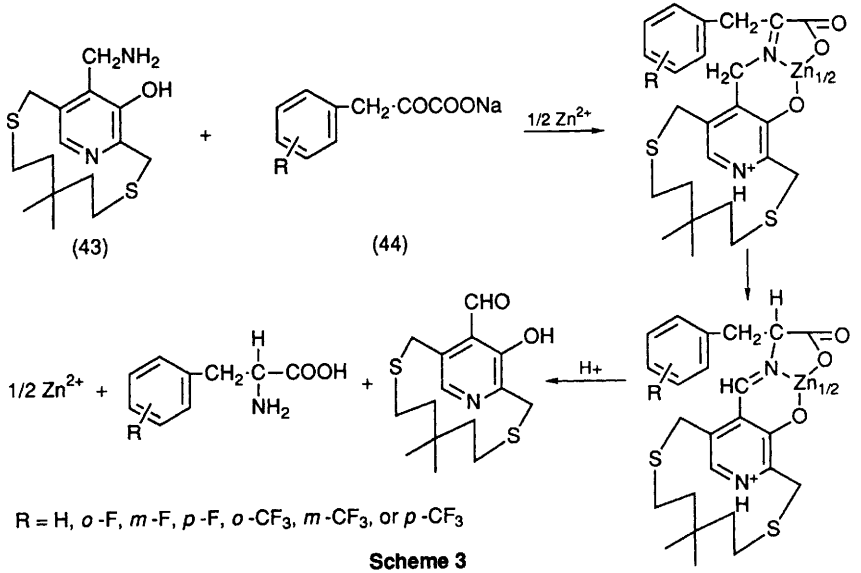


(43)

(44)

R = H, *o*-F, *m*-F, *p*-F, *o*-CF_3, *m*-CF_3, or *p*-CF_3

Scheme 3

Λ(R)-β-fac(O) ⟶ Λ(R)-β-mer(O), 100% op + Λ(S,S)-α-mer(O), 33% op

Λ(R)-β-mer(O) ⟶ Λ(R)-β-fac(O), 65% op + Λ(S,S)-α-mer(O), 40% op

Λ(S,S)-α-mer(O) ⟶ Λ(R)-β-fac(O), 50% op + Λ(R)-β-mer(O), 60%
A mechanism involving Co-O bond rupture is proposed.

Stereoisomerisation reactions of cobalt(III) complexes containing the tetra- or pentamine ligands dien, trien, cyclam and tetraethylenepentamine in aqueous solution were investigated by ^{59}Co n.m.r. spectroscopy.[59] For $[Co(dien)(NO_2)_2NH_3]Cl$ the *mer* geometric isomer predominates while trans complexes with tetradentate ligands are more labile than the *cis*.

Twelve bis(salicylideneglycinato)cobaltate(III) complexes having substituents on the 3,4,5 or 6 positions of the aromatic ring have been synthesised and C-H bond breaking reactions in the glycine gem-methylene protons studied by deuterium exchange.[60] Rates differ for the exchange of the two protons, with ratios varying between 0.81 and 0.47. Rates obey Hammett behaviour and the structural and electronic effects governing selectivity are discussed.

The catalytic oxidation of the methine group of two p-substituted benzoins (OMe, Cl) by air or pyridine N-oxide in the presence of $MoO_2(Cys\text{-}OMe)_2$ and $MoO_2(S_2CNEt_2)_2$ was studied kinetically and the involvement of dioxygen in the catalytic process has been studied using ^{18}O-enriched dioxygen.[61] The catalytic oxidation rates follow the order OMe>H>Cl. Complex formation between Mo(VI) and cysteine was studied in aqueous solution using c.d. and n.m.r. spectroscopy.[62] In neutral or basic solution the only Mo(VI) species present is $[MoO_3Cys]^{2-}$ while in acidic solution a second 1:1 complex resulting from the reaction of this with acid is also present. A single complex $[WO_3Cys]^{2-}$ is formed in aqueous solutions of sodium tungstate and cysteine at pH>6.7.

The copper(II) complex of a macrocyclic ligand (**42**) has been examined as a viable metalloreceptor and carrier for α-amino acid anions.[63] This complex is chiral and contains a strongly bound ligand with both hydrophobic and hydrophilic groups capable of interacting with a weakly coordinated amino acid anion. The apical interaction of Pro with this complex in D_2O at pD 11 was studied using n.m.r. relaxation techniques and structural information regarding interatomic distances deduced. The ability of the complex to act as a phase transfer carrier for the anions of Phe, Leu, Pro or Pro-OH is also reported.

The reaction of $Cu(Gly)_2$ with methyl free radicals in aqueous solution gives an intermediate $(Gly)_2Cu^{III}\text{-}CH_3$ species which decomposes to short lived $Cu(Gly)_2^+$ and methane.[64]

Transamination reactions between the pyridoxamine analogue (R)- or (S)- 15-aminomethyl-14-hydroxy-5,5-dimethyl-2,8-dithia[9]pyridinophane (**43**), which has planar chirality and o-, m-, or p- fluoro or trifluoromethyl-phenylpyruvic acid (**44**) gave in the presence of Zn(II) moderate yields of the corresponding substituted phenylalanines, with 33-66% enantiomeric excess, Scheme 3.[65] The rate constants for these transamination reactions were determined and found to obey the Hammett relationship.

The reactions of cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ with 2-aminomalonic acid (Ammal), Asp and Glu have been studied by ^1H, ^{13}C, ^{195}P and ^{15}N n.m.r.[66] Asp and Glu initially give carboxylato bonded complexes such as cis-$[Pt(NH_3)_2(H_2Asp\text{-}O)H_2O]^{2+}$ in which at pH<2 the α-COO^- groups are bonded predominantly but at pH 4-5 both COO^- groups are involved to the same extent. At pH 1.5 over a 2-3 day period N,α-COO^- chelates such as $[Pt(NH_3)_2HAsp\text{-}N,O]^+$ form. With Ammal the O,O chelate $[Pt(NH_3)_2Ammal\text{-}O,O]^+$ is initially formed but over 2-3 days at pH 1.5 this converts to a 5 membered N,O chelate $[Pt(NH_3)_2Ammal\text{-}N,O]^+$. In acidic solutions this undergoes decarboxylation to $[Pt(NH_3)_2Gly\text{-}N,O]^+$. In the above complexes the uncoordinated carboxylate groups react with excess cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ to give binuclear complexes.

In order to investigate the interaction of organic arsenicals with biological sulfhydryl containing molecules the reaction of phenyldichloroarsine (PDA) with L-cysteine was studied in d^4-methanol.[67] The adducts PhAs(Cl)Cys and $PhAs(Cys)_2$ both containing S-coordinated cysteinate were obtained in solutions containing 1:1 and 2:1 mole ratios of reactants respectively.

2.3 Formation Constants.- Complex formation between Ca(II) and the amino acids glycine, DL-alanine, β-alanine and DL-aspartic acid was investigated potentiometrically using calcium ion selective and glass electrodes.[68] ^{14}N and ^{17}O n.m.r. spectra were also obtained for the Gly and Ala complexes in order to ascertain the ligand binding sites. In the case of these ligands the only species detected was CaL^+ in which the amino acids act as bidentate N,O donors. In the case of Asp the species $Ca(HX)^+$ and CaX where H_2X represents Asp were identified.

The interaction between VO^{2+} and L-aspartic acid in aqueous solution $1.5 \leq pH \leq 11$ has been studied by potentiometric and spectroscopic (e.s.r., electronic absorption and c.d.) methods and formation constants and spectra are reported for the species $VOLH_2$, VOLH, VOL, VOL_2H_3, VOL_2H, VOL_2 (LH_2 = Asp) and several hydrolysis products.[69] A similar study of the VO^{2+}/L-cysteine and D-penicillamine (H_2L) systems at pH 1.8-13.5 identified the species $VOLH_2$, VOL_2H_4, VOL_2H_2, VOL_2H, VOL_2, VOL_2H_{-1} and $(VO)_2L_2$.[70] Plausible isomeric structures for each of the stoichiometries are presented.

Equilibrium constants, equation 1, have been determined for mixed ligand complexes of cobalt(III) which contain a D-amino acid, (AA = Gly, Ala, Val, Leu, Thr, Phe, Trp, Pro, Asp,

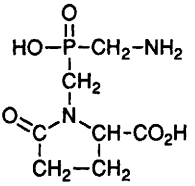
Asn, Glu) and a tetradentate Schiff base ligand derived from salicylaldehyde and R,R-1,2-cyclohexanediamine, sal-R,R-chxn, equation (1).[71]

$$\text{trans-[Co(sal-R,R-chxn)(H}_2\text{O)}_2\text{]}^+ + \text{AA} \overset{K_1}{\rightleftharpoons} \Delta\text{-}\beta_2\text{-Co(sal-R,R-cyhxn)AA} \quad \ldots.(1)$$

The fact that the values of K_1 for the Phe and Trp complexes are much greater than for the other amino acids is attributed to interligand stacking involving the aromatic groups of the Schiff bases and the amino acids.

Complexes of amino acid and peptide hydroxamic acids in aqueous solution have also been the subject of a thorough investigation. A combination of pH-metric and ^{13}C n.m.r. spectroscopy has been used to determine the microscopic and macroscopic pK_a values for α-, and β-alaninehydroxamic acids.[72] Formation constants and likely bonding modes are reported for complexes of cobalt(II), nickel(II), copper(II), zinc(II) and iron(III) with D,L-aspartic acid-β-hydroxamate.[73] The species $M(HA)^+$ with the exception of Fe(III) contain NH_2, CO_2^- bonded bidentate ligands which on deprotonation give the species MA in which the ligand is tridentate involving the hydroxamate nitrogen in addition to the above sites. Iron(III) forms 1:1 and 2:1 complexes in which the ligand is tridentate via the hydroxamate and carboxylate oxygens. Complexes of histidinehydroxamic acid with copper(II) both with and without histidine as co-ligand have been investigated by a combination of potentiometric and e.s.r. methods.[74] Protonation and complex formation equilibria have been investigated for the ligand 2-amino-N-hydroxy-n-butamide using potentiometric and spectrophotometric methods.[75] The complexes studied were those of Co(II), Ni(II) and Cu(II).

The biological activity of phosphonic and phosphinic derivatives of essential amino acids in many cases results from inhibition of metalloenzymes having amino acid substrates. In order to assess the complexation ability of aminophosphinates a detailed study has been carried out on the protonation (microscopic and macroscopic) and copper(II) complex formation equilibria involving the N-phosphorylmethylated derivatives of 5-oxo-L-proline (**45**) and leucine (**46**) at 25°C, I = 0.2 mol dm^{-3} KCl using a combination of pH metric and spectrophotometric (visible, e.s.r. and n.m.r.) techniques.[76] Both ligands were found to be ambidentate with complex formation occurring at the amino acid end initially giving $[Cu(HA)]^+$ an N,O chelate in the case of the leucine derivative (**46**) but a monodentate O bonded complex in the case of the oxoproline derivative (**45**). The aminophosphinate group complexes at higher pH giving CuA, in which (**45**) acts as an N,O bidentate ligand and (**46**) as a 2N,2O tetradentate ligand. At higher pH the species $Cu(A)OH^-$ and CuA_2^{2-} have been detected. The greater denticity of the leucine residue relative to the oxoproline residue makes the former the better complexing agent of the two towards copper(II).



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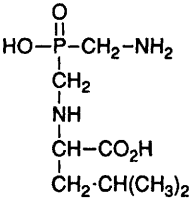

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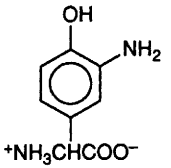

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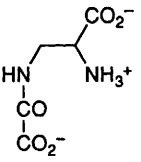

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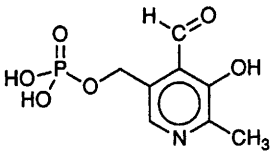

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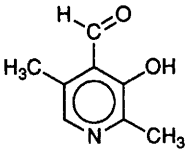

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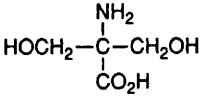

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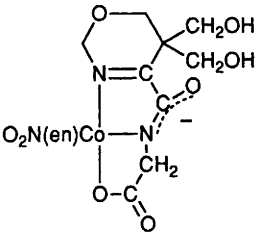

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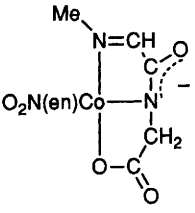

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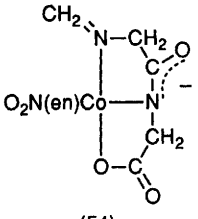

(54)

The tyrosine derivative 3-amino-L-tyrosine (**47**) is formed in the degradation of pheomelanin in living organisms and is known to exert antibacterial and antifungal activity. The macroscopic and microscopic pK_a values of this compound and its complex formation constants with copper(II) have been determined at 25°C, I = 0.2 mol dm^{-3} KCl.[77] The ligand shows ambidentate properties forming monomeric aminocarboxylate and aminophenolate type complexes as well as dimers involving both metal coordination sites. In the complex the ligand is tridentate using the amino and carboxylate coordination sites. Potentiometric and calorimetric methods have been used to investigate acid base and complex formation equilibria involving α-aminomalonic acid $NH_2CH(CO_2H)_2$.[78] In the copper(II) complex this amino acid acts as an N,2O tridentate ligand. The non protein amino acid β-N-oxalyl-L-α,β-diamino propionic acid (**48**) which occurs in the seed of Lathyrus Sativus is a neurotoxin which causes a spastic condition affecting the lower limbs. The compound has been isolated from the seed in the presence of added copper(II) and its coordination chemistry in the presence of copper(II) and zinc(III) has been investigated.[79]

As a model for metal ion assisted molecular recognition the thermodynamic selectivity of mixed ligand copper(II)-histidine complexes with various L-amino acids have been examined.[80] Hence formation constants have been determined for ternary complexes of copper(II)/D or L-histidine with the amino acid ligands Gly, L-Ala, L-Val, L-Leu, L-Trp, L-Phe at 25°C, I = 0.1 mol dm^{-3}. Ternary complexes in which the amino acids have aromatic side chains are more stable if the ligands are of opposite chirality; the opposite is the case for aliphatic amino acids. Enthalpies and entropies of complex formation were also obtained. Ternary systems of copper(II)-histamine with L-Ala or L-Phe have also been investigated. Linear thermodynamic relationships have been found between enthalpy changes accompanying the formation of the ternary complexes copper(II) / 5-substituted 1,10-phenanthrolines/α-amino acid ligands (2-Me-Ala, Ile, Val, Ser) and those for protonation of the ligands.[81] Similar relationships have been established for binary and ternary complexes of Cu(II) with N-p-substituted phenyliminodiacetic acids and amino acids (L-Pro, L-Ile, L-Val, L-Ser, Gly, 2-aminoisobutyric acid) as coligands.[82]

Binary and ternary complex formation between copper(II), D- or L-alanine and a chiral polymer of (-)-trans-1,2-diaminocyclohexane (dachx) which is used as a chromatographic resolving agent has been investigated by e.s.r. and potentiometric methods.[83] Below pH 5 a binary 1:1 complex is formed but at pH>8.5 the predominant species is a ternary species similar to that formed in the dachx-Cu(II)-L-Ala and en-Cu(II)-Gly systems. Both dachx and polydachx act as N,N chelating agents towards copper(II) and these complexes show no selectivity in their binding to the enantiomers of alanine.

Binary and ternary complex formation equilibria involving copper(II) and the amino acids Gly or Ala and the dipeptide Gly-Gly in 5.0 M NaCl solutions have been investigated.[84] Formation constants of N-alkyl-β-alanine complexes of copper(II) have been determined by potentiometry (H^+, Cu^{2+}) and found to increase with alkyl chain length up to n-butyl.[85]

Metal complexes of Schiff base ligands derived from pyridoxal-5'-phosphate have been investigated as model systems for the complexes formed anaerobically in vitamin B_6-amino acid systems.[86] The Schiff bases were formed from the vitamin B_6 derivatives, pyridoxal-5'-phosphate, **(49)**, and 5'-deoxypyridoxal, **(50)**, with arylglycines (phenyl, p-methoxyphenyl and p-sulfophenyl) as amino acid substrates. Protonation constants for these synthetic amino acids are reported and also the stability constants of their 1:1 and 2:1 complexes with Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}. Both pyridoxal derivatives form 1:1 metal complexes. Schiff base formation constants and stability constants for the 1:1 and 2:1 complexes of the Schiff base ligands with the above metal ions are reported.

The interaction of zinc(II), calcium(II) and magnesium(II) with 3,6,9,12-tetraazadecanedioic acid, a ligand which when complexed to copper(II) shows potential as an antirheumatic drug, in aqueous solution at 25°C, I = 0.1 mol dm^{-3}, has been investigated by potentiometric and n.m.r. spectroscopic methods.[87] From the results the effect of this ligand on blood plasma metal ion distribution in vivo has been examined by computer simulation.

Stability constants of complexes of amino acids with the cationic water soluble porphyrin tetramethylpyridiniumporphyrin and its zinc(II) derivative have been determined by ^1H n.m.r. spectroscopy at pH 10.5.[88] The amino acid-metalloporphyrin complexes are stabilised by stacking or electrostatic interactions and stability constants follow the order -

Tyr > Phe ~ Glu > Asp > Ile > Val > Gly.

The interactions of the amino acids with the free base porphyrins follow the order -

Phe < Tyr < Trp.

In order to examine the factors which influence sulfur binding to cadmium(II) in proteins such as metallothioneins and phytochelatins, complexes of this metal with 15 sulfur containing amino acid and peptide ligands in aqueous solution were investigated by potentiometric and polarographic methods.[89] While thiol groups such as those in cysteine and D-penicillamine are the most effective donors towards cadmium(II) thioamide groups also appreciably stabilise the complexes formed. Generally stability constants obey the following order of donor sets -

(S,N,O) > (S,N) > (S,O,O) > S,O.

In the presence of amino acid and peptide binding sites the disulfide group is ineffective in complexing to cadmium(II).

Stability constants have been determined for complexes of Mg^{2+}, Ca^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} with L-Ser and L-Leu at 298K in ethanol-water media.[90] Formation constants have also been determined for the mixed ligand complexes Cd(AA)imidazole where AA = Gly, Ala or Val and compared with those for the binary complexes of cadmium(II) with both these ligands.[91]

3 Peptide Complexes

Metal peptide complexes continue to attract considerable attention and an interesting selection of papers has appeared.

3.1 Synthesis, Structure and Reactivity.- There is considerable current interest in the molecular species involved in the carcinogenicity and mutagenicity of chromium(VI). It is generally accepted that chromate ion CrO_4^-, the dominant form of chromium(VI) in neutral solution, can readily cross cellular membranes via non-specific anion carriers. A material which analyses as $Na_4Cr(GSH)_4.8H_2O$ (GSH = glutathione) can be reproducibly precipitated from the reaction of glutathione with chromate.[92] Spectroscopic evidence suggests that this is predominantly a chromium(V) complex of glutathione, involving carboxylate and thiolate coordination to the metal. Polarographic and e.s.r. data obtained for the reduction of chromate in the presence of glutathione and sugars has also been studied.[93] The results indicate that in the binary GSH-Cr(VI) system, glutathione binds chromate forming a thioester species which can be reduced by free tripeptide. In systems containing chromate, sugars and glutathione, chromium(VI) interacts with the sugar (or with sugar and GSH) to give esters which are readily reduced by GSH. The Cr(V) so formed is then stabilised by coordination to the sugar. Sugars having pairs of *cis*-hydroxyl groups are the most effective in the formation firstly of Cr(VI) esters and then Cr(V) complexes.

Cobalt(III) complexes with dipeptides containing an L-methionine residue, $[Co(dipeptidato\text{-}N,N,O)_2]^-$,$[Co(dipeptidato)(diamine)]^+$ and *cis*-$[Co(dipeptidato)(NH_3)_2]^+$ have been prepared where dipeptidate is L-methionyl-glycinate, glycyl-L-methioninate, L-methionyl-L-alaninate or L-alanyl-L-methioninate, and diamine is 1,2-diaminoethane or 1,3-diaminopropane.[94] In the diamine and diammine complexes, the dipeptide is quadridentate via the NH_2 group, peptide N, CO_2^- and the sulphur atom. The 500MHZ ^1H n.m.r. spectra indicate that the N-S chelate rings of the L-Met residue adopt a chair conformation and the S-methyl groups have the S(S) configuration for the C-terminal L-Met.

The reaction of formaldehyde under basic conditions with glycine coordinated to cobalt(III) gives the corresponding complex of α-(hydroxymethyl)serine (**51**).[95] Three new products (**52**) - (**54**) result from the reaction of *mer*-$Co(NH_2CH_2CONCH_2CO_2)NO_2(en)$ with formaldehyde in basic solution.[96] The structure of (**52**) has been confirmed by X-ray crystallography. The 1,3-oxazine derivative is tridentate, coordinated via the carboxylato oxygen, peptide nitrogen and imino nitrogen atoms.

Multinuclear (^{15}N, ^{195}Pt, ^1H, ^{13}C) n.m.r. spectroscopy has been used to study the reactions of *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ with $GlyNH_2$, Gly-Gly and Gly-Gly-Gly.[97] With glycinamide near pH 5 the N,O-chelate $[Pt(NH_3)_2(NH_2CH_2CONH_2)]^{2+}$ is formed. Attempts to deprotonate this complex with base leads to rapid hydrolysis to $[Pt(NH_3)_2(NH_2CH_2CO_2)]^+$ and

NH_3. With glycylglycine the initially formed complex was *cis*-$[Pt(NH_3)_2(H_2Gly\text{-}Gly\text{-}O)(H_2O)]^{2+}$ in which the ligand is bound only via the carboxyl oxygen. When the solution was allowed to stand near pH 5, the complex $[\{Pt(NH_3)_2\}_2(digly)]^{2+}$ is formed, in which one *platinum* is bound to the ligand via CO_2^- and peptide nitrogen and the second platinum is chelated by the peptide oxygen and NH_2 group. The crystal structure of $[\{Pt(NH_3)_2\}_2(digly)](SO_4)1.35H_2O$ has been determined and confirms this stoichiometry. With excess glycylglycine near pH 4, n.m.r. data indicates a complex in which the terminal carboxylate and peptide nitrogen chelate to $Pt(NH_3)_2$. In strongly acidic solution (pH<1) this complex converts to another N,O-chelate in which terminal nitrogen and peptide oxygen coordinate. The peptide bond in the latter complex slowly hydrolyses in acid to give $[Pt(NH_3)_2(Gly\text{-}N,O)]^+$. The complex initially formed in the case of triglycine is *cis*-$[Pt(NH_3)_2(H_3Gly\text{-}Gly\text{-}Gly\text{-}O)(H_2O]^{2+}$ which then converts to the trinuclear species $[\{Pt(NH_3)_2\}_3(Gly\text{-}Gly\text{-}Gly)]^{3+}$, with three platinum atoms bound via N,O-chelate rings.

The reactions of $PtCl_4^{2-}$ with oligoglycyl peptides in aqueous solution proceeds by amine coordination, followed by sequential deprotonation and coordination of available peptide nitrogens.[98] Complexes of $[PtLCl_3]^{2-}$, $[Pt(H_{-1}L)X_2]^{2-}$, $[Pt(H_{-2}L)X]^-$ and $[Pt(H_{-3}L_4)]^{2-}$ where L is triglycine (G_3), triglycinamide (G_3a) or tetraglycine (G_4), X is Cl^- or OH^- and H_{-n} designates the number of deprotonated peptide groups were characterised by use of ^{195}Pt n.m.r.. Several bis(peptide) complexes were also identified.

A number of electron transfer reactions involving peptide complexes have been investigated. The rates of electron transfer reactions can vary by more than 18 orders of magnitude from subpicoseconds to several hours or days. Recent studies have focussed on intramolecular electron transfer reactions where the electron transfer step in a donor-acceptor complex occurs without complications from diffusion and other molecular interactions. A series of binuclear $[(NH_3)_5Os(Pro)_nCo(NH_3)_5]^{5+}$ complexes have been prepared (n = 0-4).[99] Long range intramolecular electron transfer reactions in these polypeptides was studied by the formation of the $Os^{II}(Pro)_nRu^{III}$ precursor complexes using reducing radicals (CO_2^- and e^-_{aq}) generated by pulse radiolysis techniques. For the n=0 complex, the intramolecular electron transfer rate was very fast (k ca. $5 \times 10^9\ s^{-1}$) at 25°C. For the n=1-3 complexes, the rate constants and activation parameters for electron transfer were determined as $3.1 \times 10^6\ s^{-1}$ ($\Delta H^{\dagger}$=4.2 kcal mol^{-1}; $\Delta S^{\dagger}$=−15 e.u.) $3.7 \times 10^4 s^{-1}$ ($\Delta H^{\dagger}$=5.9 kcal mol^{-1}; $\Delta S^{\dagger}$=−19 e.u.) and $3.2 \times 10^2 s^{-1}$ ($\Delta H^{\dagger}$=7.4 kcal mol^{-1}; $\Delta S^{\dagger}$=−23 e.u.) while for the n=4 complex, $k=50\ s^{-1}$ at 25°C. The results indicate that rapid rates of electron transfer across polypeptides can be observed for a metal-metal separation of > 20Å. Fast rates of electron transfer over a metal-metal distance of 40Å are predictable, if the driving force and reorganisational energy are appropriately controlled.

The reactivity of $O_2^{\cdot-}$ towards copper(II)-peptides containing glycine and histidine for which E^o for Cu(III))/Cu(II) ≤ 1.08V has been studied.[100] The ability of the various complexes to

catalyse the dismutation of $O_2^{\cdot-}$ ($2O_2^{\cdot-} \longrightarrow O_2 + O_2^{2-}$) depends inversely on the redox potential of the Cu(III)/Cu(II) couple. Copper(II)-peptides containing histidine which have higher redox potentials than that of the couple O_2^-/H_2O_2 do not catalyse the reaction. Although direct evidence was not obtained for the formation of the copper(III)-peptide, the results obtained suggest that catalysis involves alternate oxidation and reduction of the metal by $O_2^{\cdot-}$.

The selective recognition of nucleic acids by proteins requires direct interactions between the chemical groups constituting each of the two macromolecules. However, indirect interactions mediated either through space or via metal ions could also be involved in the formation of protein-nucleic acid complexes. The formation of ternary copper(II) complexes of α-amino acids and dipeptides with adenosine has been studied by electronic and e.s.r. spectroscopy.[101] A characteristic difference between Gly-Pro and other dipeptides is attributed to the lack of the peptide proton in Gly-Pro. It is suggested that, at near physiological pH values, the nucleoside binds at an equatorial site by displacing a water molecule from the copper(II) ion.

FAB and tandem mass spectrometry has been used to study the gas-phase interactions of lithium ions and dipeptides.[102] Lithiated dipeptides decompose as metastable ions producing two amino acid ions, those corresponding to the N-terminus and the C-terminus. The interactions of sodium and potassium ions with peptides are similar, however, the lower polarising power of K^+ dramatically reduces the formation of the N-terminus amino acid ion.

3.2 Formation Constants, Species in Solution.- Complexation of Cu(II), Ni(II) and Co(II) with four L,L-dipeptides containing weakly or non-coordinating side chains (Phe-Leu, Leu-Phe, Phe-Met and Met-Phe) has been studied by potentiometric, calorimetric and spectroscopic measurements.[103] For species $[MH_{-1}A]$ (A denotes the conjugate base form of the ligand) an increase in stability is observed with respect to glycylglycine or dipeptides containing one non-glycine residue. This effect is attributed to the hydrophobic interactions between the non-coordinating side chains. Another stabilising effect is observed with a C-terminal Phe residue, which is attributed to the interaction between the metal ion and the aromatic ring. The enthalpy of this non-covalent effect is evaluated as -9.5 kJ mol^{-1} and is not observed with N-terminal Phe residues. Spectroscopic measurements (e.s.r. and u.v.-visible) suggest the presence of a CuN_2O_2 chromophore in $[CuH_{-1}A]$ and formation constants follow the order Cu(II)>Ni(II)>Co(II). Cobalt(II) does not deprotonate the peptide below pH 8. For a given species for example [MA], the complexes with the three transition metals appear to adopt a common structure.

The synthesis of the tetrapeptides Ala-Gly-Gly-His, Boc-Ala-Gly-Gly-His, Ala-Gly-Gly-BomHis (Bom = N^{π}-benzoxymethyl), Ala-Gly-Gly-HisOMe and Ala-Gly-Pro-His has been described,together with the results of a potentiometric and spectroscopic (electronic, c.d.

and e.s.r.) study of their complexes with H^+ and Cu(II).[104] The results show that the π-N of the imidazole ring of the histidyl residue is the primary anchoring site for copper(II) coordination, and that the next nitrogen to bond can be the terminal N, forming a macrocyclic chelate ring.

Recent investigations have shown that the formation of peptides from their amino acid constituents takes place in aqueous solution in the presence of 3-5M NaCl and 0.4-0.8M Cu(II).[105] As a result the complex equilibria between the copper(II) ion and the amino acids glycine, alanine and the peptide glycylglycine have been studied in aqueous solution where condensation of glycine to peptides has been observed.[106] The species distribution under these conditions suggests that copper(II) is already complexed by chloride and glycine at very low pH values. The presence of chloride in the complexes and its influence on complex stability is discussed in detail. A potentiometric study with some supporting spectroscopy (u.v.-visible, c.d. and e.s.r.) has been made on copper(II) complexes of L-Phe-L-Tyr, L-Tyr-L-Phe, L-Lys-L-Tyr and L-Tyr-L-Lys at 25°C and I = 0.2 mol dm^{-3} KCl.[107] In addition to the metal-ligand coordination characteristic of simple peptides, there are interactions between copper(II) and the side-chain phenolate group of the tyrosine residue and/or the ε-amino group of the lysine residue. In these dimeric complexes, both the lysine and the tyrosine moieties can act as bridges between monomeric complexes.

The formation constants of the ternary complexes [Cu A(L)], where A refers to the monoanions of glycylglycine, glycyl-L-alanine or glycyl-L-leucine and L to the dianions of catechol, pyrogallol, 4,5-dihydroxybenzene-1,3-disulphonic acid or naphthalene-2,3-diol have been determined by potentiometric titration at 30°C and I = 0.2 mol dm^{-3} $NaClO_4$.[108] The enhanced stability of the ternary complexes is attributed to hydrogen bonding between the two ligands via a water molecule. Ternary complexes involving dipeptides and substituted catechols provided a basic model system for the enzyme laccase which catalyses the oxidation of o- and p-dihydroxyphenols to quinones.

Tyrosine is a constituent of many neuropeptides and appears to play a fundamental role in the activity of these compounds. Recent studies on tyrosine containing oligopeptides has established that the direct participation of the side chain phenolate groups in metal ion binding depend on the position of tyrosine in the peptide molecule. A recent paper discusses proton and copper binding in Tyr-Tyr and Tyr-Tyr-Tyr.[109]

Hydroxamic acid derivatives of amino acids and peptides can inhibit metalloproteinases. Complexes of Co(II), Ni(II), Cu(II), Zn(II) and Fe(III) with Pro-Leu-NHOH and Pro-Leu-Gly-NHOH have now been studied by potentiometric, spectrophotometric and e.s.r. methods.[110] Complexes of moderate stability are formed in the systems containing Co(II) and Zn(II) in the pH range 6.0 - 8.5, where there is no deprotonation of the peptide nitrogen. Stable complexes are formed in the copper(II)/Pro-Leu-NHOH and Pro-Leu-Gly-NHOH systems above pH 4. It is suggested that the hydroxamate nitrogen, peptide carbonyl oxygen and terminal amino

groups are initially involved as donors in the Cu(II)/Pro-Leu-NHOH complex. Coordination is primarily "hydroxamate-like" in the copper(II)/Pro-Leu-Gly-NHOH system in this region. Deprotonation of the peptide nitrogen occurs in both systems, after which only the nitrogen donor atoms (amino, amide and hydroxamate) are involved in coordination. Planar complexes predominate above pH 6 with nickel(II). In the Fe(III) systems, complex formation is appreciable even below pH 3.

Cadmium complexes with 15 sulphur containing amino acid and peptide ligands have been investigated by potentiometric and polarographic methods.[89] Thiol donors were the most effective in cadmium binding, following the order (S,N,O)>(S,N)>(S,O,O)>(S,O) donor sets. Thioamide groups also enhance the stability of the complexes formed, while the disulphide group is ineffective in the presence of amino acid or peptide binding sites.

The mechanism of action of Cisplatin, *cis*-$PtCl_2(NH_3)_2$, as an antitumour agent is believed to be due to the interaction of the $Pt(NH_3)_2^{2+}$ moiety with the nucleobases of DNA. A limitation of *cis*-platin in its use as an antitumour drug is its concentration-dependent nephrotoxicity apart from a variety of other side effects. The nephrotoxicity can be reduced by using the reagents sodium diethyldithiocarbamate Na(ddtc) or thiourea. Borch et al.[111] have sugested that nephrotoxicity is due to the inactivation of certain enzymes due to the binding of the metal to the -SH groups of cyteine residues. Na(ddtc) and thiourea may be effective by removing the Pt from the sulphur atoms, restoring the original structure of the enzyme. Model adducts for platinum-protein binding i.e. at cysteine and methionine sites have been synthesised starting from [PtCl(dien)]Cl, *cis*-$[Pt(NH_3)_2Cl_2]$, *trans*-$[Pt(NH_3)_2Cl_2]$ and $[Pt(NH_3)_3Cl]Cl$. Glutathione (GSH) and S-methylglutathione (GS-Me) were used to mimic the sulphur atoms in the proteins.[112] At pH 11 both *trans*-$[Pt(NH_3)_2Cl_2]$ and $[PtCl(NH_3)_3]^+$ form *trans*-$[Pt(NH_3)_2(GS)_2]$ upon reaction with two equivalents of GS. Only the intermediate $[Pt(NH_3)_3GS]Cl$ was found to be relatively stable. The Pt-sulfur bonds in $[Pt(dien)GS]^+$ and *trans*-$[Pt(NH_3)_2(GS)_2]$ could not be broken by sodium diethyldithiocarbonate or thiourea. However the methylglutathione complexes $[Pt(dien)(GS\text{-}Me)]^{2+}$ and *cis*-[Pt(GS-Me)] react rapidly with Na(ddtc) ($t_{1/2}$<2mm) and more slowly with thiourea ($t_{1/2}$ = 30mm-2 hr). It thus appears that Na(ddtc) and thiourea are only effective in removing platinum from methionine-type sulfur.

A new series of mixed cobalt(II) complexes with dipeptides (Gly-Gly, Gly-L-Leu, L-Ala-Gly and L-Ala-L-Ala) and imidazole have been found to be dioxygen carriers.[113] The oxygen-free, high spin octahedral form of the active complex Co(Himid)(dipeptH_{-1}), the reversible dimeric complex with a μ-peroxo bridge as the oxygenated form, and the irreversible complex with a μ-superoxo bridge have been studied by u.v.-visible, near i.r., potentiometric and gas volumetric methods. Molecular structures have been proposed for each species.

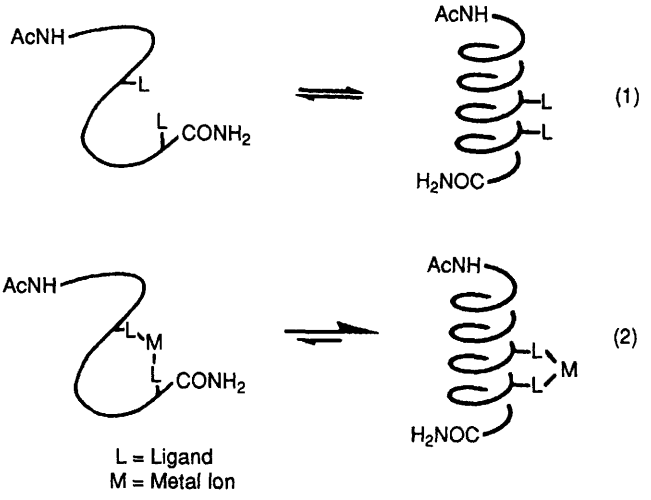

Figure 1 *The coil-to-helix equilibrium of a peptide (Equation 1) bearing two side chains capable of metal coordination should in theory be shifted to the right by simultaneous coordination to a single metal (Equation 2), the result of reduction of entropy of the metal-coordinated coil conformation*
(from *J. Am. Chem. Soc.*, 1990, **112**, 9403)

Many oligopeptides show very high biological activity, particularly in the central nervous system where many of them act as releasing factors, neurotransmitters or as opiates. Previous work has indicated that specific interaction of copper(II) with proline or/and tyrosine residues in oligopeptides can influence the conformation of the peptide, promoting the formation of β-turns.[114] Such β-turns are known to be particularly important in the biologically active conformations of many peptides. The α-helical conformation adopted by 40% of all residues in proteins is not, in isolation, energetically favoured, as indicated by the existence of most short peptides in aqueous solution as random coils. Protein helices and rare helical peptides apparently owe their existence to exogenous stabilising factors.[115] A recent paper reports on the use of metal ions as peptide side chain "cross-linking" agents.[116] For peptides containing metal-ligating residues, the position of the coil-to-helix equilibrium is strongly dependent on the number and spacing of ligating residues, the tether length between backbone and ligand, and the metal ion, Fig 1. In one remarkable case, an 11-residue peptide is converted from random coil to ca 80% helix content by the addition of Cd(II) at 4°C. The use of an exchange-inert Ru(III) complex *cis*-$[Ru(NH_3)_4L_2]^{3+}$ (L_2 are the side chains of two histidines in positions i and i+4 of a peptide) for constraining the intervening chain in an α-helical conformation and effecting helix nucleation has been described.[117] A 17-residue polypeptide functionalised in this way has a melting temperature of 35°C and exhibits 80% α-helicity at 21°C. The use of labile transition metal complexes in the formation of a-helical peptides has also been described.[118]

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