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Amino Acids and Peptides

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Amino Acids and Peptides
Volume 24

A Review of the Literature Published during 1991

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Preface

In taking over after Dr John Jones's 10-year tenure as Senior Reporter, the philosophy has been to preserve continuity through reliance on the expertise of authors who have done this series proud over many years. The experience of having to read each author's manuscripts has highlighted again the unstinting efforts of my colleagues in reviewing such a wide 'swath' of papers and moulding them into useful treatises. Sincere thanks, therefore to Graham Barrett, Donald Elmore and Colin Frydrych for agreeing to soldier on, and to Christine Bladon for accepting and fulfilling the challenge of producing her first Chapter in this series. Continuity for the Chapter on metal complexes of amino-acids and peptides had also been planned, but pressure of work on Professors Nolan and Hay necessitated the decision to include their Chapter as a biennial contribution which will now appear next year.

The year 1991 saw consolidation rather than any quantum leaps in development in most of the areas reviewed. Quite detailed probing of biological systems with high field nmr is now possible and in the field of molecular recognition involving immunosuppressive agents, it has prompted an eminent practitioner to state that "the fog is slowly lifting on the understanding of immunosuppression". Over the years, within the criteria of purity used for inclusion of peptide syntheses in these Volumes, the size of peptides have continued to increase. But the success of the solid phase approach has seen the burgeoning of techniques which enable "multiple peptide syntheses" to be carried out. 'Biologically-required' sequences are selected from the products of "multiple peptide synthesis". This will inevitably demand editorial consideration soon to ascertain whether our criteria for reviewing these developments will have to be re-defined.

The trend towards larger peptides has increased the use of single letter amino acid abbreviations to report the peptide sequences. As a service to all of us who still find the occasional letter designation elusive, a list has been included in the Abbreviations section. The structures of the closely related coupling agents developed as alternatives to the BOP reagent are also included as the trend is to refer to them only by their abbreviations.

Finally, sincere thanks to John Jones for his 10-year tenure and to Colin Frydrych who has intimated his wish to make this his last Chapter for the present.

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Abbreviations

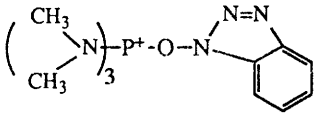
The abbreviations for amino acids and their use in the formulation of derivatives follow in general the 1983 Recommendations of the IUB-IUPAC Joint Commission on Biochemical Nomenclature, which were reprinted as an Appendix in Volume 16 of this series. Chapter authors have been encouraged to include new abbreviations in their texts.

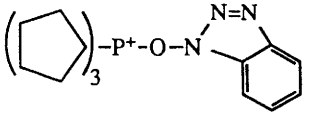
The synthetic peptide chemist has over the years been a strong supporter of the three-letter amino-acid abbreviation. However, with the increasing sizes of the peptides being produced the single-letter code has found increasing use so below is a timely reminder of the single-letter code, listed in alphabetical order.

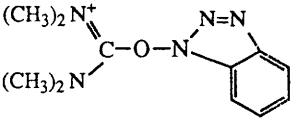
Amino Acid (3-letters)	*Single Letter*	*Amino Acid* (3-letters)	*Single Letter*
Ala	A	Leu	L
Arg	R	Lys	K
Asn	N	Met	M
Asp	D	Phe	F
Cys	C	Pro	P
Gln	Q	Ser	S
Glu	E	Thr	T
Gly	G	Trp	W
His	H	Tyr	Y
Ile	I	Val	V

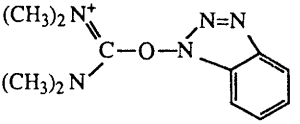
Acronyms for coupling agents are spreading fast and the recent development of "BOP" analogues highlights the trend. On the next page is listed the structures of some recently developed reagents.

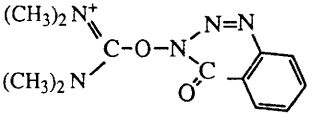
Structures of some recently developed coupling reagents

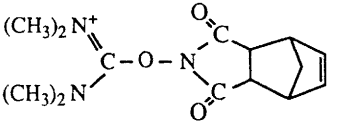
BOP  · PF_6^-

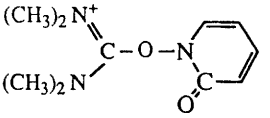
PyBOP  · PF_6^-

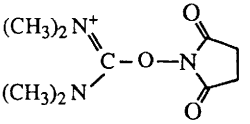
HBTU  · PF_6^-

TBTU  · BF_4^-

TDBTU  · BF_4^-

TNTU  · BF_4^-

TPTU  · BF_4^-

TSTU  · BF_4^-

1
Amino Acids

BY G.C.BARRETT

1 Introduction

The chemistry and biochemistry of the amino acids, as featured in the 1991 literature, is reviewed in this Chapter. The targeted material could be categorized as the occurrence, chemistry, and analysis of the amino acids, and with the exclusion of routine literature covering the natural distribution of well-known amino acids. As before, the term 'amino acids' is taken to mean [ω]-amino-alkanoic acids, and there is therefore no coverage of amino-phosphonic, -sulphonic, -boronic acids and others of these types.

There continue to be themes in this literature that will be familiar to regular readers of this Specialist Periodical Report, and papers developing these long-running themes are usually given only brief coverage here. However, more thorough discussion is offered for papers where more significant synthetic work, and mechanistically-interesting results, are reported. Patent literature is almost wholly excluded, but this is easily reached through Section 34 of *Chemical Abstracts*, and other Sections (e.g. Section 16: Fermentations etc).

This Chapter is arranged into sections as used in all previous Volumes of this Specialist Periodical Report, and major Journals and *Chemical Abstracts* (to Volume 116, issue 11) have been scanned to reveal the material to be reviewed.

2 Textbooks and Reviews

Most of the citations of textbooks and reviews are located within appropriate Sections of this Chapter. Some books and monographs[1,2] having broad relevance to several Sections of this Chapter, are collected here.

3 Naturally Occurring Amino Acids

3.1 *Methodology of Isolation of Amino Acids from Natural Sources*

This Section covers a number of topics of increasing importance (though mostly simple in themselves). The generation of artefacts through

extraction procedures applied to natural samples, and the ever more sensitive analytical methods used with amino acids, all factors that increase the scope for erroneous conclusions concerning the presence (or absence) of amino acids in natural sources.

Aqueous acidic hydrolysis of peptides can be accelerated by microwave irradiation,[3] and large-scale separation of amino acids from hydrolysates can be achieved using appropriately-designed ion-exchange columns[4] or by reverse-phase flash chromatography.[5] Large-scale crystallisation of L-asparagine from aqueous solutions has received detailed attention.[6]

3.2 *Occurrence of Known Amino Acids*

Topics in reviews include non-protein amino acids,[7] the role of D-amino acids in the biosphere,[8] and N-acylamino acids as components of bacterial lipids.[9] An issue of *Advances in Enzymology and Related Areas in Molecular Biology* includes several reviews relevant to this Chapter [e.g. N^5-(1-carboxyethyl)-L-ornithine and related opines in crown gall tumours, marine invertebrates, microorganisms,[10] and ovothiols[11]].

Perhaps the most spectacular example of the occurrence of a known amino acid is the presence of alanine in the Murchison meteorite – though known for many years, refined analytical methods now allow the additional, even more spectacular, knowledge to emerge, that the amount of the L-enantiomer exceeds that of D-alanine by about 18%.[12] The result needs independent confirmation in another laboratory,[13] but also needs independent proof that the amino acids in a meteorite (or in a fossil,[14] for that matter) are indigenous; this is partly solved by stable isotope analysis, the ^{13}C-content of the meteorite amino acid indicating extraterrestrial origin.[12] The ^{15}N-content of amino acids in fossil samples can be a useful monitor of indigeneity since this isotope is increasingly enriched up the food chain.[14] These are welcome analytical checks on the authenticity of spectacular inferences made, based on the appearance of well-known amino acids in ancient samples – evolution of protein content being one such controversial topic – and another consideration is the chemical stability of the amino acids over such time-spans. The environmental decomposition of aspartic and glutamic acids, serine, alanine, and glycine in 1500y-old molluscan shells has been discussed.[15]

Further examples given later (Section 6.1: Racemization) describe studies of protein amino acids in fossils, but several recent papers report the presence of some uncommon, but known, amino acids in contemporary natural sources. These include L-aminobutyric acid as C-terminal residue in nazumamide A, a thrombin-inhibitory peptide from the marine

sponge *Theonella* sp.,[16] L-thiazolidine-5-carboxylic acid combined with L-proline in a new di-oxopiperazine (1) found in the Bermudan sponge *Tedania ignis*,[17] another new di-oxopiperazine (2), a germacranolide – valine condensation product (the first of its type) from aerial parts of *Centaurea aspera*,[18] and α-methyl-L-serine as a constituent of conagenin (3) from *Streptomyces roseosporus*.[19] Lactacystin (4) is a new microbial metabolite that induces differentiation of neuroblastoma cells.[20]

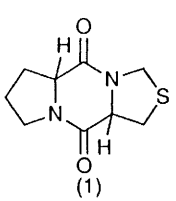
Further bromotyrosine – cysteine condensation products have been found in a marine sponge already shown to be rich in such psammaplins.[21] Far-reaching revision has been necessary for structure assignments made to radish hypocotyl constituents, the raphanusins, thought to be piperidine-2-thiones (see Vol.23, p.3). Raphanusin B is now established to be the pyrrolidinethione (5).[22,23]

3.3 *New Natural Amino Acids*

Relatively simple aliphatic amino acids emerging for the first time include trans-4-methoxypipecolic acid (6) from the tropical legume, *Inga Paterno*,[24] and trans-4-hydroxy-β-proline (7) from the red marine alga *Furcinellaria lumbricalis*.[25] Phenol ring-opening (at C-2 – C-3, and at C-4 – C-5) of L-DOPA by an enzyme from the red peel of *Amanita muscaria* yields two (hitherto hypothetical) intermediates 2,3-secodopa and 4,5-secodopa (8) and (9) respectively. They must be regarded as still elusive since their existence was proved in this study on the basis of the isolation of reaction products muscaflavin and betalamic acid.[26]

A novel addition to the natural biphenyl family is the aldose reductase inhibitor (10) from the fungus *Humicola grisea*,[27] while the similarly-expanding bromotyrosine family has gained two new derivatives (11; R = H) and its ethyl ester.[28]

Heterocyclic systems are represented by L-3-(2-carboxy-4-pyrrolyl) alanine (12) from the poisonous mushroom *Clytocybe acromelalga*,[29] and near relatives (13), a novel fungal antibiotic (TAN-950A) [with (14) as minor component; structural proof supplied by synthesis from L-glutamic acid],[30] and (15),[31] the oxidative adduct from N-acetyl-L-histidine and N-acetyldopamine, but from very different sources. TAN-950A was isolated from *Streptomyces platensis* A-136, while (15) was formed *in vitro* through the action of the cuticle of silkmoth larvae as an enzyme source [(15) is suggested to be widespread in Nature though as yet not recognized to be a natural product]. S-[2-Carboxy-1-(1H-imidazol-4-yl)ethyl]cysteine (16) has been located in normal human urine.[32] It is suggested to be the precursor of its reductive de-amination product, recently discovered also in normal urine.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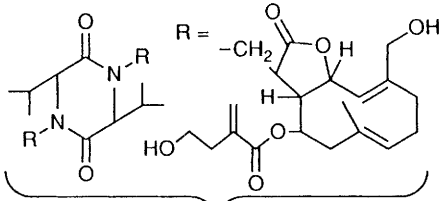
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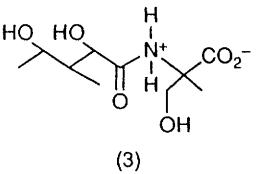
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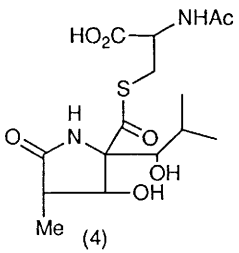
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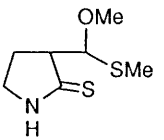
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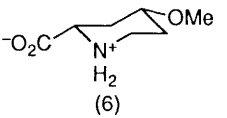
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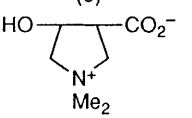
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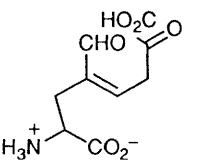
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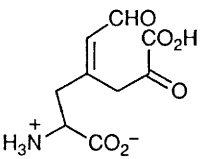
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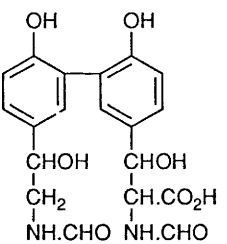
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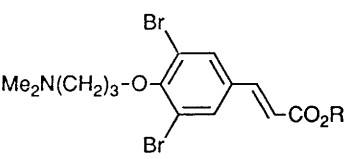
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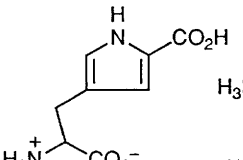
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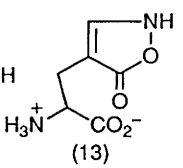
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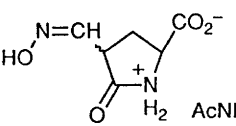
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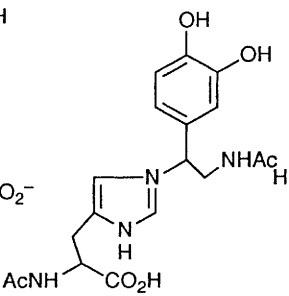
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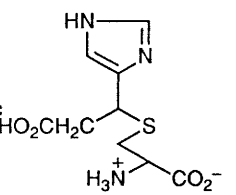
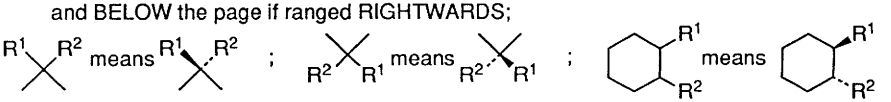

(16)

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

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

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



3.4 *New Amino Acids from Hydrolysates*

As in previous Volumes, this Section is intended to include new amino acids that would be released from condensed structures (i.e. peptides and proteins, mostly) by hydrolysis (in principle if not readily achievable in practice).

Full details are available (*cf.* Vol.23, p.3)[33] of the new protein crosslink allodesmosine (from bovine lung, aorta and skin hydrolysates, as well as from elastin). As the name implies, this pentafunctional amino acid is structurally related to well-known crosslinking amino acid residues, and, like desmosine, contains a pyridinium moiety, being formed from one lysine and four allysine residues in the proteins.

Another reference back to earlier-published material[34] is a correction of the structure of the antibiotic FR900148, revised from the pyrrolone isomer to (17). The opportunity was also taken to establish additional stereochemical details for (17), including the L-configuration shown).

The marine sponge *Theonella* (see also, preceding Section 3.2) biosynthesizes thrombin-inhibitory factors cyclotheonamides A and B made up of proline, phenylalanine 2,3-diaminoptropionic acid, as well as a modified arginine residue (-CO- between the α-methine and COOH groupings) and a modified tyrosine residue (-CH=CH- between the α-methine and COOH groupings).[35] The sea urchin *Tripneustes gratilla* produces o-, m- and p-bromophenylalanine-containing peptides, the p-isomer being the only previously-known isomer.[36]

New cyclic anti-tumour peptides trapoxins A and B (18) contain the surprising α-amino 6-epoxyacylhexanoic acid residue. These differ in the adjacent prolyl or pipecolyl residue.[37]

More complex aliphatic amino acids (often heavily disguised) are represented in the newly-studied pyoverdin-type peptide siderophores (19) from *Pseudomonas fluorescens* E2.[38]

4 Chemical Synthesis and Resolution of Amino Acids

4.1 *General Methods of Synthesis of α-Amino Acids*

This Section offers representative examples from the 1991 literature of mostly well-established general methods. Later sections often reinforce the merits of some of these methods, by giving further examples, and no attempt is made to rank them here; but over the years reviewed in this Specialist Periodical Report, readers will have noticed the growing distinctions between the perennials and the annuals.

Amination reactions, e.g. the reaction of 2-bromopropanamide enantiomers with amines to give alanine amides,[39] and conceptually-

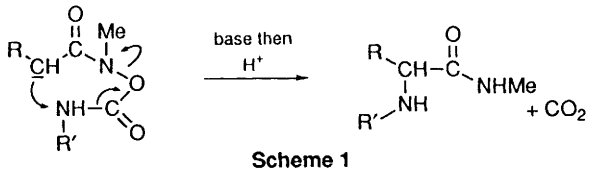
related azidation (3-fluoro-alanine from $BrCH_2CHBrCO_2Me$ by BrF_3, then NaN_3 followed by catalytic hydrogenation)[40] a similar approach to all isomeric 3-phenyl-serines and -iso-serines;[41] and a very useful, long sought – but extremely hazardous! – regiospecific S_N2 ring-opening of an alkoxycarbonyl epoxide using HN_3-di-isopropylethylamine at room temperature (20→21)[42] are conventional ways of introducing a nitrogen functional group into an aliphatic substrate. They are joined by a new reagent, p-Me-C_6H_4-SO_2-O-NHBoc, that may be converted into the N-lithio-derivative so as to offer a Boc-NH^+ equivalent for α-amino acid synthesis; thus, reaction with the zinc enolate $PhCH{=}C(O^iPr)ZnMe$ gives isopropyl N-Boc-phenylglycinate but in only 35% yield.[43] Time will tell whether the methodology can be improved (and simplified) so as to turn this promising method into a generally useful procedure.

A new α-amination method for aliphatic carboxylic acids, would be a suitable way of describing the rearrangement of N-acyl-N-methyl-hydroxylamine O-carbamates to α-amino acid N-methylamides under basic conditions.[44] Yields are in the range 34 – 76% in the cases so far tried for this anionic hetero[3,3]-rearrangement (Scheme 1). Mercury-catalyzed cyclization of chiral amidals is also a new α-amination method, applied to αβ-unsaturated aliphatic carboxylic acids (Scheme 2).[45]

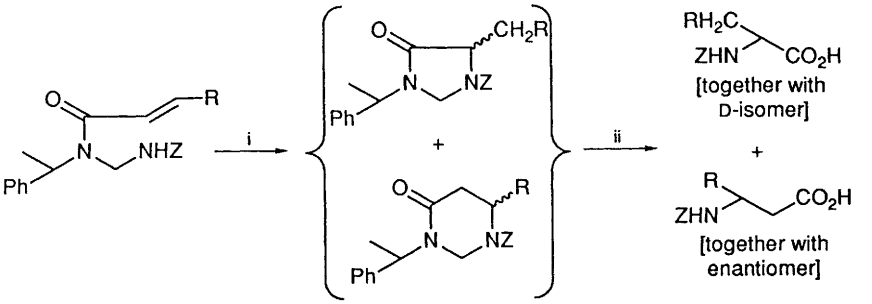
Cobalt-catalyzed aminocarbonylation processes using $Co_2(CO)_8$ with CO and aldehydes,[46,172] or equivalent gem-dihalogenoalkanes,[47] continue to provide effective entry to α-amino acids. Use of acetamide as substrate leads to N-acetyl β-cyclopropylalanines and its β-methyl homologue,[46] and use of diethylamine gives NN-diethylamino acid NN-diethylamides.[47]

Alkylation of glycine derivatives is as popular as ever as a route to target α-amino acids. Diethyl acetamidomalonate has been employed in many laboratories,[e.g.,221] including use in the synthesis of cis- and trans-pyrrolidine-2,4-dicarboxylic acid.[48] These targets are viewed as cyclic analogues of glutamic acid,[48] and similar objectives and methods are involved in the synthesis of all stereoisomers of related substituted prolines.[49] Alkylation of diethyl acetamidomalonate has yielded 4,4-difluoro-threonine,[50] and similar methodology has been applied, to syntheses of 3,6-dimethyldioxopiperazines (from dioxopiperazine and methyl magnesium carbonate),[51] to ethyl α-azidoacetate [aldol reaction with 4-{(bis-t-butoxy)phosphonylmethyl}benzaldehyde and reduction of the resulting cinnamate],[52] and to α-aminonitriles (readily alkylated by epibromhydrin, in contrast with acylated glycine esters, to give 2,3-methanoserines).[53]

Schiff base alkylation is much used, especially in asymmetric synthesis (next Section), notable examples being based on $Ph_2C{=}NCH_2CO_2Et$ [synthesis of a "-CH=CH- for -S-S- replacement", *viz.* 6,6-penta-

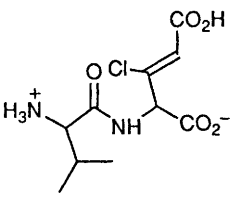


Scheme 1



Reagents; i, $Hg(OTFA)_2$; ii, H_3O^+ with separated products

Scheme 2



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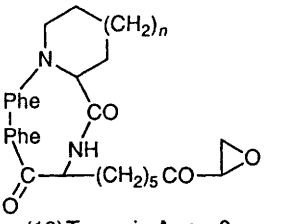
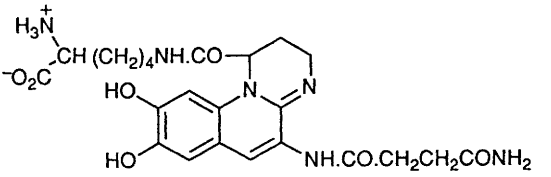


(18) Trapoxin A; $n = 0$
Trapoxin A; $n = 1$



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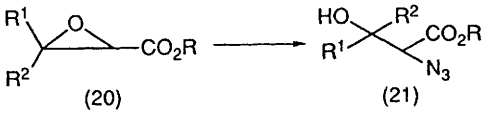

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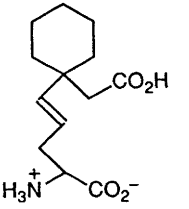


(22)

methylene-2-amino-$\Delta^{4,5}$-suberic acid (22) in a cystine analogue],[54] or on $PhCH{=}NCH_2CO_2Et$ (alkylation in an aqueous organic medium).[55] The imidate $PhC(OEt){=}NCH_2CO_2Et$[56] and the corresponding nitrile $PhC(OEt){=}NCH_2CN$[57] undergo aldol condensation with an aldehyde, elaboration of the resulting oxazoline in conventional ways giving β-hydroxy-α-amino acids[56] and α-hydroxymethylserines.[57]

Interesting applications of the ring expansion of azetidin-2,3-diones to N-carboxyanhydrides, which amounts to a general synthesis of α-amino acids from β-amino acids, have perhaps been slow in coming. The example in Scheme 3 (see also Scheme 30) is representative of the method.[58]

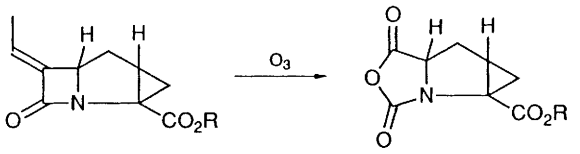
4.2 *Asymmetric Synthesis of α-Amino Acids*

Many of the methods that are familiar to regular readers are found again here. They have, in their own way, become an aspect of general methods of synthesis of α-amino acids, and the text of this Section could be combined with that of the preceding section for readers seeking information on the broader overall current situation.

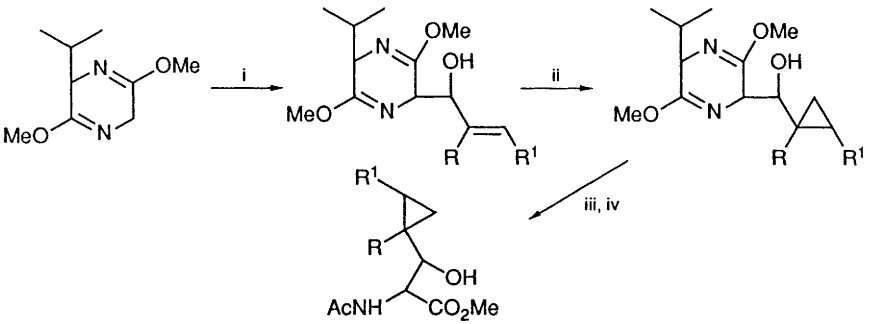
Numerous reviews have appeared: the use of carbohydrates as chiral auxiliaries in asymmetric synthesis of α-amino acids[59-62] including synthesis of prolines and pipecolic acids[60] and β- and γ-amino acids (including polyoxins);[62] amino acids from chiral lithiated amides;[63] and asymmetric hydroformylation.[64] A brief general review of asymmetric synthesis of amino acids is available,[65] accompanied by a review of asymmetric synthesis of statine.[66]

Standard methods are being exercised in a number of laboratories. Alkylation of the bislactim ether illustrated in Scheme 4, and its enantiomer, for the synthesis of (2R)- and (2S)-2-amino-2-methylmalonic acid (chiral on account of ^{13}C-isotopic substitution at one of the carboxyl carbon atoms), has been fully described following last year's preliminary account (Scheme displayed in Vol.23, pp.9,10).[67] The method has also been used in syntheses of (2R,3S)-3-hydroxy-3-(2′,3′-substituted-cyclopropyl)alanines through diastereoselective Simmons-Smith cyclopropanation of the appropriate 1-hydroxy-2-alkenyl bis-lactim ether (Scheme 4).[68] Corresponding (2R,3S)-3-substituted serines have been obtained similarly, exploiting the $ClTi(NEt_2)_3$-catalyzed addition of the bis-lactim ether (*via* 23) to a ketone.[69] Both enantiomers of each member of a series of 2-alkyl-2,3-diaminopropanoic acids,[70] and substituted phenylglycines,[71] have been prepared by bis-lactim ether alkylation, the latter case involving arene-Mn complexes as nucleophiles.

Asymmetric alkylation of Schiff bases also features in several recent papers. The prodigious output continues,[72-78] of examples based on alky-

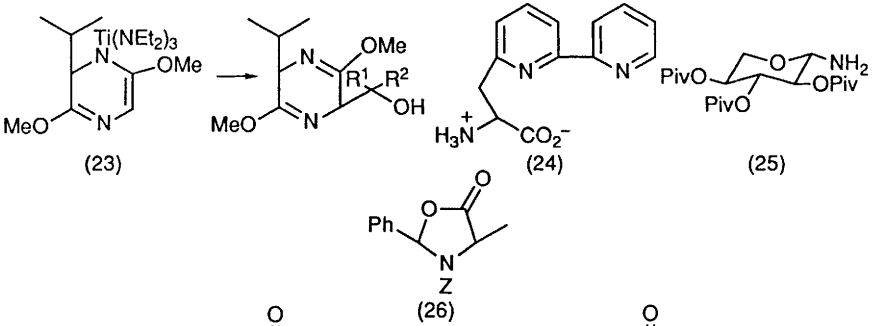


Scheme 3



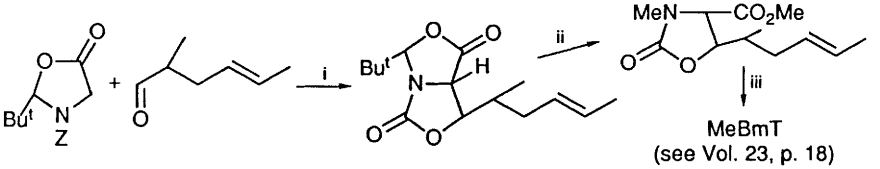
Reagents: i, $R^1CH{=}CR.CHO$; ii, R^1CuI_2 and Et_2Zn; iii, hydrolysis; iv, protection strategy

Scheme 4



Reagents: i, $Pb(OAc)_4$ in boiling toluene

Scheme 5



Reagents: i, LDA, then $COCl_2$; ii, oxazolidinone cleavage, O,N–methylation;
iii, established methods

Scheme 6

lation of the Ni(II) complex of the (S)-2-[N(N′-benzylprolyl)aminobenzaldehyde] Schiff base of glycine or alanine ethyl ester (*cf.* Vol.23, p.15). Phenylalanine or α-methylphenylalanine obtained in this way through (S)-(2-aminomethyl)pyrrolidine catalysis of benzylation, are obtained in 33-87% yields but only 3-21% optical purity.[72] Hydroxyalkylation using benzaldehydes gives (2S,3R) and (2R,3R)-β-phenylserines from isomeric starting materials.[73] Curiously, while (2S,3S)-perfluoralkylserines are obtained in this way with perfluoroalkanals, the (2R,3S)-alkylserines are obtained when non-fluorinated alkanals are used under otherwise identical conditions with the same starting material.[74] Fluorine-substituted benzaldehydes give a range of (2R,3S)-phenylserines carrying F-, F_2CHO-, F_3CO-, and F_3C-substituents when used in this process.[75] α-Methylserine has been obtained from the process based on the alanine Schiff base,[76] similarly applied to the asymmetric synthesis of α-methylvaline and α-methylglutamic acid through conventional alkyl halide alkylation;[77] however, although aspartic acid and its α-methyl analogue were prepared analogously using ethyl bromoacetate as alkylation agent, the latter target could not be obtained from α-allylalanine.[78]

Related Schiff base alkylation routes established many years ago are also of continuing interest in asymmetric synthesis of α-amino acids. These routes involve substrates with a chiral alkylidene moiety (Schiff bases generated from 2-hydroxypinan-3-one and an amino acid ester[79]), those with a chiral ester or amide function [Schiff bases derived from chiral sultams (Vol.23, p.15) and N-benzophenylideneglycine[80]] in sytheses of L-diphenylalanine and L-9-fluorenylglycine,[80] and those derived from TWO chiral moieties [*e.g.*, the (−)-menthyl ester of the (+)-camphor ketimine of glycine[81-84]]. These studies explore factors determining diastereoselectivity, which is usually not high, though the "double asymmetric induction" study[81-84] includes examples of syntheses of L-amino acids from lithium enolate alkylations in 72-96% optical yields.

Asymmetric alkylation of benzylideneglycine t-butyl ester by α-13-bromomethylbipyridine in the presence of (8S,9R)-(−)-N-benzylcinchonidinium chloride gives modest (53%) enantiomeric excess in favour of the (S)-enantiomer of the novel metal-chelating amino acid (24).[85]

Schiff bases may be used in another way for asymmetric synthesis of α-amino acids, again illustrated in this year's literature in the context of the development of established methods. Asymmetric Strecker synthesis of D-amino acids using imines derived from tetra-O-pivaloyl-α-D-galactosylamine (see Vol.23, pp.28,34)[86] or from tri-O-pivaloyl-α-D-arabinopyranosylamine (25),[87] and an asymmetric Ugi synthesis based on the latter imine,[87] are efficient processes. A diastereoselective Strecker

synthesis the other way round – imines of achiral aldehydes and (−)-α-phenylglycinol – yields chiral N-substituted α-aminonitriles that can be cleaved with lead tetra-acetate (though, unfortunately, with destruction of the chiral auxiliary).[88] Asymmetric synthesis of cyanohydrins [$Me_3SiCN + RCHO \rightarrow RCH(OH)CN$] employs the AlR_3 or $Ti(OR)_4$ complex of the 2-hydroxy-1-naphthylidene Schiff base of L-valyl-L-tryptophan methyl ester as chiral catalyst.[89] The enantiomer excesses achieved are good [71% in one case, better than 94% in the case of (R)-mandelonitrile], and cyanohydrins are suitable for amino acid synthesis in a modification of the Strecker synthesis.

Enantioselective hydration of racemic α-aminonitriles in basic aqueous media has been observed in the presence of a homochiral monoterpene-derived nitrile, reaching 42% enantiomeric excess at half-completion.[90] Although the mechanism is as obscure as the thinking that led to the choice of catalyst, the demonstration by the same group that α-chymotrypsin brings about the same result and completes the overall process by catalyzing the hydrolysis of the amide (the hydration product) is more easily rationalized.[91]

Asymmetric alkylation processes in which the glycine moiety is rendered the electrophilic partner through α-halogenation, are illustrated in a synthesis in high optical yield of (S)-[2-^{2}H]glycine from N-Boc-glycine (−)-menthyl ester, through reaction with N-bromosuccinimide followed by radical formation with Bu_3Sn^2H,[92] and related enantioselective α-substitution of the same substrate with alkenyl- and alkynylstannanes.[93] These results are important in a wider context, since they demonstrate asymmetric induction in radical reactions. Methyl arylacetate – $Cr(CO)_3$ complexes are readily alkylated by N-benzyloxycarbonyl α-halogeno-α-amino acid esters in the presence of sodium hydride, to give β- and δ-arylated α-amino acids.[94] α-Aryl α-amino acids are obtained in optically-pure form in this way using fluorobenzene – $Cr(CO)_3$ with a chiral Schiff base [from L-alanine methyl ester] and (1R,2R,5R)-2-hydroxy-3-pinanone in the presence of LiN^iPr_2 or lithiated 2-t-butyl-4-methyl-1,3-oxazolidin-5-one.[95]

An example of amino acid synthesis by amination,[96] in addition to those described in the preceding Section, describes asymmetric amination of αβ-unsaturated amides (Scheme 5).

Aldol reactions of a conventional type leading to β-hydroxy-α-amino acids from N-protected glycine derivatives and aldehydes or ketones give modest excess of the threo-isomer (33-39%) and poor enantioselection (3-12% excess) when conducted in the presence of a chiral phase transfer catalyst.[97] The chiral trans-oxazolidin-5-one (26), formed from D-alanine and benzaldehyde followed by N-benzyloxy-

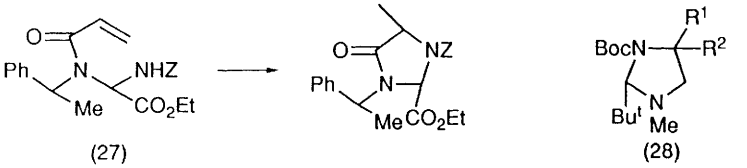
carbonylation, can be used as a source of homochiral α-methyl-α-amino acids through alkylation using an alkyl halide after carbanion formation.[98] X-Ray crystal analysis demonstrates that inversion of configuration occurs.

Numerous studies of alkylation of the glycine homologue of (26), with opposite chirality with t-butyl in place of phenyl, have been reported, mostly from Seebach's group. This chiral synthon, readily resolved by preparative scale chromatography (and readily racemized in boiling MeCN),[99] has been used to prepare threonine analogues by the aldol route, using $LiN(SiMe_3)_2$ for chiral enolate formation.[99] This method is suitable for a synthesis of MeBmT, the threonine homologue that is a component of cyclosporin A (Scheme 6), relying on the high diastereoselectivity of the alkylation step for achieving good optical yields with the correct stereochemistry.[100]

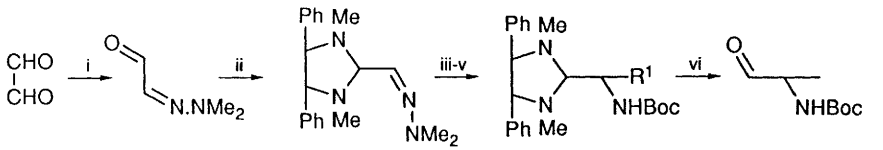
The corresponding imidazolidin-4-one (26; MeN in place of ring O) has been used in a synthesis of threo-3-alkyl- and aryl-glutamic acids[101] and studied for its stereochemical requirements in a number of other simple alkylation processes.[102-105] Aldol condensation gives a 5-alkylidene derivative to which dichlorocarbene or hydrogen (in the presence of a heterogeneous metal catalyst) add completely stereoselectively to the face opposite the t-butyl group.[102] N-Bromosuccinimide-AIBN bromination of the imidazolidin-4-one gives the trans-bromination product, from which the 5-allyl derivative can be prepared using $CH_2{=}CHCH_2SiMe_3$ and $ZnCl_2$, involving inversion of configuration.[103] A careful study of the stereochemical features of Hg-promoted cyclization of chiral unsaturated amidals (27), the basis of a new α-amino acid synthesis (see Scheme 2) to give 2,5-trans-imidazolidin-4-ones, has been reported.[104] The 2R-configuration favours the induction of the 5R-configuration at the new chiral centre.

An efficient way of using the potential for enantioselective alkylation of the racemic 2,5-trans imidazolin-4-ones (26; MeN in place of ring O) as well as recovering one enantiomer of it, requires only a chiral base for deprotonation of the racemic synthon.[105] Thus, (R,R)-$(PhCHMe)_2NLi$ followed by MeI gives the (S,S)-2-t-butyl-5-methylimidazolidinone together with (R)-2-butylimidazolidinone.[105]

Related chiral heterocycles studied in the present context include the imidazolidine (28; R^1 or $R^2 = H$; R^2 or R^1 = alkyl), which is amenable to alkylation after deprotonation with Bu^t Li and used (in the case of R^2 or R^1 $= CO_2Me$; R^1 or $R^2 = H$) to prepare (R)- and (S)-$MeNHCH_2CH(NH_2)CO_2H$.[106] A route from glyoxal to a multi-chiral imidazolidine (Scheme 7) is part of an enantioselective synthesis of α-amino aldehydes.[107] The thiazoline (29) from L-cysteine undergoes

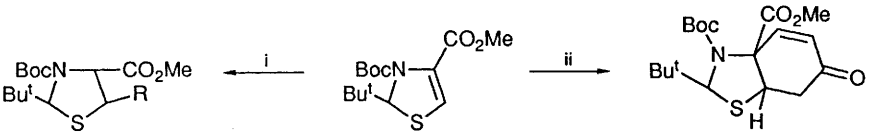


(27) (28)



Reagents: i, $NH_2.NMe_2$; ii, $(MeNHCHPh)_2$; iii, R^1M; iv, H_2/Ni; v, $(Boc)_2O$; vi, 2% HCl

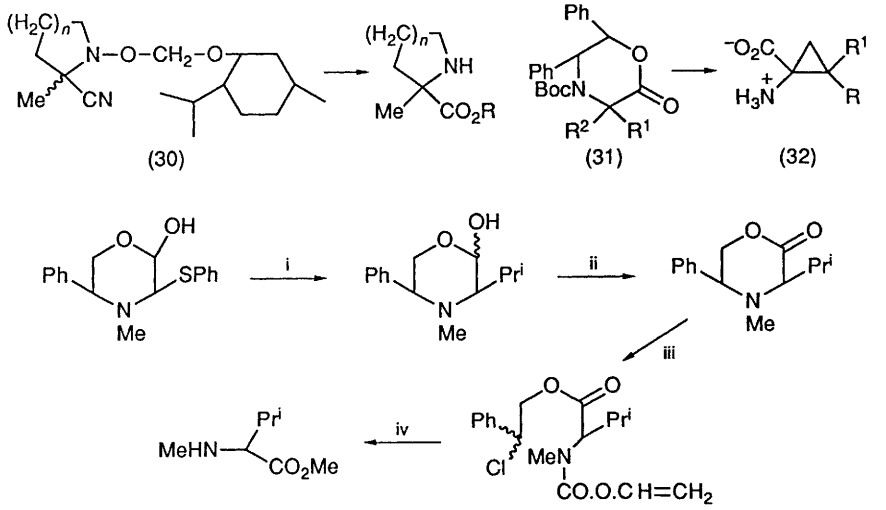
Scheme 7



(29)

Reagents: i, $Bu_2Cu(CN)Li_2$-$BF_3.OEt_2$/–30 °C, then $NH_4Cl/NH_3/H_2O$;
ii, $CH_2{=}C(OSiMe_3)CH{=}CHOMe$ in PhMe/reflux

Scheme 8



(30) (31) (32)

Reagents: i, Pr^iZnI; ii, $COCl_2$/DMSO/Et_3N; iii, $Cl.CO.OCH{=}CH_2$; iv, HCl/MeOH

Scheme 9

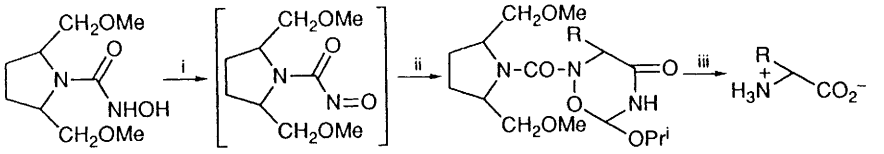
stereoselective trans addition of BuCu and enamines and has been shown to undergo cycloaddition to Danishefsky's diene to give 2-amino-5-hydroxy-3-mercaptoalkanoic acid derivatives after routine work-up (Scheme 8).[108] Chiral oxazolidin-2-ones[109] and thiazolidin-2-thiones[110] seem to be destined for more limited applications. The 4-hydroxymethyl derivatives of the former family [from (R)-glycidol and 2,3-epoxy-carbamates] offer access to homochiral serinols, and 5-methoxycarbonyl derivatives of the sulphur analogues can lead to homochiral α-hydroxy-ethyl-β-lactams. Pyrrolines (30) built up from nitrones, KCN, and chloro-methyl ether, with chirality based on (−)-menthol, can be elaborated into (S)- and (R)-α-methylprolines.[111]

The use of chiral enolates of (5S,6R)- and (5R,6S)-oxazin-3-ones is accompanied with high enantiomeric excesses when used as substrates for alkylation [anti-mono-alkylation of (31; R^1 and $R^2 = H \rightarrow R^1 =$ alkyl, $R^2 = H$) has been established.[112] Further alkylation is feasible; work-up involves Li-ammonia reduction to give Boc-amino acids. (2S,6R)- and (2S,6S)-2,6-di-amino-6-hydroxymethylpimelic acid (a component of the dipeptide antibiotic from *Micromonospora chalcea*) have been synthesized in this way using $ICH_2CH_2CH{=}CH_2$ for alkylation and $MeOCH_2Br$ for inserting the hydroxymethyl group.[113] These synthons give dimethoxy-phosphoryl derivatives (31; $R^1 = PO_3Me_2$, $R^2 = H$) that undergo Wadsworth-Emmons alkylidenation with aldehydes, giving substrates for cyclopropanation with $PhS(O)(N^+Et_2)CH_2^-$ to result in coronamic acid (32; R = Et) and its nor-analogue (32; R = Me) and ^{2}H-analogue.[114]

Homochiral α-(N-methylamino) acids result from nucleophilic dis-placement of thiophenoxide from the tetrahydro-oxazines (Scheme 9), giving an 84:16 diastereoisomer ratio at C-6, with retention when PrZnI is used, but with inversion with alkylcopper reagents.[115]

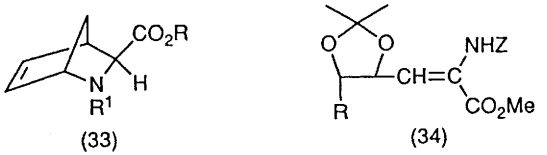
More complex chiral heterocycles are accessible through cyclo-addition reactions of 2-azadienes to chiral nitrones (Scheme 10).[116] The α-amino acid resulting from the particular starting materials shown is of L-configuration and of greater than 98% optical purity, so establishing a novel asymmetric amination procedure.

Aza-Diels-Alder reactions leading to 1-azabicyclo[2.2.1]heptene-2-carboxylic esters (33) based on a chiral iminium ion formed *in situ* from glyoxal and a chiral amine, approach 90% diastereoisomeric excess for exo-isomers, when a non-hindered moiety is introduced with the chiral amine.[117] Related chiral 1-acetamidobicyclo[2.2.1]heptene-1-carboxylates formed by Diels-Alder addition of cyclopentadiene to N-acetyldehydro-alanine (−)-cis-2-neopentyloxyisoborn-3-yl esters exhibit a preference for the isomer with exo-disposition of the carboxylate moiety.[118] A cognate study (that strictly does not fall within the terms of reference of this



Reagents: i, $NEt_4^+IO_4^-$;
ii, $RCH_2COCl + Pr^iOCH{=}\overset{+}{N}H_2Cl^- \rightarrow\rightarrow$ R.CH=C(OTBDMS)CH=N.OPr^i;
iii, $Mo(CO)_6$ then H_3O^+

Scheme 10



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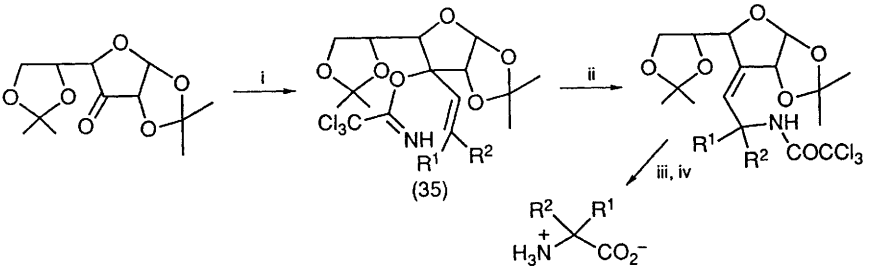
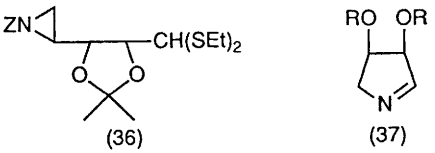
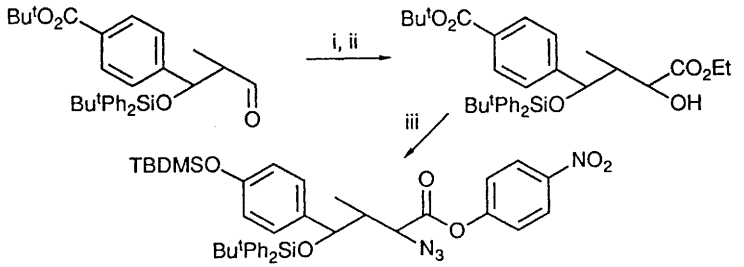


Reagents: i, established methodology; ii, xylene/135 °C; iii, $RuCl_3/NaIO_4$; iv, H_3O^+

Scheme 11



(36) (37)



Reagents: i, $CH_2{=}CLi\text{-}OEt$; ii, O_3; iii, routine protection strategy, NaI; iv, NaN_3;
v, ester interchange Et → 4-nitrophenyl for coupling to give nikkomycin B

Scheme 12

Section, but is mechanistically related), succeeds in explaining the diastereofacial selectivity observed in Lewis-acid catalyzed cycloaddition of cyclopentadiene to N-acryloyl-L-amino acids.[119]

Asymmetric hydroformylation of N-acetamidodehydroalanine ethyl ester has been achieved using $HRh(CO)(PPh_3)_3$ as catalyst with a chiral chelating diphosphine [e.g. (−)-DIOP] as chiral selector, giving the protected α-formylalanine.[120] Related studies with acrylate esters of N-acylamino acids have been reported.[121] Chiral rhodium or ruthenium catalysts effect dynamic catalytic resolution during hydrogenation of 2-acylamino-3-oxobutanoates to yield D- or L-threonine.[122]

The substantial record of results on asymmetric (homogeneously catalyzed) hydrogenation of α-acetamidocinnamic acid esters continues to be augmented, studies this year including a report of better than 92% enantiomeric excess with chiral RhX(bichep)(nbd) catalysts [X = Cl, ClO_4; nbd = norbornadiene; bichep = 2,2′-bis(dicyclohexylphosphino)-6,6′-dimethyl-1,1′-biphenyl].[123] Better than 95% stereoselectivity is attributed to the use of a zeolite anchor for the nitrogen-based chiral ligands of the Rh catalyst for the same substrate,[124] and very high stereoselectivity is also achieved with a chiral cyclopentane-1,2-diphosphine.[125] All four stereoisomers of tripalmitoyl γ,δ-dihydroxyamino acids have been prepared through this methodology using an acylamino dehydroamino acid (34) already containing a chiral moiety.[126] More routine studies have appeared,[127,128] in one of which[128] there is mechanistic interest in the fact that a decisive role exists for water, present in a two-phase (EtOAc – H_2O) medium used in deuteration (20 – 70% incorporation) of N-acyldehydroalanine methyl esters.

Better than 95% optical yield, is the extraordinary outcome for hydride transfer from (S)- or (R)-N,N′,1,2,4-pentamethyl-1,4-dihydronicotinamide to the Schiff base $MeO_2CN{=}CPhCO_2Me$ to give the derivatized phenylglycine enantiomers.[129] This provides an excellent model for the *in vivo* action of NADH.

Elaboration of the carbonyl group of the chiral lactone from diacetone-D-glucos-3-ulose provides a versatile intermediate (35 in Scheme 11) that is susceptible to the imidate rearrangement.[130] The resulting αβ-unsaturated amine gives the appropriate α-amino acid enantiomer through $RuCl_3$ – $NaIO_4$ cleavage, and the novel variation of known methodology has been exemplified in the case of a synthesis of (2R)-[2-^{2}H]glycine.

4.3 *Synthesis of Protein Amino Acids and Other Naturally Occurring α-Amino Acids*

The substantial literature on fermentative production of protein

amino acids continues to be represented here only in representative citations, given the availability of authoritative reviews and the accessibility of the literature through *Chemical Abstracts* (mainly within Section 16 – Fermentation and Bio-industrial Chemistry). The reviews this year cover amino acid production mediated by transaminases,[131] by microbial eukaryotes and prokaryotes other than coryneforms,[132] and by acylases, aminopeptidases, and hydantoinases.[133] One of these[133] also covers α-alkyl-α-amino acids. L-Aspartic acid production using the L-aspartase in immobilized microbial cultures,[134] and L-aspartic acid and D-alanine production in pressurized reactors connecting immobilized *Pseudomonas dacunhae* and *Escherichia coli* mediated reactions,[135] have been reviewed.

Representative topics featured in primary research papers include L-phenylalanine production employing *Citrobacter freundii* with L-glutamic acid as NH_2-donor for the transaminase-based process with phenylpyruvic acid[136] (a mathematical model is reported[137] for the corresponding L-tyrosine production from phenol, pyruvate, and NH_3 employing the same organism immobilized on macrocyclic gel granules), L-tryptophan production, from indole-resistant *Corynebacterium glutamicum*,[138] and from tryptophan synthase using L-serine and indole.[139] Less familiar α-amino acids covered, include norvaline and O-ethylhomoserine that accompany L-isoleucine produced by *Brevibacterium flavium* AB-07 (these can be suppressed by using mutant strains),[140] and S-adenosyl-L-methionine and -L-homocysteine production in animal tissues after inactivation of methionine synthase by N_2O.[141]

A number of illustrations of general laboratory methods and asymmetric synthesis methods described in preceding sections have used with protein and other natural amino acids as synthetic targets. Further examples that could have been located here, have instead been collected in a later Section (6.3: Specific Reactions), because they illustrate the synthesis of one amino acid starting from another.

Aliphatic, alicyclic, and saturated heterocyclic examples of particular interest concern polyoxamic acid [enantiospecific ring opening by PhS^-, of an aziridine (36) derived from a protected L-arabinose],[142] and a related synthesis of the γ-hydroxy-β-methyl-α-aminobutanoic acid moiety in nikkomycin B [starting from (−)-(E)-crotyldi-isopinocampheylborane; Scheme 12].[143] Leucinostatine constituents (2S,4S,6S)-2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid and (4S,E)-4-methylhex-2-enoic acid have been synthesized; the amino acid wss prepared through alkylation of a chiral glycine Schiff base with (S)-$CH_2{=}CH.CH_2CH$-$Me.CH_2I$, followed by routine elaboration].[144] A norcoronamic acid synthesis (see also an example in the preceding Section) builds the cyclopropane ring on to a Schiff base, prepared following the illustrative

simpler sequence starting from $ClCH_2CH_2CH(OH)CN \rightarrow ClCH_2CH_2CH(N{=}CHPh)CN \rightarrow$ 1-benzylideneamino-1-cyanocyclopropane.[145] A related natural product, (2S,3S)-(+)-aziridine-2,3-dicarboxylic acid has been synthesized from the corresponding 2,3-dicarbethoxyoxirane (Me_3SiN_3 followed by Ph_3P).[146]

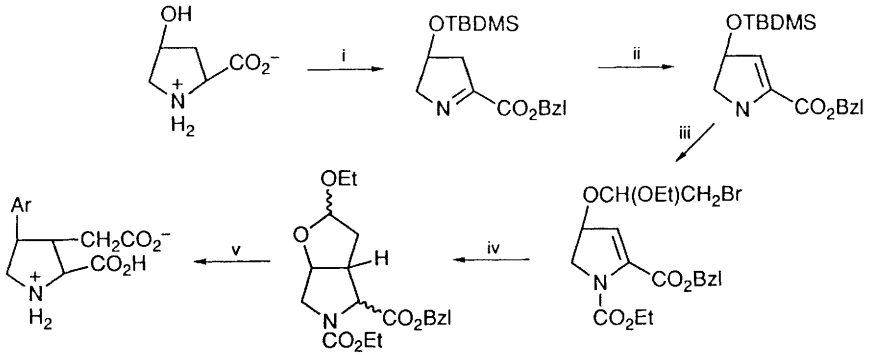
The natural stereoisomer of dihydroxyproline, configuration (2S,3S,4S), and a new isomer [(2R,3S,4S)], have been synthesized from L-tartaric acid by cyanosilylation and routine elaboration of the derived Schiff base (37; R = Bz, TBDMS).[147] These are relatively simple targets compared with members of the kainoid family and near relatives, which continue to stimulate applications of modern synthetic methods. Acromelic acid congeners (carrying an aryl group in place of the 3-pyrid-2-onyl grouping at the 4-position) are accessible through methodology applied to hydroxy-L-proline (Scheme 13; see also Vol.23, p.20).[148] Electrochemical C-5 methoxylation of hydroxy-L-proline carbamates followed by C-5 stereospecific radical homologation ($OMe \rightarrow CH_2OH$) are the crucial steps in a bulgecinine synthesis.[149]

Approaches to kainoids continue to illustrate the best of strategies using interesting new methodology. The Nicholas reaction (Scheme 14) applied to the purpose gives a pyrrolidine carrying all essential substituents.[150] Total syntheses of racemic α-allokainic acid, one involving two allylsilane – N-acyliminium ion reactions (shown in part in Scheme 15),[151] and another based on $Zn(OAc)_2$-catalyzed cyclization of γ-isocyanosilyl enolates $RC(NC)(CO_2Me)CR^1{=}C(OSiMe_3)Me$, use of 6-(TBDMSO)-3-hexen-2-one giving (38), amenable to functional group modification by standard methods.[152]

The interest in a synthesis published for tyrosine[153] lies in its exploration of the feasibility of synthesizing the more complex isodityrosine diaryl ether moieties of the vancomycins. Cycloaddition of Danishefsky's diene (Scheme 16) is possible and the equivalent diene $CH_2{=}C(OTMS).C(OAr){=}CHOTMS$ will be likely to be successful for the more important target. A synthesis of (S)-3,5-dihydroxyphenylglycine (a constituent of the vancomycins and related antibiotics) starts with an apparently routine synthesis of the dihydroxyphenylacetic acid, used to acylate Evans' chiral oxazolidin-2-one followed by α-azidation (trisyl azide).[154]

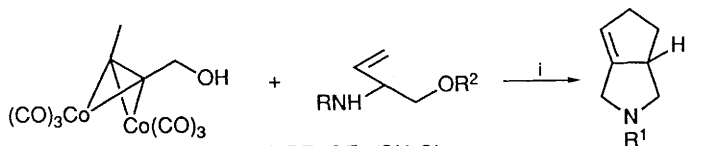
4.4 *α-Alkyl Analogues of Protein Amino Acids*

The long-running interest continues in these homologues, seen as highly hindered compounds potentially capable of modified biological activity in comparison with their natural counterparts. Implicit in this structural feature is the fact that general methods of α-amino acid synthe-



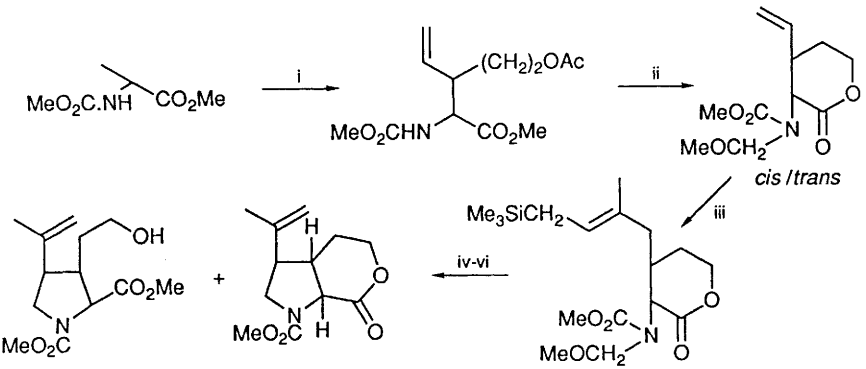
Reagents: i, routine steps; ii, $ClCO_2Et$, py; iii, TBAF, then 1, 2-dibromoethyl ether; iv, $Bu^n{}_3SnH$; v, H_3O^+, then TsCl, then Ar_2CuLi and functional group elaboration

Scheme 13



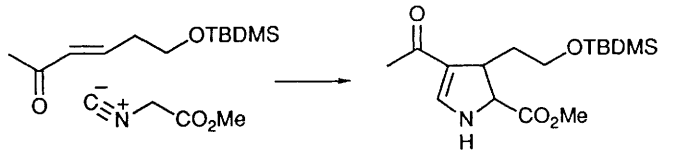
Reagents: i, reactants as shown, with $BF_3.OEt_2/CH_2Cl_2$

Scheme 14



Reagents: i, $Me_3SiCH_2CH{=}CHCH_2CH_2OAc$, and $BF_3.OEt_2$ or $SnCl_4$; ii, TsOH—MeOH, reflux; iii, NaH then $ClCH_2OMe$ with *cis* -isomer; iv, O_3; v, CHO → $CH_2CMe{=}CHCH_2SiMe_3$; vi, $BF_3.OEt_2$

Scheme 15



(38)

sis, with the notable exception of the Strecker synthesis, often fail when applied to these homologues.

Alkylation of heterocycles categorises many of the successful routes, and a review of the use of 3-amino-2H-azirene in this way has appeared.[155] A 4,4-diaralkyl-2-phenyloxazol-5-one is obtained from the parent heterocycle through reaction with the aralkyl bromide in the presence of $MeMgCO_3$.[156]

A spectacular achievement is the first synthesis of αα-di-isopropylglycine, through a modified Ugi synthesis ($HCO_2H/PhCH_2N{=}C^iPr_2/C_6H_{11}NC$) but requiring 0.9GPa pressure.[157] All four isomers of α,β-dimethylphenylalanine and 4,5-dimethyl-1,2,3,4-tetrahydroisoquinolin-3-carboxylic acid have been synthesized as "constrained" phenylalanine analogues, through alkylation of the chiral imidazolidin-4-ones (*cf.* 26, NMe in place of ring O) prepared from alanine enantiomers.[158] A similar approach, α-alkylation of an α-amino acid through various means, underlies syntheses of α-benzylproline (hetero-Cope rearrangement of N-trifluoroacetylphenylalanine allyl ester, as established by Steglich many years ago,[159] and subsequent elaboration),[160] and of α-carboxymethyltryptophan (prepared from the isonitrile analogue of N^{im}-Boc-tryptophan benzyl ester by alkylation).[161] A longer route to α-alkylated tryptophans, based on cyclization of suitably protected carbamates followed by enolate alkylation, has been described.[162]

α-[β-(D-C-Allosyl)]-L-alanine (39) and its altrosyl isomer represent a rare type of side-chain-glycosylated amino acid, prepared by Claisen rearrangement (*cf.* Ref 159) and hydration of the resulting unsaturated sugar (Scheme 17).[163]

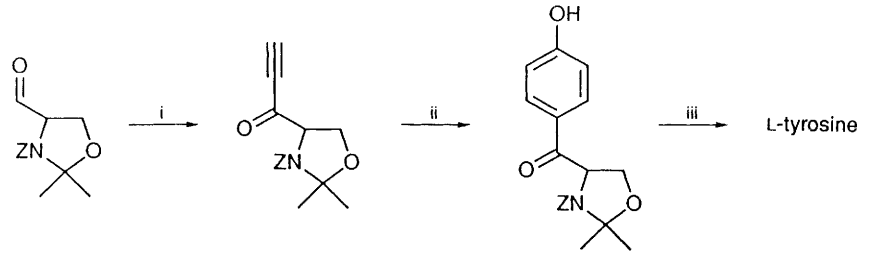
2,6-Di-amino-2-fluoromethylhept-3-ene-1,7-dioic acid, a diaminopimelic acid analogue, has been synthesized by alkylation of fluoroacetonitrile by propenylmagnesium bromide and routine elaboration.[164] Further uses for 5-fluoro-2-phenyl-4-trifluoromethyloxazole, a "hidden" trifluoroalanine synthon, have been found, alkadienylation and ring-opening giving $(E)\text{-}CH_2{=}CH\text{-}CH{=}CH\text{-}CH_2C(CF_3)(CO_2Me)NHCOPh$.[165]

Fluorobenzene chromium tricarbonyl complexes react with chiral esters of Schiff bases of L-alanine, L-leucine, and L-valine, to give the corresponding α-aryl-substituted amino acids.[166,cf.94,95]

4.5 *Synthesis of C-Alkyl and Substituted C-Alkyl α-Amino Acids*

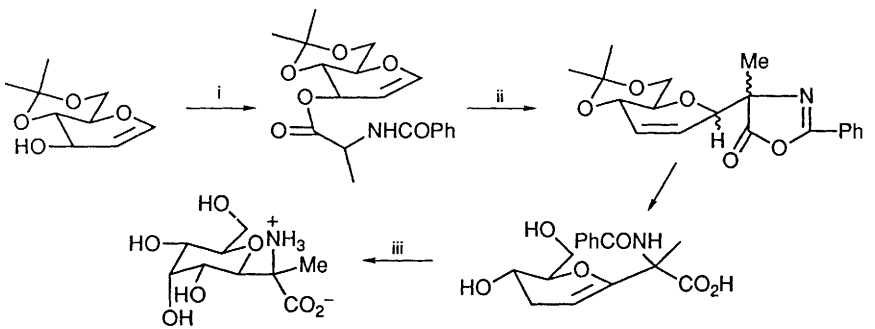
This Section collects examples of syntheses of near-relatives of the familiar natural aliphatic α-amino acids.

Acyclic examples are 3-alkylglutamic acids (potential kainic acid analogues), prepared by moderately diastereoselective alkylation of



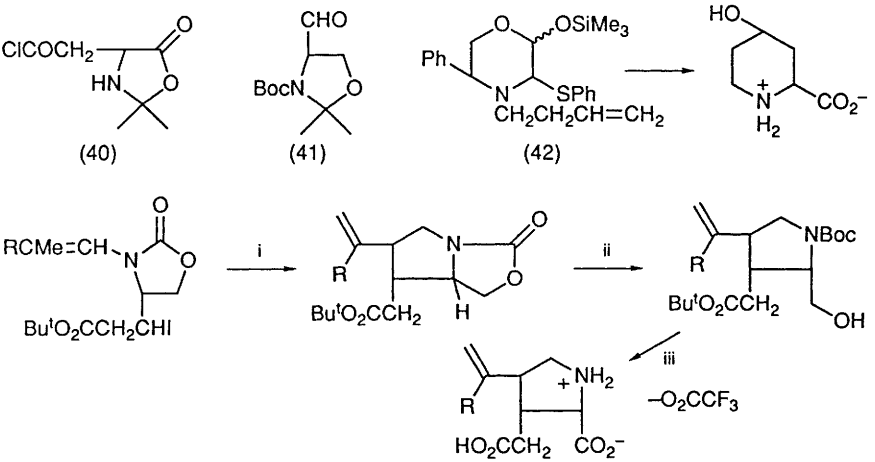
Reagents: i, CHO → CO.C≡CH; ii, CH_2=C($OSiMe_3$)CH=CHOMe/PhMe/110 °C/24h; iii, hydrolysis after C=O → CH_2

Scheme 16



Reagents: i, PhCO.Ala.OH/DCCI; ii, $Ph_3P/CCl_4/Et_3N$; iii, 6M HCl

Scheme 17



Reagents: i, cobaloxime (I); ii, aq. NaOH, then $(Boc)_2O$; iii, [O], then TFA

Scheme 18 (R = Me_2C=$CHCH_2CH_2$—)

(S)-N-Boc-2,2-dimethyl-5-(2′-methoxycarbonylethenyl)oxazolidine using R_2CuLi.[167] (R)- and (S)-2,3-Di-aminopropanoic acids have been prepared through iodocyclization of (S)-RR′C=CH.CH_2.N(CHMePh)CONHTos (chiral on account of the N-phenylethyl moiety) to the N-tosyl-N′-4-α-iodoalkyl-phenylethylimidazolidin-2-one.[168] $HClO_4$-Catalyzed Friedel-Crafts acylation of arenes using the L-aspartic acid derivative (40) yields γ-oxoaralkyl-α-amino acids.[169]

Aliphatic α-amino acids with conformational constraints built in to otherwise flexible acyclic side chains, include the four diastereoisomeric L-α-(carboxycyclopropyl)glycines prepared by cyclopropanation of (S)-CH_2=CH.CH(NHBoc)$CO_2SiMe_2Bu^t$.[170] trans-2-(Phenylcyclopropyl) glycine enantiomers have been synthesized from t-butyl (E,4R)- and (E,4S)-2,2-dimethyl 4-(2′-phenylvinyl)-3-oxazolidinecarboxylates [prepared from the corresponding aldehyde (41)→-CH=CHPh→phenylcyclopropyl].[171]

A substantial collection of papers on the synthesis of members of the proline and pipecolic acid families can be seen in this year's literature, partly covered in earlier sections of this Chapter. This activity is perhaps mainly stimulated by the potential pharmacological activity of the targets, bearing in mind the importance in this context of the kainoids as well as conformationally-constrained analogues of protein amino acids. Following the method used in new syntheses of lysine and ornithine,[46] $Co_2(CO)_8$-catalyzed carboxylation of N-benzoylpyrrolines and -tetrahydropyridines under hydroformylation conditions gives moderate yields of N-benzoylprolines and -pipecolic acids.[172] The mechanism of this process, already exemplified some time ago,[173] is tentative but may involve C – Co bond formation at the C=C group followed by CO insertion and (even more tentatively) may involve participation by the benzoyl oxygen atom to give the carboxy function after hydrolytic work-up.

The Schiff base route using Ph_2C=N.CH_2.CO_2R as nucleophile in a Michael addition to an α,β-unsaturated ketone with 10 mol% Cs_2CO_3 as catalyst gives the adduct, cyclisation leading to 2,5-disubstituted 1-pyrrolinecarboxylates and -prolines.[174] α-Acetoxy-[175,176] and α-chloro-[177] N-methoxycarbonylglycine esters carrying an N-alk-3-enyl grouping undergo cationic π-cyclisation ($SnCl_4$ or HCO_2H)[175,176] or Cu_2Cl_2- 2,2′-bipyridine cyclisation [attempted radical cyclization (Bu_3SnH) merely gives H in place of Cl][177] to give substituted pipecolic acid esters[175,176] and proline esters.[177] There are curious stereochemical results, *viz.* that cis-4-hydroxypipecolic acid esters are formed when the temperature is at −78° throughout the reaction and subsequent quenching, but trans-isomers result if the reaction mixture is allowed to warm before quenching.[176] The formic acid mediated reaction gives 4-formyloxypipecolates with low

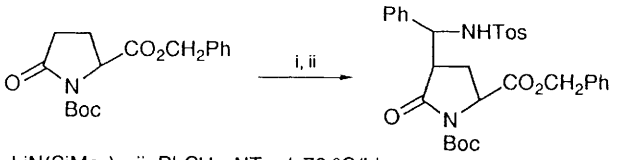
stereoselectivity at the newly-created chiral centre (C-4).[175] Cyclization of the ene-iminium ion derived from the (R)-2-phenylglycinol derivative (42) gives optically-pure 4-substituted pipecolic acids after routine deprotection and hydrolysis stages.[178] Cobaloxime(I)-mediated cyclization of homochiral 2-(α-iodoalkyl) N-alk-1-enyl oxazolidin-2-ones gives the C-8 side-chain analogues of domoic acid shown in Scheme 18.[179] Radical cyclization (Ph_3SnH-AIBN) of N-allyl-N-Boc-L-serine lactone gives 4-alkyl- and 4,4-di-alkylprolines.[180] The (2S,9S)-2-amino-8-oxo-9,10-epoxydecanoic acid moiety of the trapoxins (18) has been prepared by homolytic homologation of protected (S)-2-amino-5-iodopentanoic acid.[181]

Other syntheses of proline derivatives reported this year also start from familiar amino acids. (S)-Pyroglutamic acid can be elaborated into optically-pure homologues containing three chiral centres through addition of the derived enolate to activated imines (Scheme 19).[182] The chiral centre in the side chain is of the configuration shown, in the major (75%) diastereoisomer. The same starting material has been used in a relatively straightforward synthesis of (2S,3R)-2-carboxy-3-pyrrolidine-acetic acid, a simple kainic acid analogue.[183]

Hydroxyproline isomers are convenient as starting materials in a synthesis of all stereoisomers of (43), a conformationally-restricted arginine analogue.[184] The eight-step procedures used in this study, amount to relatively straightforward elaboration of the secondary alcohol chiral centre in the starting material. Azomethine ylide formation from the secondary amine (Scheme 20) derived from L-valine provides a partner for use in asymmetric cycloaddition; e.g. to N-methylmaleimide, giving a homochiral α-isopropyl D-proline derivative though with loss of the L-valine chiral auxiliary (discrepancies exist between absolute configurations in formulae and names in text).[185]

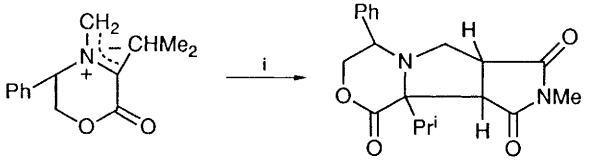
A new synthesis of racemic piperazine-2-carboxylic acid has been described, based on Schmidt rearrangement of N-ethoxycarbonyl-piperidin-4-one to give the seven-membered azalactam. αα-Dibromination at the amide carbonyl followed by Favorskii rearrangement effects the required ring-contraction and simultaneous creation of the α-carboxy group.[186] Azepane-2-carboxylic acid enantiomers are available through Schmidt rearrangement of homochiral 2-substituted cyclohexanones prepared from D- and L-valine-based enamines (Scheme 21).[187]

1-Aminocyclopropanecarboxylic acid is easily prepared from cyclopropanone in a modified one-pot Strecker synthesis.[188] If the ethyl trimethylsilyl acetal of the ketone is treated with NaCN and a chiral amine (phenylethylamine), an asymmetric synthesis opportunity is created. β-Chloroaldimine – HCN adducts prepared using acetone cyanohydrin



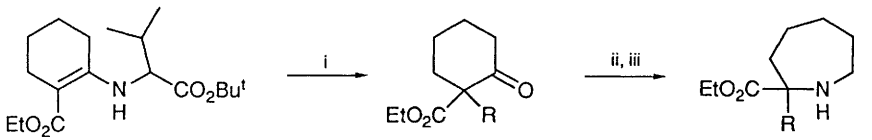
Reagents: i, $LiN(SiMe_3)_2$; ii, PhCH=NTos/–78 °C/l h

Scheme 19



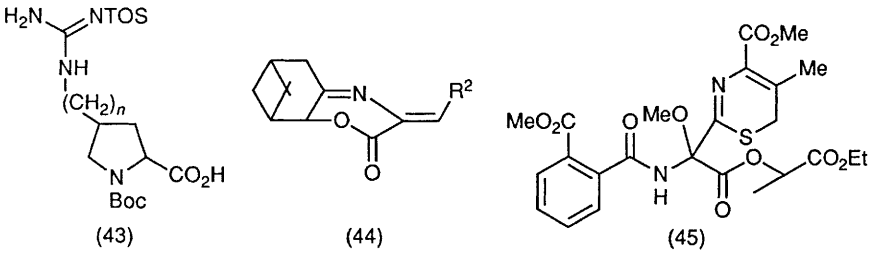
Reagent: i, *N*-methylmaleimide

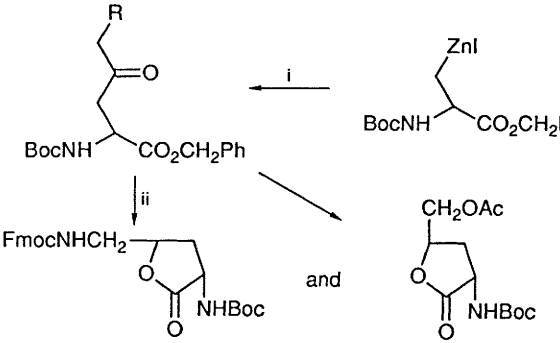
Scheme 20



Reagents: i, RBr; ii, HN_3; iii, $BH_3.Me_2S$

Scheme 21





Reagents: i, $R.CH_2COCl/Pd(PPh_3)Cl_2$; ii, [H]

Scheme 22

have been converted into α-amino-γ-chloronitriles that can be cyclized to 1-amino-2,2-dimethylcyclopropanecarboxylic acid.[189] More conventional synthesis routes based on cyclopropanation of alkenes using diazomethane, and giving more flexibility as far as patterns of substituents are concerned, have been described for the Schiff base (44),[190] giving cis- and trans-2-methyl- and -2-ethyl-1-aminocyclopropane carboxylic acids, and for 4-ethylidene-2-phenyloxazol-5-one (giving DL-allocoronamic acid).[191]

First syntheses of 1-amino-3-aza-, 3-oxa-, and 3-thia-cyclobutane-1-carboxylic acids have been announced, starting from 1-chloro-2,3-epoxypropane.[192] These are considered to have potential as NMDA receptor modulators.

A tested procedure for the synthesis of a mixture of cis- and trans-4-aminocyclohexyl-D-alanines, through catalytic hydrogenation of Boc-D-phenylalanine, has been described.[193]

4.6 *Prebiotic Synthesis Models for Amino Acids*

Relevant reviews of the broader topic, within which environmental synthesis of amino acids may have occurred, have been published. One[194] is aimed more at the layman than the other.[13]

Amino acids have been shown to be present in reaction mixtures consisting of 1.0 or 2.2M aqueous KCN kept over kaolinite at 70° during 20 days (glycine, alanine, and aspartic acid),[195] of nitrogen, carbon monoxide, and water subjected to electric discharge over a pool of water (glycine in 5.6% yield based on available carbon, and trace amounts of other amino acids; together with HCN, HCHO, and urea).[196]

Amination of aliphatic carboxylic acids in water occurs under nitrogen with glow discharge, the best yield being seen for maleic acid.[197] This reductive fixation of nitrogen is enhanced by HCl, apparently suffering oxidation to ClO_3^- in the process.

4.7 *α-Alkoxy α-Amino Acids and Related α-Hetero-Atom-Substituted α-Amino Acids*

A useful new synthesis of N-benzyloxycarbonyl α-acetoxyglycine methyl ester from the threonine analogue employs lead tetra-acetate in benzene as reagent.[198] Substitution of acetoxy by thiolate can be effected by a thiol in the presence of DABCO.[198] Twenty-six examples of α-hetero-atom (O, N, S) substituted N-acetylglycine benzylamides have been synthesized to follow up the discovery that the alanine derivative has potent anticonvulsant activity.[199]

The N-methoxyamino- and N-methoxy-N-methylamino- deriva-

tives showed the highest activity. The α-methoxy α-amino acid (45) has been resolved through diastereoisomer formation with ethyl (S)-lactate.[200]

4.8 *α-(Halogenoalkyl) α-Amino Acids*

Aliphatic fluorinated amino acids have been reviewed.[201] Fluorinated analogues, $EtO_2C.CH{=}C[(CF_2)_nCF_3]$-X-$(CH_2)_m.CH(NH_2)CO_2H$, of lysine, arginine and cysteine (X = NH; m = 4,6; X = S; m = 1, n = 4,6) have been prepared from $CF_3(CF_2)_n.C{\equiv}C.CO_2Et$ through Michael addition processes.[202] Trifluoroalanine is obtainable from hexafluoroacetone or trifluoropyruvates, but conveniently from 5-fluoro-2-phenyl-4-trifluoromethyloxazole (see also Refs. 165, 169, 515) by conversion into the 5-t-butoxy analogue and hydrolytic fission, a procedure that allows easy [2 – ^{2} H]-labelling.[203]

D-γγγ-Trichloro-allo-threonine, prepared from the oxazoline from N-benzyloxycarbonyl-L-serinal (*cf.* 41), gives D-allo-threonine by catalytic hydrogenation, a route suitable for preparing the ^{3}H-labelled amino acid.[204]

4.9 *Synthesis of Aliphatic α-Amino Acids Carrying Side-Chain Hydroxy Groups*

Stereoselective reduction of α-([ω]-oxo-alkyl) amino acids is a somewhat neglected route, and is given a useful stimulus in the establishment of methods illustrated in Scheme 22, for γ-hydroxy-compounds.[205] Alternatively, αβ-dehydro-α-amino acids are appropriate starting materials for these targets, demonstrated with an improved route to (2S,4R)-4-hydroxyornithines.[206]

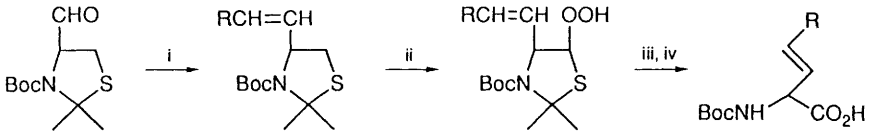
DL-5,5′-Dihydroxyleucine and it 4-fluoro-analogue have been prepared by alkylation of the Schiff base $Ph_2C{=}NCH_2CO_2Et$ with 2,2-dimethyl-5-iodomethyldioxan and the corresponding 6-fluoro-compound.[207] Alkylation of diethyl 2-acetamidomalonate by the same compounds was not so satisfactory.

4.10 *Synthesis of Aliphatic α-Amino Acids Carrying Unsaturated Side-Chains*

As seen in the immediately preceding Section, and elsewhere in this Chapter, alkenyl amino acids are valuable in synthesis and show potential as biologically-active compounds.

Synthesis of βγ-unsaturated amino acids,[208] and synthesis of α- and γ-amino acids containing an acetylenic moiety,[209] have been reviewed.

αβ-Dehydroamino-α-amino acids have been prepared by the time-honoured aldol condensation method, illustrated with the unusual sub-

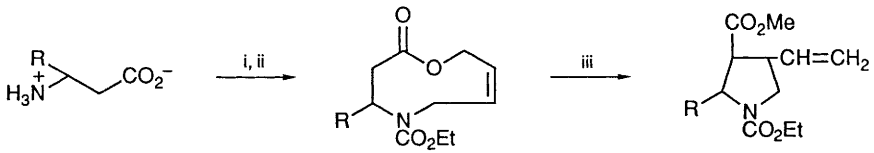


(46)

Reagents: i, Wittig reaction; ii, O_2/*h* v, *meso*-tetraphenyl porphyrin;
iii, Ph_3P reduction (–OOH → –OH); iv, LiOH

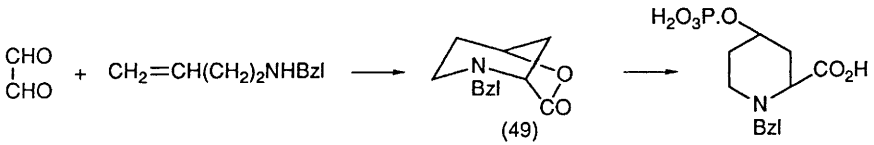
Scheme 23

(47) (48)



Reagents: i, $ClCO_2Et$; ii, (*Z*)-$ClCH_2CH{=}CHCH_2OTHP$;
iii, excess LDA, TBDMSCl, THF/–100 °C

Scheme 24



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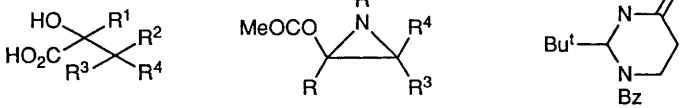
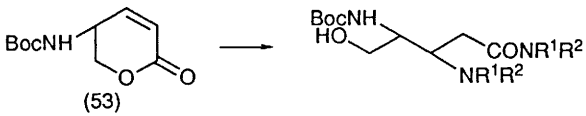


(50) (51) (52)



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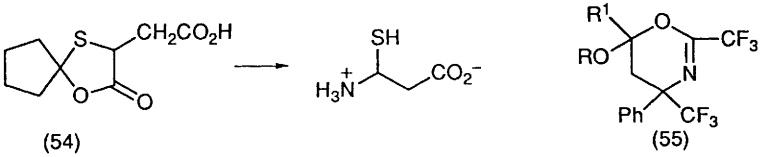


(54) (55)

strate $(HCO)_2NCH_2.CO_2Et$ which requires a strong base (NaOEt in EtOH) for the purpose when benzaldehydes are used.[210] A mixture of (E)- and (Z)-dehydro-compounds is obtained on working up the products of radical bromination (N-bromosuccinimide; there are several examples of this reaction in the year's literature[427,496,497]) of phthaloylphenylalanine t-butyl ester.[211] X-Ray crystal analysis was used to assign stereochemistry to the separated products.

Regioselective α-amination of di-anions of αβ-unsaturated alkanoic acids has been established, employing $H_2N.O.PPh_2$; yields of αβ-dehydro-amino-α-amino acids are modest.[212] The special case situation of L-DOPA is shown in many of its properties covered elsewhere in this Chapter, and in the present context too; the N-acetyl ethyl ester derivative gives the αβ-dehydro-analogue with $NaIO_4$ or with catechol oxidase, through rearrangement of the initially-formed dopaquinone.[213]

Vinylglycine enantiomers are available through effective stereo-selective routes, from the (R)- or (S)-serinal derivative (41), already found to be a valuable synthon in other contexts (Sections 4.5, 4.9, and 4.15),[214] or from the cysteinal analogue (46).[215] Wittig elaboration of (41) gives alkenes that are converted oxidatively into the D-vinylglycines. Similar treatment of the sulphur analogue (46), followed by photo-oxidation (O_2, meso-tetraporphyrin) then Ph_3P reduction to the hemithioacetal, gives D-βγ-unsaturated-α-N-Boc-amino acids (Scheme 23).[215]

Allylation of Schiff bases is also a feasible route, using a Pd catalyst with allylic carbonates, esters or halides,[216] or through Michael addition to 1-alkenes in the case of benzylideneamino nitriles.[217] In the former study, it was shown that the reaction could be biased to the extent of 70% in favour of one enantiomer when $Pd(OAc)_2$ – (+)-DIOP was used as catalyst.[216] N-Benzyl αα-divinylglycine ethyl ester was obtained in an application of the latter route.[217]

Two representative α-amino acids with γ-thioenol ether side-chains have been prepared through a Pummerer-type reaction with S-alkyl-homocysteines effected using N-chlorosuccinimide.[218]

4.11 *Synthesis of α-Amino Acids Carrying Aromatic and Heteroaromatic Side-Chains*

The routine nature of synthetic routes to near-relatives of the aromatic protein amino acids, is accounted for both by the effectiveness of standard methods of α-amino acid synthesis and by the simplicity of methods needed to create the aromatic side-chain precursor from which the α-amino acid is to be prepared. Some natural products in this category, however, offer more challenging problems (e.g. vancomycins,[154])

and examples of these, and of more routine work,[71,94,95,166] have been located elsewhere in this Chapter.

A review of fluorine-containing aromatic amino acids has appeared.[219] Direct fluorination is possible in some cases, e.g. m-tyrosine gives 2- and 6-fluoro-compounds in anhydrous HF;[220] this permits easy ^{18}F-labelling and further examples are collected in the later Section 4.15. DL-2′-Fluoromethyl- and -difluoromethyltyrosines have been prepared from 3,4-dimethylanisole through side-chain radical bromination and use of diethyl acetamidomalonate in the former case, and routine elaboration of ethyl 5-hydroxy-2-methylbenzoate, in the latter case.[221] Iodination of L-DOPA has been achieved through halogen replacement of the 6′-chloro-compound, prepared using $Cl(CH_2CH_2O)_2P^+(NMe_3)_2PF_6^-$.[222] 4′-Substituted phenylalanines are accessible from the N-Boc-4′-iodo-compound, *e.g.* 4′-aminomethyl-L-phenylalanine by Pd-catalyzed carbonylation, followed by oxime formation and catalytic reduction.[223]

Heteroaromatic side-chain creation and manipulation is also often challenging [*e.g.* preparation of D- and L-pyridylalanines from corresponding bromopyridines by Pd(0)-catalyzed substitution of N-acetyldehydroalanine methyl ester, followed by asymmetric hydrogenation,[224] and the 2-pyridiniomethyl-alanine (47) prepared by alkylation of a 5-methyl-2-t-butylimidazolidin-4-one (*cf.* Section 4.2).[225] Standard methods have been used to prepare β-(3-quinolinyl)alanine and its lysine analogue, and 3-pyridylcarbonyl-lysine (by alkylation of diethyl acetamidomalonate),[226] and the fluorescent amino acid, DL-2-amino-1-(7-methoxy-4-coumaryl) propionic acid.[227] The heterocyclic moiety has been built on to the amino acid framework in the cases of β-(4-thiazolyl)-L-alanine [from the L-aspartic-acid derived chloromethyl ketone (48) by Hantszch synthesis – condensation with thioformamide],[228] and of novel analogues of the pharmacologically-interesting β-(3-carboxyalkyl-isoxazol-5-yl)-alanines.[229]

Fischer indole synthesis of 7-fluoro-DL-tryptophan,[230] and routine alkylation methodology [1-hydroxytryptophan from methyl 1-hydroxyindole-3-acetic acid;[231] and 5-bromo-DL-tryptophan from 5-bromoindole and the bromoalanine Schiff base $HON{=}C(CH_2Br)CO_2Et$[232]] have been described.

4.12 *Synthesis of N-Substituted α-Amino Acids*

While this Section excludes peptides, it includes non-routine examples of side-chain N-acyl- and N-alkyl derivatives.

Easy methylation of the ring nitrogen atom in (S)-phthalimido- and -tritylamino -lactams derived from L-lysine and ornithine, has been established using MeI and Ag_2O in DMF.[233] Side-chain N-(α-halogenoacetyl)

derivatives (S)-$RCH_2CONH(CH_2)_nCH(NH_2)CO_2H$ R = Cl, Br, I; n = 1-3 have been prepared for study as enzyme inhibitors.[234] Synthesis of the same iodo-acetyl derivatives of ornithine and lysine (n = 3, 4 respectively) has been reported simultaneously from a different laboratory.[235]

A new synthesis of N^{im}-hydroxytryptophan has been published.[236] N-Amination of α-amino acids under mild conditions using N-methoxycarbonyl phenyloxaziridine offers a useful entry to new carbazates.[237]

4.13 *Synthesis of α-Amino Acids Carrying Phosphorus-Containing Side-Chains*

Organic synthesis is responding more noticeably to the biological importance of glycosylated, phosphated and sulphated side-chains. The enhanced response is also stimulated for other reasons, and has been significantly helped by simplified synthesis methodology.

Together with other examples collected elsewhere in this Chapter, current papers include cis-4-(phosphonoxy)-pipecolic acid (a conformationally-restricted potential antagonist of the NMDA subtype of the glutamate receptor), synthesized from N-benzyl but-3-enylamine and glyoxal through alkene – iminium ion cyclization and ring-opening of the resulting lactone (49).[238] (2R,3S)-β-(Phosphonoxyacetyl)pipecolic acid has been synthesised from N-(3-chloropropyl)-D-aspartic acid for the same purpose.[239]

4.14 *Synthesis of α-Amino Acids Carrying Boron-Containing Side-Chains*

This short new Section is introduced for the first time this year in this Specialist Periodical Report series. This is not intended to imply that these are in any way novel types of α-amino acid, but that their study continues to be recognized as offering useful biological rewards. Uneventful alkylation of the Schiff base $Ph_2C{=}NCH_2CO_2Me$ with 3-[2-methyl-1,2-dicarba-*closo*-dodecaborane(12)-1-yl]propyl iodide gives the corresponding carboranyl amino acid.[240]

4.15 *Synthesis of Labelled α-Amino Acids*

The topic has all the relevance and fascination in this year's literature, that has been illustrated in all recent Volumes of this Report. References are arranged in order of increasing atomic number of the substituting isotope.

^2H- and ^{13}C-Labelling of L-cysteine has been accomplished through tryptophan synthase-catalyzed condensation of L-[3-^{13}C]serine with toluenethiol.[241] The corresponding method with 1-^{15}N- and 2-^{13}C-indoles gives labelled tryptophans.[242]

$^2H_2$ or $^3H_2$-Solid-state labelling of amino acids by isotopic exchange

has been extensively studied recently, following a large number of similar earlier studies. Uniform labelling with 80-90% substitution is possible, with retention of stereochemical configuration.[243] The most recent of the Russian studies have concentrated on catalysis[244] and other parameters[245] involved in the $^3H_2$ version of the process, concentrating particularly on L-valine.[245] General $^3H - {}^1H$ exchange using a Pd catalyst with $^3H_2$ is also featured in a preparation of ^{3}H-α-amino-γ-butyrolactone, work-up with HBr-AcOH giving the hydrobromide of labelled α-amino-γ-bromobutyric acid as main product (57% yield) with 23% of the γ-hydroxy-analogue.[246] [4,4-^{3}H]-γ-Aminobutyric acid has been prepared from glutamine by Chloramine-T oxidation to the nitrile, followed by reduction with $^3H_2$.[247] A report on [3-^{3}H]-labelling of S-ribosyl-L-homocysteine has been published,[248] and stereochemical control has been exerted in syntheses of (5R)- and (5S)-[5-^{3}H]-L-ornithines, through crucial stages involving asymmetric reduction of $CH_2{=}CH(CH_2)_3C^3HO$ followed by Evans' azidation methodology,[249] and in a synthesis of [3-$^3H_3$]-L-threonine involving [$^3H_2$-Pd] tritiolysis of the trichloromethyl alcohol derived from the Z-D-serinal oxazoline [41; $CHO \rightarrow CH(OH)CCl_3 \rightarrow CH(OH)C^3H_3$].[250]

^{11}C-Labelling continues to provide valuable derivatives for diagnostic medical studies, but applications are limited by the need for deft organic chemistry in view of the short half-life of the isotope. $^{11}CH_3Cl$ has been used in a synthesis of labelled (R)-carnitine in this context.[251] An improved synthesis of [1,1′-$^{13}C_2$]-L-cystine from $Na^{13}CN$ includes *Aspergillus* acylase resolution.[252] Specific ^{13}C-labelling of each carbon atom is featured in the synthesis of [3-^{13}C]-, [4-^{13}C]-, [5-^{13}C]-, and [3,4-$^{13}C_2$]-2-oxoglutaric acids for use in the synthesis of correspondingly-labelled L-[^{15}N]glutamic acids.[253] Less spectacular are the syntheses of L-[1-^{14}C]phenylalanine (in seven steps from $^{14}CO_2$),[254] and of L-[^{14} C-methyl]-methionine, converted into the S-adenosyl derivative using methionine adenosyl tranferase and ATP in better than 90% yield with relatively high labelling efficiency.[255]

^{18}F-Labelling, referred to elsewhere in this Chapter,[220] is featured in syntheses of 4-[^{18}-F]fluoro-m-tyrosine by regioselective fluorodemercuration of the 4-trifluoroacetoxymercurio derivative using $AcO^{18}F$,[256] and of the isomeric L-[6-^{18}F]fluoroDOPA by the Schiff base alkylation route using similar methods applied to 6-nitroveratraldehyde, to synthesise the labelled 6-fluorobenzyl bromide.[257]

A new synthesis of N-bromoacetyl-[3′-^{125}I]3,3′5-tri-iodo-L-thyronine has been published.[258]

4.16 *Synthesis of β- and Higher Homologous Amino Acids*

Attention continues to be paid to gaps in synthetic methodology in this topic area, so that it can approach the level of sophistication established for the α-amino acids.

The chemistry of β-alanine has been reviewed.[259] Standard methods for the synthesis of β-amino acids exemplified this year for asymmetric synthesis, include addition of nitrogen nucleophiles to αβ-unsaturated esters.[260,261] The presence of baker's yeast achieves up to 60% enantiomeric excesses, enhanced further by the presence of cyclodextrin.[260] N-Lithio (R)-N-benzyl-α-methylbenzylamine adds highly diastereoselectively (better than 95% enantiomeric excess) to (E)-t-butyl but-2-enoate in giving (R)-3-aminobutanoic acid and (S)-β-tyrosine.[261] Efficient and practical syntheses of (R)-3-aminobutanoic acid starting from L-asparagine have been developed,[262] through elaboration of the derived N,N-dibenzyl-L-asparaginol methanesulphonate *via* the nitrile. D-Aspartic acid acts as starting material in a new synthesis of the β-amino acid ADDA, the chiral centres in the derived (4R,5S)-4-methyl-5-phenyloxazolidin-2-one (*cf.* Scheme 18) becoming the (8S)- and (9S)-chiral centres of the synthesis target.[263] Further syntheses in the general category of synthesizing one amino acid from another, include (2S,3R)-3-amino-2-hydroxy-4-(4′-hydroxyphenyl)butanoic acid from Boc-D-tyrosine methyl ester by DIBAL reduction to the aldehyde, and cyanohydrin formation and conventional elaboration,[264] and specifically deuteriated isoserines (50; $R^2 = NH_2$) by Curtius rearrangement of 3-deuteriated malic acid (50; $R^2 = CO_2H$), itself prepared through enzyme-catalyzed methods. Cyclization of an isoserine to the aziridine (51) creates a key intermediate in a versatile route to labelled D-amino acids.[265] An enolate Claisen rearrangement of a β-amino acid allyl ester shown in Scheme 24 has been used for β-proline synthesis.[266]

Examples of homochiral β-amino acid synthesis, building on methods established for α-amino acids, include homoallylamines prepared by addition of allylsilanes or -stannanes to O-pivaloyl-galactosylamine imines (*cf.* the arabinose analogue 25) catalyzed by $SnCl_4$,[267] alkylation of the homologue (52) of the well-established imidazolin-4-ones (Section 4.2) with high diastereoselectivity, and in good yields,[268] and BINAP-Rh(II)-catalyzed asymmetric hydrogenation of β-substituted (E)-β-acylamino-acrylic acids.[269] The Boc-serine-derived δ-lactone (53) undergoes highly diastereoselective 1,4-addition with amines combined with lactone aminolysis.[270]

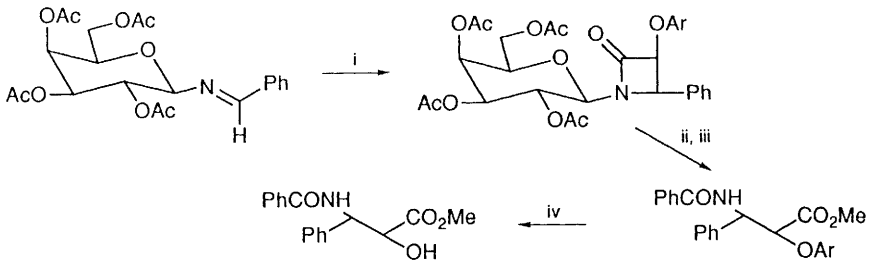
A general asymmetric β-amino acid synthesis exemplified by syntheses of natural β-leucine, β-lysine, and β-phenylalanine, involves

dipolar cycloaddition of nitrones to vinyl acetates, keten acetals and α-chloroalkenyl-nitriles.[271]

Methods reminiscent of standard α-amino acid syntheses are illustrated in a number of recent papers. Ammonolysis of 2-bromo-3-deoxy-D-threonic and -D-arabinoic acids to give 3-aminoalkanoic acids *via* 2,3-epoxycarboxamides.[272] The formation of isomeric α-amino acids is also a feature of this study. Curtius rearrangement of mercaptosuccinic acid oxathiolone (54) gives β-amino-α-mercaptosuccinic acid (*alias* isocysteine), isolated as its S-benzyl derivative.[273] The β-trifluoromethyl derivative of β-phenyl-β-alanine has been synthesized starting from $CF_3CPh{=}NH$, the Schiff base of trifluoroacetophenone, through N-trifluoroacetylation and addition to a vinyl ether to give the oxazinone (55) followed by hydrolysis with concentrated aqueous acid.[274] A chiral Schiff base is employed in an enantioselective Staudinger reaction to give β-lactams from which the corresponding isoserines are obtainable (Scheme 25).[275] A new homochiral β-lactam synthesis from a homochiral diazaborolidine is based on addition to simple Schiff bases as displayed in Scheme 26.[276] Birch reduction of homochiral N-Boc-phenylethylamines followed by ozonolysis and either decarboxylation (to β-amino acid esters) or β-lactam formation is shown in Scheme 27.[277] An alternative, familiar, way to create the carboxy group in the present context is represented in synthesis of 3-amino-2-arylpropanoic acids by N-pivaloylation and benzylic lithiation of 2-arylethylamines and their addition to CO_2.[278] N-Silylated enamines $MeCR^1{=}CHN(SiMe_3)_2$ are a source of 2-aminocyclopropane-1-carboxylic acid derivatives through cyclopropanation using methyl diazoacetate and $Rh_2(OAc)_4$.[279]

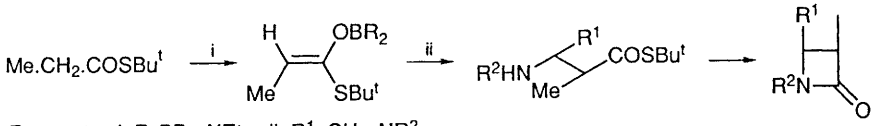
Pharmacologically-interesting β-amino acids are featured in recent synthesis studies, leading to β-proline analogues (agonists at the strychnine-sensitive glycine receptor) through azomethine ylide addition to methylpropiolate (Scheme 28),[280] *Rhodococcus equi*-mediated enantioselective hydrolysis of 6-azabicyclo[3.2.0]hept-3-en-7-one to give a precursor of the antifungal agent (+)-cispentacin,[281] and a synthesis of a series of GABA receptor-binding 2-(thien-2-yl)-3-aminobutanoic acids (56; X = S) and closely related heterocycles.[282] Points of methodological interest in these studies include an effective N-demethylation step in the β-proline synthesis (Scheme 28), using $ClCO_2CHClMe$,[280] and points of interest as far as biological activity is concerned are that 3-carboxy-3,4-dehydropyrrolidines (Scheme 28) are more active than any other isomer,[280] and that the 5-methyl- and 5-chloro-thienyl compounds were the most potent, and also specific for the $GABA_B$ receptor, from the series (56) studied.[282]

Within the γ-amino acid area, there is also considerable interest in



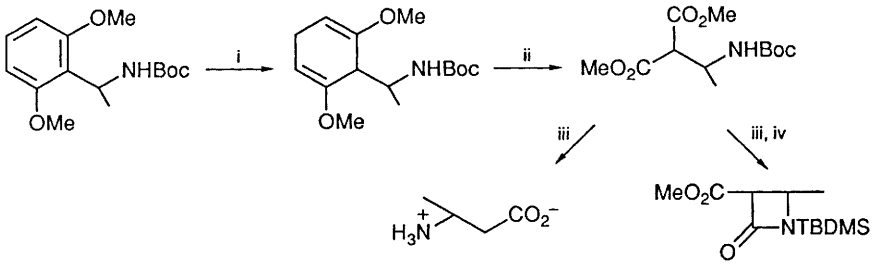
Reagents: i, $ArOCH_2COCl/NEt_3$; ii, H_3O^+, MeOH; iii, $PhCOCl/NEt_3$; iv, NH_4^+ cerium(IV) nitrate

Scheme 25



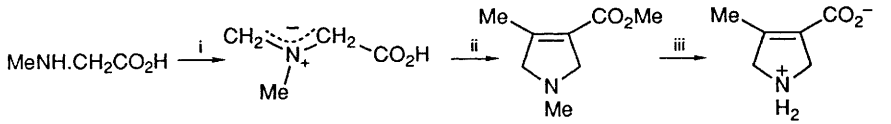
Reagents: i, R_2BBr, NEt_3; ii, R^1. $CH{=}NR^2$

Scheme 26



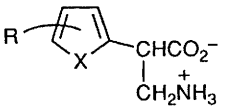
Reagents: i, $Na/NH_3/Et_2O/EtOH/-78$ °C; ii, O_3–EtOH then Pd–C/H_2/–78 °C; iii, deprotection, decarboxylation; iv, $LiN(SiMe_3)_2$, then TBDMSCl

Scheme 27



Reagents: i, paraformaldehyde; ii, $MeC{\equiv}CCO_2Me$; iii, $ClCO_2CHCl.Me$ and hydrolysis

Scheme 28



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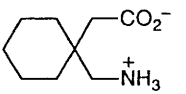


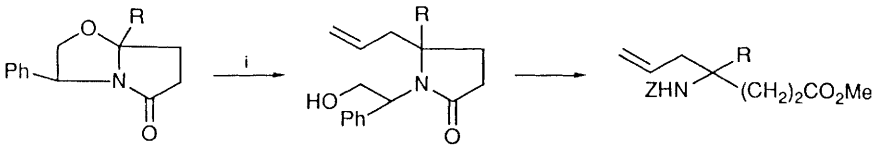
(57)

biologically active compounds. Gabapentin, the anticonvulsive 1-(aminomethyl)cyclohexaneacetic acid (57), is synthesized in a new way through addition of HCN to diethyl cyclohexylidenemalonate, followed by routine elaboration.[283] Other GABA analogues have been synthesized, including γγ-dialkyl homologues (Scheme 29) illustrating acetal alkylation with allyltrimethylsilane,[284] and both enantiomers of Baclofen [4-amino-3-(4-chlorophenyl)butanoic acid, which, unlike GABA, can cross the blood – brain barrier], prepared by α-chymotrypsin-catalyzed hydrolysis of prochiral dimethyl 4-chlorophenylglutarate di-esters to the mono-esters, followed by amination [$-CO_2H \rightarrow NH_2$ *via* azide (Curtius rearrangement), and $-CO_2Me \rightarrow CONH_2$].[285]

Dolastatin 10 components (3R,4S,5S)-dolaisoleucine and (2R,3R,4S)-dolaprine have been synthesized from an N-protected isoleucine (*via* the derived β-keto-ester $-CO_2H \rightarrow -COCH_2CO_2Bu^t$) and from N-Boc-L-prolinal [$CHO \rightarrow CH(OH)CHMeCOSPh$] respectively.[286] Several other studies extending the separation of amino and carboxy functions by similar means have been reported, particularly those based on β-keto-esters and using enzyme-catalyzed reduction of the ketone grouping. In this way, baker's yeast and (S)-Boc.NH.CHMe.CO.CH_2 CO_2 Me give the 3R-configuration required for elaboration into sperabillin and negamycin,[287] and the simplest example has been studied further – (R)- and (S)-GABOB from enantiomers of AcO.CH(CN).CH_2-CO_2Et through lipase-catalyzed kinetic resolution (*cf.* Vol 23, p.38).[287a] A different route to these β-keto-esters employs enolate alkylation by N-carboxyanhydrides, newly synthesized by the astonishing ring-expansion of 3-oxolactams (Scheme 30; see also Scheme 3).[288]

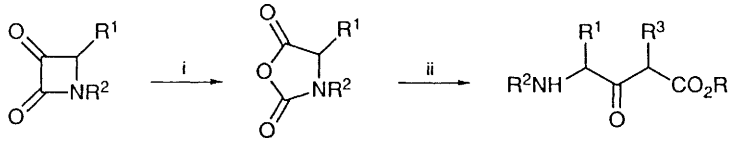
The statine group is of long-standing interest and although many syntheses have been described, still more are evidently in the pipeline. The continuing investigations in this area are of broader interest since light is cast on factors controlling stereoselectivity, especially since standard organic reactions of wide applicability are involved. The aldol condensation of silyl enolates $CH_2=C(OMe)OSiMe_3$ with homochiral aldehydes is better than 95% diastereoselective in the presence of $TiCl_4$, and (3S,4S)-statine and (3S,4S)-cyclohexylstatine have been obtained in this way from L-leucinal and L-phenylalaninal, respectively.[289] 2-Trimethylsilylethylidene triphenylphosphorane adds to Boc-amino aldehydes to give the Cram chelation-controlled product, successive hydration and oxidation of the ethenyl moiety of the adduct, to give a carboxy group providing N-Boc-statine in 36% overall yield from Boc-L-leucine.[290]

Alkylcuprate – BF_3 substitution of 5-methoxy or 5-phenylthio-groups from 4,5-disubstituted oxazolidin-2-ones with retention of configuration has been employed in syntheses of (3S,4S)-statine and (3S,4S)-



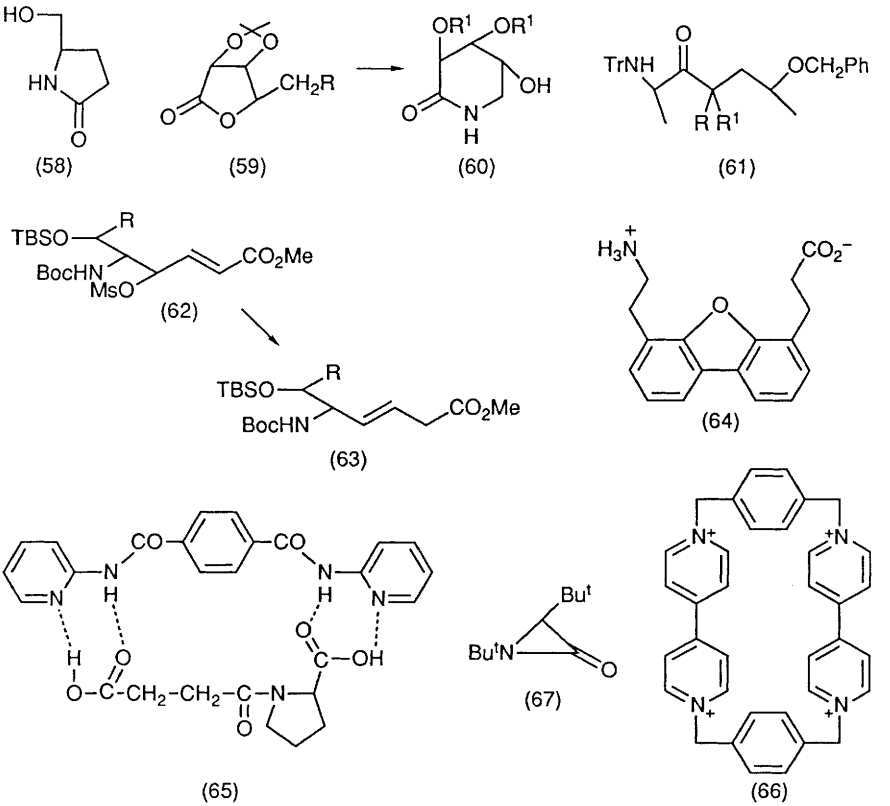
Reagents: i, described in text, and routine elaboration

Scheme 29



Reagents: i, *m*-chloroperbenzoic acid; ii, LDA, then $R^3CH_2CO_2R$

Scheme 30



cyclohexylstatine.[291] Routes *via* these oxazolidinones prepared differently, have been a feature of recent studies (Vol.23, pp.36, 37).

(3S,4S)-Statine and (3S,4R)-statine have been prepared from (S)-malic acid, already used by Bernardi's group (Vol.23, p.34) involving allylation of an α-alkoxy N-acyliminium ion.[292] An "easy" synthesis of cyclohexylnorstatine (3-amino-4-cyclohexyl-2-hydroxybutanoic acid) from D-glucose involves an electro-oxidation and Baeyer-Villiger oxidation sequence.[293] 1,3-Dipolar cycloaddition of a chloronitrile oxide to N-allyl trichloroacetamides gives a diastereoisomer mixture that is not particularly biased (1.4 – 2.2:1) in favour of the stereochemistry of DL-statine.[294] The $C_{33} - C_{38}$ portion of the calyculins is a (2R,3R,4R)-2,3-dihydroxy-4-dimethylamino-5-methoxypentanoic acid residue, and is thus closely related to the statines. Syntheses, incidentally verifying the assigned stereochemistry, have been based on building-up the serine oxazoline (41),[295] and on the pyroglutamate-derived hydroxymethyllactam (58).[296]

δ-Amino acids and higher homologues are generally accessible through standard organic synthesis methodology, as opposed to the particular methods that are recognizable to the protein amino acid chemist. D-Ribonolactone is a convenient starting material for a synthesis of (2R,3R,4R)-5-amino-2,3,4-trihydroxyvaleric acid (60),[297a] employing the key azidation step (59; R = OH→59; R = N_3 in several steps→60) so frequently used in bridging from the carbohydrates across to the amino acids [see also a synthesis of natural trans-5-hydroxypipecolic acid from (S)-5-hydroxy-2-piperidone by the same group,[297b] using the acyliminium ion method[cf.292]]. D-Ribose has been elaborated into the δ-amino acid corresponding to (59) with one fewer OH groups, after reaction of its 5-O-methanesulphonate with NaN_3.[298] Other 2,3-dideoxymonosaccharides can be manipulated in this way, a synthesis of N-Boc-O-benzyl-(4S,5S)-5-amino-4-hydroxy-6-phenylhexanoic acid being crucially dependent upon selective protection of the secondary and anomeric hydroxy groups so that azidation at the C-6 hydroxy group could be accomplished.[299] A lactone almost identical with the above-mentioned lactone (59; $NHCPh_3$ in place of OH) has been prepared from L-phenylalanine for synthetic studies in this area, through the transformation – CO_2Me→-$COCH_2$ $P(O)(OMe)_2$ followed by isopropylidenation, and ring closure.[300] Further connections with statine syntheses are noticeable in this and in a number of other syntheses of higher homologues starting with α-amino acids, such as aldolization of the phenylalanine-derived aldehyde based on (41) with $^iPrCH{=}C(OSiMe_3)SBu^t$,[301] and aldolization of a protected prolinal [reversal of stereoselectivity was observed in this case, compared with experience in the same method applied in a dola-

proine synthesis,[302a] and is ascribed to the use of excess dibutylboron triflate and NEt_3].[302b] Alkylation of diethyl 2-substituted malonates with the chloromethyl ketone derived from a protected ornithine,[303] alkylation of N-t-butyl-D-alanine pyridylthioester with (R)-1-bromopropan-2-ol benzyl ether, giving (61) for further elaboration,[304] and reduction with alkenylcopper reagents $MeCu(CN)Li.BF_3$[305] and $(vinyl)_2Cu(CN)(MgCl)_2$,[306] of δ-amino-γ-methanesulphonyloxy-αβ-enoate esters, (62)→(63)[306] have also been reported.

Full details are available[307] of support by synthesis of the revised structure of galantinic acid, a component of galantin I previously assigned a tetrahydropyran structure now shown to be readily formed from the now-established open-chain structure (see also Vol.23, pp.40,41).

An example of the synthesis of an amino acid with considerably greater separation of amino and carboxy functions, is offered by the compound (64), prepared from the corresponding dicarboxylic acid (from di-iodobenzofuran) through a route employing conversion of the corresponding mono-ester into the t-butyl carbamate with $(PhO)_2P(O)N_3$ and t-butanol.[308] Development of synthetic routes to handle such a separation of functional groups is relevant for residues in some antibiotics but particularly in syntheses of protein cross-linking moieties and their peptide models.

4.7 *Laboratory Resolution of DL-Amino Acids*

All the classical methods for resolution of racemic amino acids in the laboratory, are represented in the recent literature: separations based on diastereoisomer formation and on eutectic phenomena; on enantioselective reactions; and on chromatographic interactions. The last-mentioned category has both preparative and analytical aspects and papers dealing with the latter aspect are mostly covered in later Sections of this Chapter.

Conventional diastereoisomeric salt formation, e.g. between (RS)-2-phenylglycine and (S)-10-camphorsulphonic acid, is accompanied by asymmetric transformation in favour of one enantiomer, when the mixture is allowed to equilibrate in an alkanoic acid solution at 100°.[309] A "replacing crystallization" phenomenon (favoured crystallization of the L-enantiomer) is illustrated for solutions of ammonium salts of N-acetyl-DL-butyrine, norvaline, and norleucine in the presence of ammonium N-acetyl-L-alaninate.[310] Seeding with L-threonine gives optically-impure crystals from a melt of the racemate due to co-crystallization of the D-enantiomer at the crystal surface.[311]

Numerous examples of enzyme-catalyzed "resolution" have appeared in the recent literature for the amino acids area (e.g. papain,[40]

lipase,[41] and α-chymotrypsin[55]), and the topic has been reviewed.[312] Rates of α-chymotrypsin-catalyzed ester hydrolysis differ for (Z)- and (E)-isomers of N-benzoyldehydrophenyalanine methyl ester, with the (Z)-acid predominating in reaction mixtures approaching completion.[313] Urethane protecting groups cause a drop in the selectivity of this process.[313] A new acylase, from *Comamonas testosteroni*, has been used for enantioselective hydrolysis of N-acyl-DL-amino acids;[314] a study of immobilized enzymes for the corresponding role in the resolution of β-(1- and -2-naphthyl)alanines has been reported.[314] Moderate enantiomeric excesses are recorded for the overall process of accumulation of D-ureido acids from hydantoins of L-α[ω]-di-amino acids incubated with *Agrobacterium radiobacter*.[315]

Increasing interest is being shown in resolutions employing chiral chromatographic techniques, a subject endowed with potential commercial reward like others applicable for the resolution of amino acid racemates. Pirkle chiral stationary phases (CSP's) are well established in chromatographic resolutions, and research papers continue to record improvements in their efficiency. A review has appeared in a Symposium Volume.[316] The CSP based on N-(1-naphthyl)-D-leucine has been evaluated for the resolution of N-(3,5-dinitrobenzoyl)-DL-leucinamides,[317,318] and shows improved performance compared with N-(2-naphthyl)-D-alanine.[317] The principle is used the other way round, for the resolution of N-acetyl-DL-leucine 2-naphthylamides over an N-(3,5-dinitrobenzoyl)-L-leucine-based CSP.[319] Interactions involved have been studied, in an attempt to rationalize chromatographic elution order, through collecting physical data (including X-ray analysis) for 1:1-(S,S)- and 1:1 (R,S)-co-crystals of N-(3,5-dinitrobenzoyl)leucine N-methylamide as an analogue of the stationary phase. The spacer groups, typically long-chain aliphatic α,[ω]-diols, connecting the chiral selector group with the inorganic supports, are important in the process, as are the N-protecting groups on the amino acid moiety.[320] Four new CSP's of general form N-(3,5-dinitrobenzoyl)-L-tyrosine[-$(CH_2)_3$ X$(CH_2)_3$ Y-]NHMe have been evaluated for amino acid enantiomer recognition.[321]

Studies employing related principles are featured in separation of DL-N-(2,4-dinitrophenyl)amino acids over a β-cyclodextrin-bonded stationary phase,[322] similar application of crown-ether-bonded columns,[323] and uses of synthetic polymers imprinted through having a derivative of an enantiomer of an amino acid present from the start of the polymerization, and washed out from the polymer at the end (see Vol.23, p.43).[324] Ligand-exchange chromatographic techniques also offer chiral discrimination possibilities, shown in a multi-gram scale resolution of DL-amino acids[325] and in an analytical scale application, monitoring of

the production of L-alanine from DL-aspartic acid by *Pseudomonas dacunhae*.[326] A standard technique, exemplified in resolution of chiral β-amino acids,[327] uses N-dodecyl-hydroxy-L-proline bonded to C-18 silica with a copper(II) acetate buffer, but there are numerous variations of this protocol. Thus, copper(II) acetate and 5′-guanosine monophosphate or cyanocobalamin have been shown to effect resolution of DL-^{3}H-labelled α-amino acids,[328] and copper(II) salts of ribonucleic acids have been used as components of the mobile phase.[329] This last-mentioned study was stimulated by the possible role of homochiral polynucleotides in bringing about the dominance of L-amino acids from prebiotic times, an aspect of resolution featured in the next Section of this Chapter. This explains why there are certain other unexpected aspects to this study, such as the discovery that DNAs work as well as RNAs in this respect, and that L-amino acids seemed to give more stable complexes since they were eluted more slowly than their D-isomers.

4.18 *Models for Prebiotic Enantioselection Relating to α-Amino Acids*

This topic, formerly located within the preceding Section, has strongly established strands of enquiry, and is now given its own identity within this Chapter.

A major prerequisite for a quantum physics background might appear necessary, in coming to terms with currently-discussed models accounting for the discrimination in the contemporary biosphere in favour of L-enantiomers of α-amino acids. However, the basis of the electroweak theory (3×10^{-19} eV energy difference between D- and L-enantiomers due to parity violation) is relatively accessible to all, and has been authoritatively reviewed,[330] even if it is not accepted by all.[13] From this starting point, a specific enhancement factor (i.e. a phase transition into a condensed Bose mode) has been proposed.[331] This, with co-operative and condensation phenomena, could give rise to second-order phase transitions (including equilibration of D-isomers into their L-counterparts) below a critical temperature T_c. This would provide a novel amplification mechanism to transform racemic amino acids into their L-enantiomers. This offers a target, the determination of T_c, that might be the subject of experimental study,[332] though it is considered[331] that the value for T_c might be too low plausibly to account for the occurrence of the process on planet Earth.

The two strands – how did the first small stereochemical bias arise? how did this become amplified? – continue to be fertile fields for speculation and controversy involving different areas of science, and do not depend on the ever-more-enclosed quantum mechanical debate. Long-running theories, all based as they must be on indisputable factors in the

prebiotic environment, continue to be put forward in new forms. It has been suggested[333] that in the course of a day, the planet's surface is bathed in sunlight with a slight predominance of left circularly-polarized light in the morning. In the afternoon, the predominance switches to right circularly-polarized light and because the temperature on the planet's surface is now higher on average than in the morning, chemical reactions will proceed faster (including the destruction of the D-enantiomer within samples of racemic amino acids). The related theory, in which radiolytic or electromagnetic radiative destruction of one enantiomer is proposed to be greater than that of the other, has featured in another new contribution to the debate. Since bi-molecular interactions between two like enantiomers might be such as to suppress their photodegradation, while interactions between opposite enantiomers are likely to be at some different level, there should be a consequence in the selective destruction of one enantiomer faster than the other.[334] This has aspects reminiscent of the respected Frank mechanism (spontaneous chiral selection; see Vol.22), which has now been extended to allow for the racemization that might accompany any amplification mechanism[335] – whether any amplification might be extinguished, or enhanced, as a result of racemization, depends on relative rate constants.[335]

5 Physico-Chemical Studies of Amino Acids

5.1 *X-Ray Crystal Structures*

Familiar amino acids and simple compounds derived from them continue to be subjected to X-ray crystal structure determination, because information on their solid-state structures is relevant in a number of contexts, including the behaviour of heterogeneous systems incorporating amino acids.

Single crystal X-ray analysis of L-alanine has been interpreted to give its total electronic charge density at 23K.[336] More routine motives (solid-state conformations, especially of side-chains) lie behind X-ray studies of DL-histidinium dinitrate,[337] L-lysinamide dihydrochloride,[338] calcium bis-L-pyroglutamate and lithium L-pyroglutamate,[339] N-acetyl-DL-alaninamide and N-acetyl-DL-leucinamide,[340] N-benzoyl α-hydroxymethyltyrosine,[341] and α-hydroxymethyl aspartic acid.[342] In the two last-mentioned cases, assignment of absolute configuration [(+)-isomers have the R-configuration] was the objective, as was the case in an X-ray study of methyl (2R,3S)-N-benzoyl-3-phenylisoserinate.[343] Thialysine hydrochloride[344] provides a further example outside the immediate protein amino acid family.

5.2 *Nuclear Magnetic Resonance Spectrometry*

Proton n.m.r. studies of a non-routine nature concern the interpretation of $^{1}H - {}^{1}H$ coupling constant data for serine, cysteine, and selenocysteine in every conceivable protonation state,[345] and a similar objective for thiazolidine-4-carboxylic acid, assisted by i.r., Raman, and ^{13}C-n.m.r. data.[346] ^{1}H-, ^{13}C-, and ^{17}O-N.m.r. data have assisted X-ray structural studies of calcium L-pyroglutamate.[339] Solid-state high-resolution ^{1}H-n.m.r. of glycine, alanine, N-acetylglycine, and histidine hydrochloride using CRAMPS reveal characteristic line shapes for ^{1}H bonded to ^{14}N, explained by ^{14}N quadrupole effects on $^{14}N - {}^{1}H$ interactions.[347]

Absolute configurational assignments dependent on ^{1}H-n.m.r. measurements have been reported for methyl (S)-(+)-mandelate esters of N,N-dimethylamino acids,[348] confirmed with interpretation of corresponding data for p-nitroanilides of these amino acids in chiral solvents. A modified Mosher method has been developed further, in which shift values for protons in N-(2-methoxy-2-phenyl-2-trifluoromethyl)acetyl derivatives of amines and amino acid esters are determined. A putative extended conformation for these derivatives[349] (CF_3 coplanar with the carbonyl of the chiral acyl group) for chiral amine derivatives of known configuration accounts for consistencies seen in shift values, and establishes a method for configurational assignments that requires less than 0.1 mg of an amino acid. The n-butylamide of (S)-2-(phenylcarbamoyloxy) propionic acid is a suitable chiral solvating agent for determining the enantiomeric composition of N-(3,5-dinitrobenzoyl)amino acid methyl esters.[350]

^{2}H-N.m.r. combined with m.s. data have been used to assess isotope distribution at different locations within amino acid molecules.[351] Glutamic and aspartic acids, alanine, proline, and lysine from different origins show wide variations. ^{13}C-N.m.r. data for aqueous solutions of L-lysine together with a chiral lanthanide shift agent, confirm the adoption of an extended conformation.[352] At a more sophisticated level, ^{14}N – dipole-coupled ^{13}C-n.m.r. powder spectra have been interpreted to give the ^{13}C chemical shift tensor for the indole C-2 in tryptophan.[353]

^{17}O-N.m.r. line widths for carboxy groups of protein amino acids in ^{17}O-enriched water establish the relative hydration numbers for their cationic, anionic, and zwitterionic forms.[354] ^{19}F-N.m.r. data have been determined for diastereoisomeric inclusion complexes formed between fluorinated amino acid derivatives and α-cyclodextrin.[355]

5.3 *Optical Rotatory Dispersion and Circular Dichroism*

Routine spectropolarimetry underpins a study of the dependence of optical activity on structure for L-cysteine, L-histidine, and L-tyrosine.[356]

Chiral aggregates are concluded to be formed in ethanol solutions of N-octadecanoyl-L-glutamic acid, since the positive c.d. feature at 215 nm is replaced by a negative feature at 205 nm in aqueous sodium dodecylsulphate solutions of the same solute.[357] An ordered structure is also claimed for N-acetyl-L-prolyl-D-alanine methylamide in 2,2,2-trifluoroethanol.[358] A more targeted approach is seen in establishment by c.d. of aggregation phenomena involving the L-alanine amphiphile $H_2O_3P(O)$-$(CH_2)_5$-O-p-(C_6H_4)-N=N-(p-C_6H_4)-CO-NH-CHMe-CO-O-$(CH_2)_{11}$Me.[359] A particular feature of interest in this last-mentioned study is the helical bilayer membrane-forming propensity shown by this derivative.

Raman optical activity spectrometry is developing steadily into a technique where the collection of high quality spectra is a routine matter, a point made in a paper dealing with L-alanine.[360]

5.4 *Mass Spectrometry*

The electrospray technique dominates the current non-routine literature in this area, with much remaining to be discovered so as to clarify the physical basis of ionization achieved in this way. As a contribution to this problem, the establishment of a relationhip between log (relative intensity) for ions of protonated amino acid molecules and their standard hydration free energies, is consistent with the ion evaporation theory.[361] A later paper from the same workers[362] modifies the relationship to the difference between hydration free energies and gas-phase binding free energies. Comparison of the atmospheric pressure spray and electrospray techniques has been reported for glycine.[363] While intense ions in the molecular ion region are seen in the spectra for glycine from both methods, atmospheric pressure spray leads to $[M + Na]^+$, $[M + K]^+$, and $[M + H]^+$ ions while electrospray yields only $[M + H]^+$ ions. Electrospray techniques offer several useful advantages over classical ionization techniques for amino acids and peptides,[364] but may give charged clusters; ion masses corresponding to up to 24 molecules have been recorded for arginine.[365]

Positive ion chemical ionization mass spectra of didehydroamino acids give more satisfactory results than those from other conventional ionization modes, and are suitable for structure assignments.[366]

Chemical ionization (isobutane) mass spectra of cyclic D- and L-α-amino acids derivatized with homochiral reagents make interesting comparison.[367] Characteristic ions for diastereoisomers of one configurational type consistently appear to be more abundant in mass spectra, and this opens up a use of mass spectrometry for assignment of absolute configuration using derivatives well-known for this purpose employing

spectrometric techniques based on absorption of electromagnetic radiation.

5.5 *Other Spectroscopic and Related Studies*

Infra-red studies with a traditional objective have established intermolecular hydrogen bonding in Boc-glycine N,N-dimethylamide through its influence on amide absorption features.[368] More sophisticated u.v.-resonance Raman studies applied to N-acetylamino acid amides[369,370] and prolinamides[371] continue to develop new structural insights, including a revision of long-standing dogma that the position of the amide II′-like band is diagnostic of the cis:trans ratio for the amide bond in proline derivatives.[371] A new u.v.- resonance Raman technique used to determine relative Raman intensities as a function of the refractive index of the liquid medium has employed N-acetyl-L-tyrosinamide for comparison purposes.[372] Raman spectra of [N-^{2}H]-labelled histidine salts and C-2 – ^{2}H analogues have shown their usefulness for assessing parameters of hydrogen-bonding in the solid state,[373] information that is potentially transferable to hydrogen-bonding interactions in proteins.

L-Phenylalanine and its methyl ester, derivatized with the nitroxyl spin label 2,2,5,5-tetramethyl-1-oxypyrroline-3-carboxylic acid, yields ENDOR spectra interpreted in terms of conformational details.[374]

Electron diffraction data provide a more traditional basis for gas-phase conformational information, applied to DL-alanine (a unique conformation for the neutral tautomer)[375] and to glycine (a planar structure with OH and NH_2 groups in an anti-relationship).[376]

5.6 *Physico-Chemical Studies*

What might be called 'the thermodynamic properties of wet amino acids' have obvious biological interest since that is one of the normal viewpoints taken *in vivo* by nearby molecules, small and large. It is a growing topic area for this reason, and also because valid data are easily acquired with simple apparatus. Studies of amino acids in homogeneous media include the simplest solubility studies, e.g. of domoic acid,[377] highly relevant information because this proline derivative is a seasonally-dangerous marine toxin (this background is fully described in Ref.179). Solvation of amino acids and small peptides has been reviewed.[378] Hydrogen-bonding pairing (65) involving N-succinoyl L-proline,[379] is revealed by the 32-fold alteration of the amide cis:trans ratio after presentation of the hydrogen-bonding partner. Dissociation constants for valine and norvaline[380] and the corresponding data for the alanine zwitterion together with thermodynamic parameters,[381] have been determined by conductimetric methods; thermodynamic aspects have also been the

primary interest in related calorimetric studies of acid-base reactions in solutions of DL-threonine,[382] and of N-protonation of histidine and other imidazoles.[383]

Determinations of partial molar heat capacities and partial molar volumes,[384] and of enthalpies of interaction,[385] for N-acetylamino acid amides, are accompanied by enthalpies of dilution studies of N-acetyl derivatives of sarcosine and N-methylalanine amides.[386] The last-mentioned topic area has its own fascination in establishing chiral recognition phenomena by continuing studies[387] of ternary aqueous solutions containing two different aliphatic amino acid derivatives of the same or different chirality (see Vol.23, p.49).

A different principle is involved in a study of proton transfers involving amino acids in aqueous solutions using ultrasonic velocity and absorption data.[388]

Strong inclusion complexes are formed in aqueous solutions between amino acids such as DL-tryptophan or DL-tyrosine, or to a lesser extent, DL-phenylalanine, and the rigid cyclophane (66) carrying two acceptor paraquat groups facing each other.[389] The binding constants are two orders of magnitude greater than those involving simple electron acceptors such as methylviologen. Heterogeneous systems are represented in partition and distribution coefficient measurements for amino acids in 1-octanol – water,[390] and transfer free energies of ionic amino acid derivatives (of aspartic and glutamic acids, lysine and arginine) in the same medium.[391] From such distribution coefficient data it can be inferred that amino acids are transferred as their hydrates, from aqueous media into lipid phases.[392]

A similar, and equally important inference,[393] has been drawn, based on comparisons of water-to-vapour and water-to-cyclohexane distribution coefficients for N-acetylpyrrolidine and N-butylacetamide. Proline residues in simple N-acyl amides including peptides and proteins must be taken to be much more hydrophilic than is generally believed.

Amino acid hydrochlorides are well transported through thin sheet supported liquid membranes (polysulphone, polyacrylonitrile, or polyethylene as support in the form of hollow fibres; and long chain alkanols and a choice from various crown ethers to form the membrane).[394] Partition studies of amino acids in micro-emulsion droplets (reversed micelles) have been described, revealing amino acids to have co-surfactant properties.[395] Stable monolayers at the air-water interface, capable of specifically binding amino acids, are formed by long chain alkyl derivatives of Kemp's acid.[396] Monolayer-forming amphiphilic amino acid esters $Me(CH_2)_{17}CH(NH_2)CO_2\,CH_2R$ (R = Ph or $CHCl_2$) must exist in an ordered state since rates of self-condensation to give peptides occur at

much faster rate in the monolayers compared with the process in non-ordered media.[397]

Solid state studies deal with the adsorption of phenylalanine and tyrosine on to activated carbon from water at various pH,[398] and the measurement of latent heat of melting of the oxygen adduct of the eutectic compound formed between NaCl and water in the presence of an L-amino acid (leucine, threonine or aspartic acid).[399] It is puzzling to consider that the value obtained is 5KJ mol^{-1} NaCl lower than that found when a D-amino acid is present.

5.7 *Molecular Orbital Calculations*

The mainstay for this Section over the years continues to be the application of various self-consistent field models to N-acylamino acid N-methylamides.[400-405] However, an authoritative review has now highlighted both the efficiency and limitations of these compounds as models of protein segments, and therefore the limited relevance that such calculations might have in the conformational analysis of peptides.[406] Among the research papers, specific objectives include study of N-alkylamino acid derivatives,[400,401] comparison of hydrated and non-hydrated states from the point of view of changes in free energy and hydration free energy,[402] and consideration of statine as a peptide component.[403]

Calculations relating to the underivatized amino acids have appeared for solvates of zwitterionic glycine, alanine and proline,[406] for five plausible conformations of glycine,[407] and for various models for the interaction of an amino acid with a helical structure.[408] Of course, the latter initial study can only scratch the surface as far as the multitude of possibilities is concerned, but has already shown some features of interest in terms of chiral discrimination related to the geometry of the amino acid alignment within the helix cavity. A less obscure basis is shown by calculations for complexes formed between (S)-methyl N-(2-naphthyl) alaninate and enantiomers of N-(3,5-dinitrobenzoyl)leucine n-propylamide, since they are models for putative interactions occurring on Pirkle CSP's used in chromatographic resolution.[409]

An interesting development in this topic area is the broadening of conformational calculations to compounds resulting from isosteric replacements, and the dithio-acid analogue of N-formylglycine is one such compound.[410]

6 Chemical Studies of Amino Acids

6.1 *Racemization*

There is little to report this year, on the application of amino acid

enantiomer ratios for dating relatively young fossils and similar formerly-living materials, though the subject has been reviewed,[411] and last year's review[412] gives an account of some relevant and extraordinary applications of the method.

Research papers describe long-running mechanistic interests in aldehyde-catalyzed racemization, of proline and pipecolic acid,[413] and of a series of amino acid esters.[414] In the former study, it was shown that solvent acidity affects rates, with higher acidity suppressing racemization,[413] while an unexpected rate enhancement was seen in the other study,[414] and explained to be a consequence of immobilizing of the pyridoxal used as catalyst.

Racemization of pentachlorophenyl esters of amino acids accompanying their use in dipeptide synthesis has been shown to be less than that of corresponding p-nitrophenyl esters.[415] A curious fact that may modify current theories of causes of racemization in reactions of N-protected amino acids, is the optical purity of N-acylureas formed as side-products when dicyclohexylcarbodi-imide is used in peptide synthesis to give partly-racemized peptides.[416]

6.2 *General Reactions of Amino Acids*

This, and the following Section, divide the discussion of reactions of amino acids roughly into: reactions mainly involving amino and carboxy groups (this Section), and reactions mainly involving side-chains (next Section).

Processes causing degradation of amino acids include irradiation by 3KeV helium ions (gradual carbonization of glycine monitored by i.r. spectra)[417] and thermal self-condensation.[418] The latter topic is gaining more momentum because, as well as its 'origins of life' connection, it is becoming clear that there is no uniform pattern of thermal behaviour among protein amino acids, and because mixtures of amino acids are somewhat selective in the range of peptides they form under thermal conditions. Thus, a methionine-phenylalanine bond is not formed in condensation products from mixtures containing these and other amino acids, judging by Edman degradation of cyanogen bromide-cleavage products.[418] Metal ion catalyzed self-condensation occurs under mild conditions in aqueous solutions, glycine giving mixtures of di- and tri-glycine,[419] and alanylglycine predominates in glycine – alanine mixtures containing copper(II) salts.[420] Glycine slowly forms its cyclic dimer, 2,5-dioxopiperazine, when in aqueous solution in the presence of urea,[421] and if alanine is also present, the aminolysis product glycylglycylalanine is formed. De-amination and decarboxylation of glycine ($\rightarrow NH_3 + CO_2$) occur in aqueous solutions containing pyrogallol, a process that is

accelerated strongly by the presence of a mineral, e.g. calcium nontronite.[422]

Further details are available (Vol.23, p.53) of the preparation of quaternary ammonium salts of amino acids, which should be of considerable value in synthesis.[423]

Reactions at the amino group involving simple processes can be followed by further transformations in the special case of α-amino acids. Kinetics of chlorination of aliphatic amino acids, represented by alanine and valine, have been studied;[424] the process leads to N-chloro- and N,N-dichloro-derivatives that decompose to give aldehydes and nitriles. Independently, N-chlorination of proline and hydroxyproline,[425a] and kinetics of the decomposition of the derivatives has been studied, and the same workers have applied themselves to the kinetics of decomposition of N-bromoleucine and N-bromoisoleucine.[425b] Diazotization of (S)-tert-leucine is followed by conversion into the α-chloro- and -hydroxy-acids, as expected, but then successively into the acid chlorides and α-chloro-amides which are sources of the elusive α-lactams, e.g. 67, through treatment with Bu^t OK.[426] Small amounts of N-t-butyl-tert-leucine t-butyl ester also appear among the products.

Radical N-methylation of Boc- and phenyloxycarbonylamino acid methyl esters is achieved in 47 – 57% yields using t-butyl perbenzoate in the presence of copper(II) octanoate (Boc-valine methyl ester does not react).[427] More conventional processes are reductive benzylation of amino acids and esters using benzaldehyde and sodium hydrotelluride,[428] and Eschweiler – Clarke N,N-dimethylation using $HCHO/HCO_2H$.[429,430] These latter reports concern unexpected reaction products with β-alanine (N,N,N-trimethylation to give the betaine[429]) and describe fragmentation products formed with polyamines.[430] In contrast with these thwarted alkylation processes, an unintended N-methylation, with some N,N-dimethylation, has occurred during hydrogenolytic deprotection operations of threonine derivative in MeOH-AcOH.[431] N-(1-Ethoxycarbonyl)-1-acetonylation of amino acid esters can be effected by their NEt_3-catalyzed addition to $HO.CHMe.C{\equiv}C.CO_2Et$ and its homologues.[432] Improved N-protecting group protocols have been reported for N-benzyloxycarbonylation of γ-benzyl glutamate and β-benzyl aspartate as their O,N-bis(trimethylsilyl) derivatives, using Z-Cl and N-methylmorpholine,[433] for preparing 4-azidomethyleneoxy-Z-protected amino acids,[434] and for polymeric reagents for 3,5-dinitrobenzoylation[435] and for the introduction of Fmoc, 4-nitrobenzoyl, and acetylsalicyloyl groups.[436]

Photolysis of N-2,4,6-trinitrophenylamino acids in weakly basic alkaline solution causes cleavage into 2-nitroso-4,6-dinitroaniline, CO_2, and the aldehyde corresponding to the decarboxylated amino acid.[437]

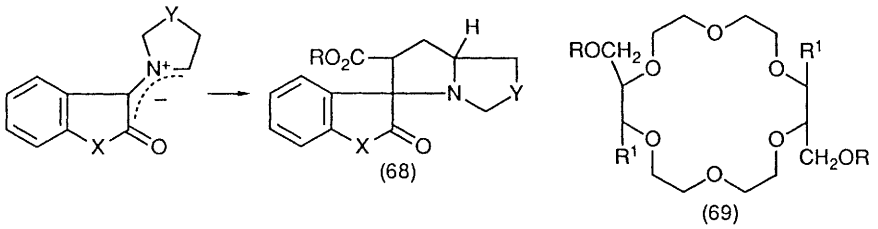
Schiff base formation between pyridoxal 5′-phosphate and L-serine, and its subsequent transamination to pyridoxamine 5′-phosphate and ketoacetate, has been subjected to kinetic study.[438] Formation equilibria for the initial step in this process have been determined for several amino acids over a range of pH.[439] In this[439] and another study,[440] spectrometric characteristics have been collected for these particular Schiff bases, including estimation by u.v. spectrometry of their tautomerization equilibria and acid dissociation constants.[439] A detailed study has appeared[441] of the oxidative deamination of (p-sulphophenyl)glycine by copper(II)-mediated Vitamin B_6 coenzymes with pyridoxal 5′-phosphate or 5′-deoxypyridoxal phosphate. The deamination product, (p-sulphophenyl) glyoxylic acid, becomes a focus in this study for a demonstration by ^{18}O-labelling that $^{18}O_2$ is a reactant and is incorporated into hydroxylamine released after conversion of its Schiff base into the oxime, followed by hydrolysis.[441] It seems reasonable from this impressive study, to assume that hydroxylamine is the precursor of the eventual product, NH_3.

[2 + 2]-Cycloaddition reactivity of chiral Schiff bases of amino acid esters have been reviewed.[442] Chiral-catalyzed addition of derived azomethine ylides to acrylic esters to give proline homologues (Scheme 31) has been explored,[443a] using $CoCl_2$ or $MnBr_2$ with (1R,2S)-N-methylephedrine; up to 96% enantiomeric excess is claimed. Isatin-derived azomethine ylides undergo decarboxylative cycloaddition to (−)-menthyl acrylate to give (68).[443b] These extend the scope of earlier results that established the wider usefulness of Schiff bases of amino acids, and Grigg's group has combined the process with a cyclization by using a mixture of N-methylmaleimide, an N-allylglycine ester, and o-bromobenzaldehyde (to provide the intermediate azomethine ylide that undergoes cycloaddition to N-methylmaleimide, but with the bromine atom to provide the point for cyclization to the allyl moiety). Catalysis by $Pd(OAc)_2$ /Ph_3 P/Et_4 NCl/K_2 CO_3) is involved, and gives a product with four chiral centres.[444]

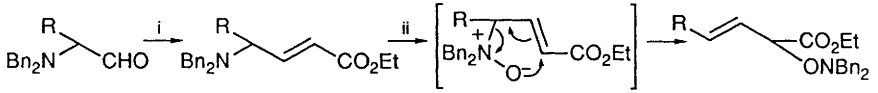
Extensive studies of Maillard reactions between amino acids and carbohydrates continue to give increasing insights into this most complex of processes. The Schiff base formed between glucose and proline rearranges in the usual way to the fructose – proline Amadori product, which is the main intermediate from which ensuing steps develop, leading to numerous products.[445] About 40 compounds were identified in this study, and a detailed study of products from glucose – N^{α}-protected lysine reaction mixtures has been published.[446] The mechanistic detail of the release of ammonia from the Amadori product is one of the difficult problems, and some attention is given to this aspect in this[445] and in other recent papers. 1,2- and 1,3-Enolization of the open-chain form of the

Reagents: i, $CH_2{=}CHCO_2R$, (1*R*, 2*S*)-*N*-methylephedrine

Scheme 31

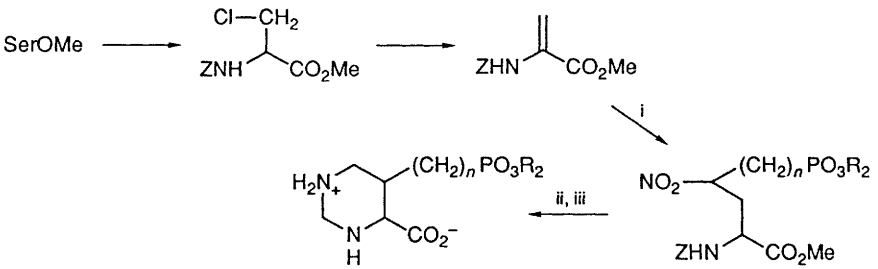


(68) (69)



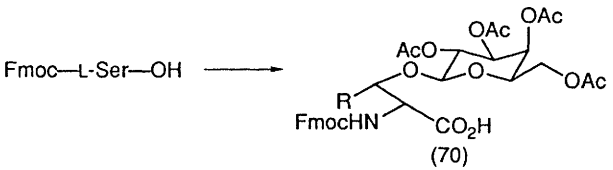
Reagents: i, see *Angew. Chem.*, 1987, **99**, 1186; ii, *m*-chloroperbenzoic acid/–50 °C

Scheme 32



Reagents: i, $NO_2(CH_2)_{n+1}PO_3R_2$-KF/Al_2O_3; ii, H_2/10 % Pd-C, HCl;
iii, $AcOCH(OEt)_2$ and routine steps

Scheme 33



(70)

Amadori compound is followed by dehydration, and then an avalanche of processes, in the currently-adopted mechanism, but it has long been realized that this does not adequately account for most of the ultimate products. An alternative dehydration mode involving cyclic forms of the Amadori compound is suggested.[447] Another interesting development is the verification of azomethine ylide behaviour by isolation of cycloadducts with the dipolarophile, norbornene, targeting the pyrylium betaines derived from initially-formed Schiff bases (Schiff bases are already known to exhibit this reactivity profile – see preceding paragraphs).[448] The original amino acids can be recovered from the fructose -β-alanine, -phenylalanine, and -N^{α}-Boc-N^{γ}-fructosyl-lysine Amadori compounds, by oxidation in the presence of copper(II) salts to release D-arabino-hexos-2-ulose.[449] The generation of fluorescent compounds and non-enzymic browning processes, based on interactions of ascorbic acid with amino acids, has been surveyed.[450] Furfural formed between L-ascorbic acid and simple amino acids accounts for the 'browning reaction' products through further condensations with the amino acids.[451] The Maillard process has been reviewed from the carbohydrate point of view, concentrating on the behaviour of 3-, 4-, and 1-deoxyosones in the presence of particular amino acids and amines.[452] The physiological role of the Maillard reaction is also important, especially in protein crosslinking processes, and the formation of the recently-discovered fluorescent crosslinking residue, pentosidine (containing the 2-amino-imidazo[4,5-b]pyridinium chromophore) in human extracellular matrix protein has been modelled with D-ribose and N^{α}-Boc-L-lysine and N^{α}-Boc-L-arginine.[453] Ribated (sic!) Boc-lysine gives a compound identical with pentosidine, which could also be obtained using glucose or ascorbic acid as carbohydrate[453] (note the link with work outside the physiological context[450]).

Relatively superficial reports still appear in the food chemistry and in the physiology contexts, but are rarer, as shown in an excellent overview of the presently greater chemical sophistication seen in the current approaches to this topic.[454]

Reactions at the carboxy group of amino acids include routine hydrolysis of Schiff base methyl esters, and re-alkylation with an alkyl halide, with the purpose of establishing the survival of the Schiff base function through these steps.[455] Crown ethers have been shown to enhance α-chymotrypsin-catalyzed transesterification of N-acetyl-L-phenylalanine ethyl ester in isopropanol,[456] while ethyl ester formation from N-protected tyrosines can be effected easily by suspending α-chymotrypsin in 95% ethanol containing an appropriate buffer.[457] Industrial-scale Fischer-type synthesis of L-phenylalanine methyl ester hydro-

chloride has been described, using DL-phenylalanine and (−)-camphor-10-sulphonic acid.[458]

In the biosynthesis of proteins, each amino acid passes from the aminoacyl adenylate to become an amino acid ester, and finally a 2′(3′)-peptidyl ester of AMP at the end of a tRNA. Because of the stereochemical situation, it would be expected and has now been established, that bis(2′,3′-aminoacyl)esters of AMP should react faster with N-acetyl-L-phenylalanine than with its D-isomer.[459] Conventional methods for preparing active esters of N-protected amino acids for use in peptide synthesis are led by dicyclohexylcarbodi-imide condensation of the acid with the alkanol or phenol. However, a way of avoiding the use of this reagent exploits the high acylation reactivity, free from side-reactions, recently established for Fmoc-amino acid chlorides.[460] While DHBt esters are made in a simple one-pot way, from the Fmoc-amino acid, thionyl chloride, and HODHBt, in the case of pentafluorophenyl esters a workable procedure requires separation of the acid chloride formation step from the acylation step.

An alternative method to that recently established ($SOCl_2$) for the preparation of Fmoc-amino acid chlorides involves reaction of an Fmoc-amino acid anhydride with anhydrous HCl, though some contamination with simple esters is inevitable, as the alkanol liberated along with the displaced anhydride grouping reacts with other components.[461] These acid chlorides are sensitive to atmospheric moisture,[462] and corresponding fluorides, which are readily prepared from Fmoc- and Z-amino acids using cyanuryl fluoride,[462] are more stable. Polymers prepared from bis(N-chloroformylmethyl)pyromellitimide bis(acid chloride)s in which the acid chlorides are based on amino acids, have been described.[463]

Cleavage by $Bu_4N^+F^-$, of 4-nitrobenzyl, 2,2,2-trichloroethyl, and phenacyl esters of N-protected amino acids,[464] and use of aqueous alcoholic alkali metal carbonates (*e.g.* Cs_2CO_3) and bicarbonates for cleavage of methyl, ethyl, and benzyl esters,[465] are useful practical procedures. The extensive studies of recent years (Vol.23, p.59) of enantioselective hydrolysis of N-acylamino acid esters continues with results using aqueous emulsions containing Z-L-histidyl-L-leucine as chiral catalyst, with a series of long chain N-acyl D- and L-phenylalanine p-nitrophenyl esters[466] and Z-L-leucine p-nitrophenyl ester[467] as substrates. A thoughtful approach to the former system[468] leads to the suggestion that the micellar interface discriminates between transition states that have different hydrophilic and hydrophobic properties. Thiolysis of hydrobromides of amino acid p-nitrophenyl esters is more effectively catalyzed by bridged crown ethers (69; 2R = p-CH_2-C_6H_4-CH_2-, R′ = Me or CH_2SH) than by unbridged analogues (R = 2-MeO-C_6H_4-).[469]

Reduction of carboxy functions is represented in the preparation of β-aminoalkanols from N-protected amino acid mixed anhydrides using $NaBH_4$ in an aqueous organic medium (see also the same results of G.Kokotis cited in Vol.23, p.58),[470] and in polarography of chiral metal complexes *fac*[Cr(L-aminoacidato)$_3$], in which the first reduction wave at the Hg surface in the presence of the tetraethylammonium ion is more positive for the (+)-isomer.[471]

Homochiral α-amino-aldehydes have been mentioned several times earlier in this Chapter, and as conveniently-available compounds nowadays, they are useful in Wittig reactions leading to γ-amino esters[472] that undergo [2,3-[σ]] rearrangement after N-oxide formation (Scheme 32) to give homochiral α-aminoxy esters.[473]

Oxidative decarboxylation of N-acylamino acids with lead tetraacetate followed by quenching with MeOH gives N,O-acetals RCONH.CHR′.CH(OMe)NHAc which can be used in α-amidoalkylation reactions with Me_3 SiCN to give α-amino nitriles.[474] There is some mechanistic interest involved in this process, since diastereoisomeric excesses in the range 5 – 72% are obtained with homochiral amino acid derivatives.[474] The ninhydrin reagent protocol has been modified for use in t.l.c., by preceding its use by a (+)-camphor-10-sulphonic acid spray, and heating the plates;[475] this produces a range of distinctive colours rather than the familiar, relatively uniform, blue-purple of the usual ninhydrin system. Since it is claimed that the colours appear in the cold, from 0.4 – 2.0 μg samples, no doubt some will be tempted to try to repeat this strange protocol. It is incorrectly claimed in this paper that secondary amines are formed from amino acids in this procedure.

α-Amino acids are useful sources of heterocyclic compounds,[476] and examples additional to those already cited above, illustrate 4-alkyloxazol-5(4*H*)-one formation from mixed anhydrides of N-formylamino acids (prepared using isopropenyl chloroformate with N-methylmorpholine),[477] and from N-acylamino acids using cyanuric chloride and triethylamine[478] (see above, for discussion of acid chloride formation with this reagent). Since oxazolones are implicated as side-reaction-introducing species in peptide synthesis, their aminolysis reactions are of considerable interest; diastereoisomer ratios disclosed for dicyclohexylcarbodiimide coupling products of N-benzoyl-L- or D-amino acids with L-amino acid esters give false indications of the racemization accompanying this standard peptide bond-forming procedure, considered to be introduced through oxazolone intermediates, unless corrected for asymmetric induction.[479] Easy assessment of diastereoisomer ratios is possible using h.p.l.c.[480] 2-Phenyloxazolones give N-benzamido-acyl 2-thiothazolidines, usable in peptide synthesis, through reaction with 2-thiothiazolidine in

boiling dichloromethane in the presence of NEt_3.[481] Oxidative dimerization of oxazolones, giving 4,4′-bis-oxazolones, can be achieved using nickel peroxide or DMSO.[482]

N-Protected oxazolidines are readily prepared from N-protected amino acids by $LiAlH_4$ reduction and *in situ* condensation of the resulting β-amino alkanols with a carbonyl compound.[483] Condensation of an N-arylalanine methyl ester with an isocyanide gives an imidazolidin-2,4-dione,[484] while N-sulphonylisocyanate gives 1,2,5-thiadiazolin-1,1-dioxides.[485] Heterocyclic synthesis from higher homologous amino acids includes β-amino acid cyclodehydration to β-lactams (methanesulphonyl chloride/$NaHCO_3$ /MeCN/80°),[486] and active ester cyclization of γ-keto-δ-amino acids to piperidin-2,5-diones.[487]

The flow of routine, often repetitive, work on simple oxidation processes involving amino acids, continues with accounts of peroxomonophosphoric acid,[488] alkaline hexacyanoferrate(II),[489] and electrooxidation at a Pt electrode.[490] Potassium permanganate continues as front-runner in this pack,[491-494] with attention to autocatalytic effects of colloidal MnO_2[491] and Mn(II),[492] Mn(III) and Mn(IV)[493] species. An exceptional interest attaches to a study of the oxidation of amino acids by Fenton's reagent [H_2O_2 and an Fe(II) salt] leading to the expected products NH_4^+, α-keto-acid, and CO_2, but also to oximes and aldehydes, and the carboxylic acid containing one fewer carbon atom than the starting amino acid.[495] The process has a dependence on bicarbonate ion and appears to involve an undefined iron chelate with an (undefined) role.

6.3 *Specific Reactions of Amino Acids*

Some reactions that are specific to a particular amino acid are occasionally extendable to homologues of that amino acid, though the distinctive functional group distribution in the familiar protein amino acids usually ensures a unique profile for each structural type. The aliphatic side-chains might have been expected to show a general group of radical substitution reactions, but there are structural influences such as the finding that phthaloylamino acids show much less α-halogen substitution than corresponding N-acylamino acids.[496] Regioselective H-transfer from β- and γ-positions of N-acetylvaline and from the N-methyl group of N-acetylsarcosine has been established by e.p.r., providing direct evidence of polar effects in radical reactions of amino acid derivatives.[497] New results indirectly demonstrating α-hydroxylation of α-amino acids, a key step in one theory for C-terminal amidation of peptides, relate to copper(II)-mediated oxygenation of N-salicoylglycine.[498] Although this substrate is a poor model for a peptide, more support is given for

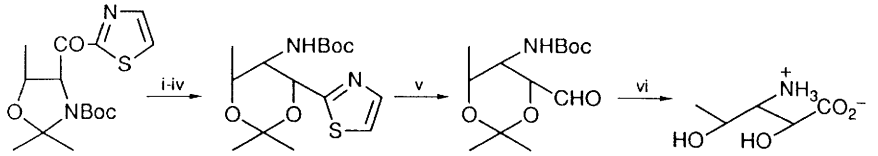
non-enzymatic processing in accordance with the original proposal for the biogenesis of peptide amides.[499]

Michael addition reactions to dehydroalanine have proved useful over the years, and another example is a synthesis of 6-phosphono-alkyl tetrahydro-4-pyrimidinecarboxylic acids (Scheme 33) as NMDA receptor antagonists.[500]

A surge of papers relating to side-chain hydroxy groups is mostly accounted for by growing interests in glycosylated amino acids, especially in protected versions suitable for peptide synthesis. β-Glucosidase from almonds, and β-xylosidase, have been employed in preparations of β-glycosides from N-acetyl-L-serine methyl ester.[501α-502] and β-Galactosidases[502,503] have been used in the corresponding transglycosylations from lactose[502,503] and raffinose,[503] employing mild deprotection procedures. Non-enzymic procedures require protection of the carbohydrate moiety, illustrated in $SnCl_4$ -catalyzed transglycosylation (Fmoc-L-serine→70),[504] and a variation of the Koenigs-Knorr process with a glycosyl bromide/AgOTfl/L-serine methyl ester Schiff base.[505] In the last-mentioned study,[505] the interesting suggestion seems to be validated, that a hydrogen bond between the side-chain OH and the imine nitrogen atom of a serine Schiff base increases the nucleophilicity of the oxygen lone pair, thereby catalyzing electrophilic attack as required in non-enzymic O-glycosylation.

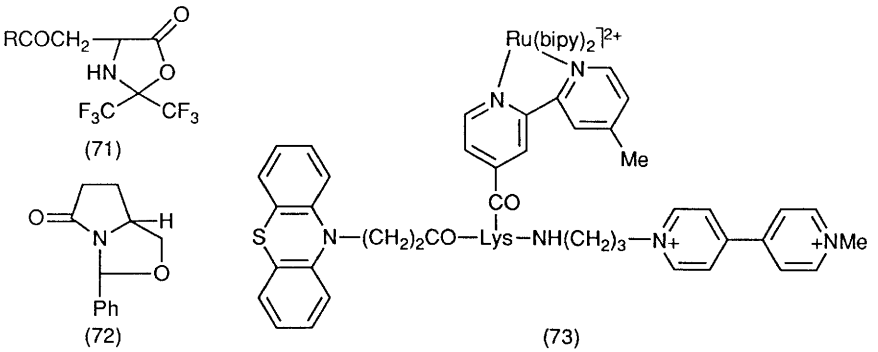
O-Phosphorylation methodology for serine, threonine and tyrosine continues to be developed for similar reasons to those stimulating glycosylation studies, a recent example being (S,S-diaryl)phosphorodithioylation.[506] O-t-Butylation of N-Fmoc derivatives has been effected for methyl esters of serine and threonine using isobutene and toluene-p-sulphonic acid (H_2SO_4 catalysis for non-esterified Fmoc-tyrosine), followed by mild, non-racemizing, ester saponification (NaOH or Na_2CO_3).[507] The overall process is suitable for large scale operations, improving on the current route based on benzyl esters.

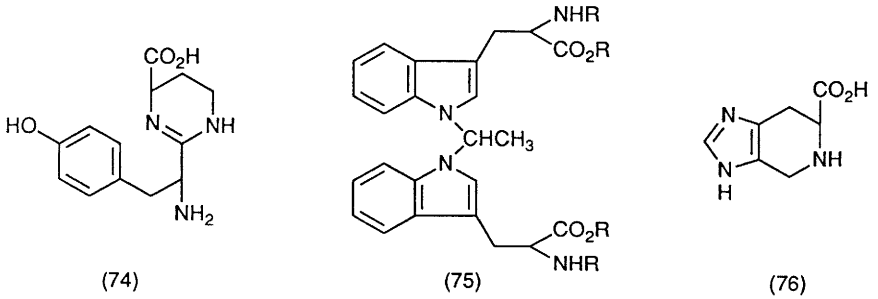
"Homologation of L-threonine", a somewhat inaccurate term used[508] for a procedure (Scheme 34) based on oxazolidin-4-yl thiazol-2-yl ketones, depends on the use of the thiazole as a latent aldehyde function.[508] Poly(ester)s $[-OCH_2\ CH(NHZ)CO-]_n$ (n [≃] 100) are obtained by heating benzotriazolyl esters of Z-serine at 105° during 3h.[509] A lengthy process involved in bridging two protected serine molecules through side-chain hydroxy groups with $-CH_2CH_2-$ starts with the conversion of N-tritylserine methyl ester into the aziridine; in this and in other steps, the synthesis is based on routine methodology.[510] L-Homoserine lactone has been prepared from L-aspartic acid through selective reduction with $LiBH_4$.[511] N-Benzoyl [3,4-$^2H_2$]-L-homoserine, subjected to 6M-HCl hydrolysis (reflux 14h) and conversion into its 3,5-dinitrobenzoate was

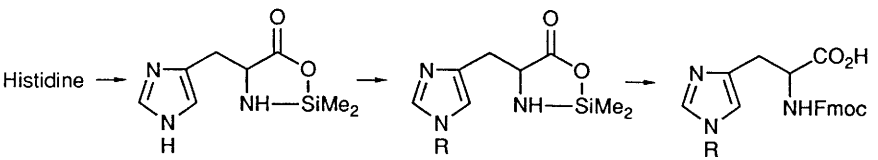


Reagents: i, TFA-CH_2Cl_2; ii, TBDMSCl; iii, L-Selectride/–78°C, then $Bu^n{}_4NF$, room temp; iv, $Me_2C(OMe)_2$, (+)-camphor sulfonic acid; v, hydrolysis; vi, [O], then TFA, then acetal cleavage

Scheme 34







Reagents: i, Me_2SiCl_2; ii, MeOTrCl, or MeTrCl (R = methoxytrityl or methyltrityl, respectively); iii, H_2O, then FmocCl

Scheme 35

surprisingly epimerized at C-4, but not at C-3.[512] The possible explanation, cyclization involving the benzoyl group (to give a 2-phenyloxazole) has been discussed.

No tritylamide derivatives of amino acids have so far been reported, but they may be prepared using triphenylmethanol with $Ac_2O/AcOH$; N-tritylasparagines and other derivatives are stable to strong mineral acids in aqueous media but may be cleaved with trifluoroacetic acid.[513] 1-Glycosylamines [easily prepared by dissolving a reducing sugar in saturated aqueous $(NH_4)_2CO_3$] have been condensed with α-t-butyl β-pentafluorophenyl Fmoc-L-aspartate to give N^{β} -glycosides of Fmoc-L-asparagine for use in peptide synthesis.[514]

L-Aspartic acid condenses with hexafluoroacetone to give the oxazolidin-4-one (71), proposed to be a useful synthon for regiospecific reactions leading to α- or β-substituted aspartates.[515] D-Isoglutamine, a component of peptidoglycans of the bacterial cell wall, has been synthesized from dicyclopentyl D-glutamate through lipase-catalyzed selective hydrolysis of the side-chain ester group followed by ammonolysis of the other ester group.[516] A useful recipe for the preparation of γ-benzyl Boc-L-glutamate that avoids the pyroglutamic acid side-reaction involves minor changes to an established procedure.[517] L-Pyroglutamic acid continues to provide the starting point in syntheses of interest to several research groups. The derived acetal (72) is readily prepared, and has been used in syntheses of 2,4- and 2,3,4-substituted pyrrolidinones.[518] The related pyroglutaminol (58), as its N-Boc O-TBDPS derivative, has been shown to be susceptible to stereoselective functionalization at C-3 and C-4.[519] After introduction of a C-3 – C-4 double bond (LDA/PhSeCl), 3,4-dihydroxy- and 4-methyl-derivatives were prepared by standard methods; alternatively, 3-hydroxylation could be accomplished (LDA/MoOPH). Methylation (Me_2SO_4/K_2CO_3) gives methyl N-methyl-L-pyroglutamate, and methyl L-pyroglutamate with $MeOCH_2$ OMe/$MeSO_3$ H gives a mixture of the N-methoxymethyl derivative and the bis[N,N-(methyl pyroglutamyl)]methane.[520]

Biogenetic interests are reflected in a study of the finer details (oxidative decarboxylation involves loss of the 3-pro-(R) proton, *i.e.* anti-geometry for the eliminated atom and group) of the conversion of (2S,6R)-(−)-S-(2-carboxypropyl)cysteine into trans-S-1-propenyl-L-cystine sulphoxide.[521] S-Protected cysteines are converted into cystines through reaction with sulphoxides and trimethylsilyl chloride in TFA.[522,523] Simpler methods are involved in reactions with NN′-diacetyl L-cystine bismethylamide (thermolysis gives trisulphides; aqueous alkali causes elimination to dehydroalanine).[524] Tyrosinase catalyzes attack by cysteine through the side-chain sulphur atom, at the 4-position of 5,6-

dihydroxytryptamine;[525] in the absence of the enzyme, 5,6-dihydroxytryptamine protects cysteine against oxidation to cystine, because it is more readily oxidized to the o-quinone.

Lysine is represented in detailed recipe for conversion into the N^{ε}-Z-derivative of its methyl ester through successive copper complexation, reaction with ZCl, and MeOH-$SOCl_2$ esterification[526] (errors in this account are corrected later[527]). Extraordinary lysine derivatives, the (εεε-tribenzyl-EDTA) ester of Boc-L-lysine,[528] and the heavily-loaded amino acid (73),[529] have been prepared. The latter derivative in MeCN displays net electron transfer between the bipyridinium group and the phenothiazine moiety when subjected to 6ns 460 nm laser pulses.

One-pot syntheses in about 50% yields, of N^{3} -Boc-$N^{\delta}N^{[\omega]}$-di-Z-arginine and of tri-Z-arginine have been reported, based on the reaction of ZCl with appropriate di- and tri-N-trimethylsilyl derivatives.[530] Established routes enjoying detailed description concern synthesis of side-chain Pmc-protected arginine,[531] and multi-gram synthesis of $N^{[\omega]}$-methyl arginine (in the classical way from ornithine and $MeNH.C(SMe)=NH_2^+ I^-$ in the presence of aqueous NaOH).[532] Lysine is the starting material in a synthesis of Boc-homoarginine through regiospecific amidation with $EtN=C(NHEt)SO_3H$.[533] Synthesis of $N^{[\omega]}$-hydroxy-arginine from N-Boc-ornithine t-butyl ester, proceeds *via* the thiourea [-NH_2 with $CSCl_2 \rightarrow$-NH.CS.$NH_2 \rightarrow$-NH.C(=NOR).NH_2],[534] or alternatively[535] -$NH_2 \rightarrow$-NHC≡N with BrCN, $\rightarrow$NHC(=NH)NHOH. This arginine derivative has created interest as the source, in tissue, of the vasorelaxing agent nitric oxide; the work of D.J.Stuehr and co-workers on this topic has been surveyed.[536]

Aromatic and heteroaromatic side-chain modifications have been mentioned in earlier Sections (4.11 and 4.15) of this Chapter, a spectacular example[38] being the biogenesis from L-tyrosine, of the pyoverdin chromophore (19) *via* (74) in *Pseudomonas fluorescens* E2.[537] Mass-spectrometric confirmation of the structure has been published.[538] The propensity to form 1,1′-ethylidene bis(L-tryptophan) (75) with acetaldehyde[539] led to the unfortunate outbreak of eosinophilia myalgia syndrome when this compound, Contaminant "97", was discovered in commercial L-tryptophan used as a nutritional additive in health foods.[540]

4-Methoxy- and 4-methyltrityl side-chain protection of histidine has been proposed,[541] removable under mild conditions compared with trityl groups, employing novel temporary protection of NH_2 and CO_2H groups (Scheme 35). Unprotected histidine readily forms spinacine (76) with aqueous formaldehyde, and several of its analogues have been prepared from N^{im} -benzyl-L-histidine.[542] The histidine side-chain can be degraded using Ru(VIII) reagents to cleave the 4,5-π-bond, leading to $N^{[\omega]}$-

carbamoyl-L-asparagine, $N^{[\omega]}$-formyl-L-asparagine, N^{α}-benzoyl-β-cyanoalanine, and aspartic acid.[543]

6.4 *Effects of Electromagnetic Radiation on Amino Acids*

Familiar theses for this Section continue to be represented, with studies of fluorescence quenching of dityrosine with boric acid and borates,[544] and similar quenching studies for 3-nitrotyrosine and N-acetyltryptophanamide.[545] Lumiflavin-sensitized photo-CIDNP study of tryptophan has to take account of accompanying irreversible photolysis processes since these reduce the intensity of the CIDNP.[546] Similarly, the presence of the anti-oxidant spermine suppresses the formation of u.v.-generated radicals in aqueous tryptophan,[547] as shown by e.s.r. monitoring. Generation of the azide radical, and radical anions from bromine and dithiocyanogen, has been assessed in relation to the formation of the tryptophan radical and the protonated tryptophan radical cation.[548]

7 Analytical Methods

7.1 *General*

Reviews have appeared covering recent advances[549] and broad areas: liquid chromatographic methods of amino acid analysis,[550] determination of L-DOPA in physiological fluids.[551] Certain drugs interfere with analytical methods for amino acids, particularly when blood and urine samples are involved.[552]

7.2 *Gas-Liquid Chromatography*

The topic has been reviewed.[553] Pre-treatment of amino acid samples for g.l.c. analysis has received attention,[554] and so it should, to improve reliability which so often depends on rigid protocols and calibration. n-Propyl esters are best extracted from an NH_4Cl/NH_4OH buffer with propan-1-ol – chloroform mixtures, increased extraction efficiency accompanying higher proportions of propan-1-ol. This is the typical first step in g.l.c. analysis of amino acids, and esterification is followed by N-derivatization with a variety of reagents. N-Trifluoroacetyl-,[555] pentafluoropropionyl-,[556,557] and heptafluorobutyroyl-,[558] derivatives are continuing in use after many years, and are being shadowed by N-alkoxycarbonyl derivatives[556,559-561] that have the benefit of being formed from an alkyl chloroformate very rapidly.[560,561]

Different workers distribute their favours differently as far as esterifying groups are concerned for g.l.c. analysis of amino acids, and isopropyl,[556] isobutyl,[558] propyl,[557] and methyl[561] esters are typical.

Configurational assignments based on enantioselection over chiral

g.l.c. columns have been made to N-methylphenylalanine using Chirasil-L-Val capillary g.l.c.[556] The topic of enantioselective g.l.c. of amino acids has been reviewed.[562] Other points of interest in these accounts include m.s. monitoring,[555] and a special derivatization protocol for g.l.c. analysis of cysteic acid [N-isobutoxycarbonylation followed by methylation (MeI/Me_2SO_4/Ag_2O) of the silver salt to give the dimethyl ester].[561] One study[557] focuses on α-aminoisobutyric acid and isovaline, non-protein amino acids that are, in fact, quite common in the biosphere.

7.3 *Ion Exchange and Related Forms of Chromatography*

New techniques are being assessed for ion-exchange separation of amino acids, employing poly(hydroxyethyl methacrylate) modified with various weak acid and strong acid functional groups such as carboxyalkyl and sulphobutyl, respectively,[563] employing hollow fibres made from a perfluorinated ion-exchange membrane of the Nafion type,[564] and using a strong cation exchange polymer gel (Polyspher PE-A).[565]

Ion-pair chromatography has been used for the analysis of N-oxalylcysteine through conversion into a highly-fluorescent derivative using monobrombimane.[566]

7.4 *Thin-Layer Chromatography*

A routine subject for evermore, perhaps, in the context of amino acid analysis, but valid new papers continue to appear that are welcome when they describe improvements, however small, such as ICT-Empore silica gel plates capable of separating glutamic and aspartic acids, and serine and glycine.[567] Mention could be made of a modified ninhydrin spray reagent (prior spraying an heating with (+)-camphor-10-sulphonic acid) that is claimed to give distinctively different blue colours for the different common amino acids.[475]

7.5 *High Performance Liquid Chromatography*

This too might almost have become a routine topic by now, like the subject of the preceding Section, but the number of relevant papers exploring new aspects shows no sign of declining. Most of the current papers reflect benefits associated with newer derivatization regimes that are proposed to take the place of older, but not necessarily displaced, methods.

The o-phthaldialdehyde (OPA) – mercaptoethanol reagent,[568-574] and its highly sensitive relative, naphthalene-1,2-dialdehyde,[575] are still dominant in amino acid analysis, as far as the volume of current literature is concerned. Comparisons are being made with Fmoc derivatives, often in favour of the latter, and with N- phenylthiocarbamoyl derivatives.

The favoured OPA derivatization routine continues to have among its supporters a number of research groups which strive to expunge some of its problematical details. Good housekeeping shows up many of these problems to be somewhat illusory,[568] and attention to sample preparation, and the incorporation of nitrilotri-acetic acid to stabilize the OPA reagent,[569] are beneficial. Better fluorescence response accompanies the sequential use of dithiothreitol and iodoacetic acid in OPA derivatization-based assays of cysteine and cystine.[570] Among studies of general application of the OPA procedure in amino acid analysis,[571-573] accurate assay of 3-methylhistidine[571] continues to have special clinical importance. Commercial samples of L-amino acids estimated for their D-enantiomer content, as OPA derivatives separated in an achiral – chiral coupled column configuration, all show at least traces of the 'wrong' enantiomer.[574] In some of these cases, the levels of D-amino acids reach several percent.

A combination of OPA with Fmoc-Cl derivatization using piperidine-4-carboxylic acid as internal standard allows estimation of both primary and secondary amino acids (the OPA protocol cannot derivatize amino acids in the latter category) with improved quantification of secondary amino acids compared with classical ion exchange techniques,[576] though h.p.l.c. shows lower reproducibility levels for some amino acids. Other assays based on fluorescence measured at 315 nm (excitation at 265 nm) for Fmoc-amino acids[577,578] include an assay of basic amino acids extracted from mixtures with the use of a weakly acidic cation exchanger.[577]

Automated derivatization of amino acid mixtures using phenyl isothiocyanate (PITC) has been compared with Fmoc-Cl derivatization,[579] with the finding that Fmoc-Cl is more sensitive to the deleterious effects of buffers and solvents. Routine uses of PITC derivatization continue to be reported, for quantifying amino acids in hydrolysates,[580] in aminopeptidase – carboxypeptidase – mineral acid[581] or trypsin[582] digests, and in wine.[583] Use of the PITC method for estimating the crosslinking amino acids desmosine and isodesmosine in elastin hydrolysates[584,585] illustrates the growing realization, that h.p.l.c. analysis offers a reliable method for the assay of trace components in tissue samples, from which clinically useful conclusions can be drawn. Assays of collagen cross-linking amino acids pyridinoline and deoxypyridinoline in physiological fluids gives early diagnosis of bone loss in osteoporosis.[586]

Enantiomer ratios are quantifiable from the use of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate as a chiral reagent, recently applied to α-methyl α-amino acids with the finding that the elution order

for enantiomers of these is the opposite of that for their parent α-amino acids of the 'same' configuration.[587]

The PITC method has been found to compare favourably with the OPA – Fmoc-Cl combination for h.p.l.c. analysis of amino acids.[583] However, PITC and OPA methods are unsatisfactory for asparagine, glutamine, N^{γ}-methylasparagine and N^{γ}-methylglutamine (protein constituents that are the products of post-translational processing). Indeed, careful studies of deamidation of these compounds in solutions at various temperatures and pH (glutamine reacts [≃] 12 – 14 times faster than N^{γ}-methylglutamine, and N^{γ}-methylasparagine undergoes cyclisation in preference to de-amidation) suggests that there must be several errors in the literature due to previous lack of this knowledge.[588]

Ninhydrin derivatization of asparagine and glutamine and their methylation products mentioned in the preceding paragraph is, however, the basis of a satisfactory analytical procedure, and this faithful standby has been used for estimating S-methylmethionine, methionine, and lysine in corn,[589] and 2,6-di-aminopimelic acid as a marker for bacterial protein.[590]

A review has appeared covering the role of h.p.l.c. in identifying phenylthiohydantoins (PTHs) for protein sequence analysis.[591] DOPA-PTH co-elutes with alanine-PTH, but such overlaps can be dealt with by varying chromatographic parameters.[592]

Isolated examples of alternative derivatization reagents arise with an assay of homocysteine in plasma, using 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate,[593] and of general uses for dabsyl chloride, claimed to be a simple, stable reagent, capable of offering sensitive assays for amino acids.[594] No derivatization is involved in assays of histidine, tyrosine and tryptophan based on their u.v. absorption at 220 nm;[595] of cystine, arginine, lysine and ornithine based on intense $[M + H]^+$ ions formed using a mass spectrometer equipped with an atmospheric pressure ion interface;[596] and of cysteine and N-acetylcysteine using indirect amperometric detection based on I_2 oxidation.[597] N-Acetylamino acids have been determined at 0.5 nmol levels using a mobile phase containing trisphenanthrolinyliron(II) salts.[598]

Examples have been given in foregoing paragraphs of reports of analytical enantiomer separation by standard method. A more experimental approach is illustrated in an application of an 18-crown-6 silica gel stationary phase for analyzing unusual amino acids.[599]

7.6 *Other Analytical Methods*

High performance capillary electrophoresis (h.p.c.e) is now an established technique in many areas, and especially in the field of amino

acid analysis. Less than one picomole of phosphotyrosine PTH can be detected,[600] and similar levels can be reached for the analysis of underivatized amino acids.[601] Enantiomer composition of amino acid samples can be determined by h.p.c.e., after derivatization with Marfey's reagent.[602]

7.7 *Assay of Specific Amino Acids*

Many examples have been given elsewhere in this Chapter, of analytical information being acquired for particular amino acids. However, this Section's title as used over the years, is not meant to have hidden meaning; its purpose is to collect papers describing methods based on specific amino acid-modifying enzymes by whose action some simple compound is released in amounts indicative of the level of the particular amino acid.

A flexible redox polymer biosensor responsive to L-glutamic acid has been developed, employing immobilized L-glutamate oxidase.[603] Improvements to details of a standard hydroxyproline assay capable of detecting levels in urine, have been announced.[604]

Instrumentation for flow-injection analysis has reached levels of sophistication that allow reliable analysis of specific L- and D-amino acids in the presence of all other L-amino acids in serum samples, based on immobilized enzymes.[605,606] In one of these accounts,[605] an assay for L-lysine is described in detail; the other[606] indicates the scope of the technique, which allows the analysis of nineteen 50μL samples per hour. A sensitive flow-injection analysis, in which L-aspartic and L-glutamic acids are enzymatically coupled with NADH that activates immobilized bacterial bioluminescence enzymes, has been described.[607] The aspartate assay depends on the aspartate aminotransferase – malate dehydrogenase combination, while glutamate is quantified through the corresponding glutamate aminotransferase – dehydrogenase system.

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2
Peptide Synthesis

BY D.T. ELMORE

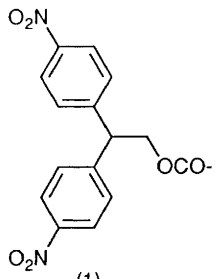
1 Introduction

The format of this report is the same as that used last year[1]. A new textbook on the chemical synthesis of peptides has been published[2]. Three books[3-5] are orientated towards peptide-based drugs, emphasizing the increasing importance of this multidisciplinary field. A plethora of other reviews[6-42] cover all aspects of the chemical and enzyme-catalysed synthesis of peptides.

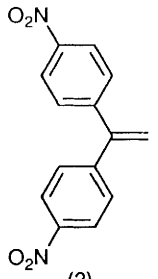
2 Methods

The arrangement of the main body of the report is identical to that used in recent years.

2.1.1 *Amino-group protection* - Automated equipment with artificial intelligence for the synthesis of *N*-(carboxyalkyl)-amino acids has been described[43]. Three syntheses can be run in a working day with minimal manual intervention. The Boc group can be introduced by sonication of a mixture containing the amino acid, $(Boc)_2O$ and $NaHCO_3$ in suspension in ethanol or methanol[44]. Cessation of CO_2 evolution provides a visual indication of the completion of reaction. Improved methods for the synthesis of Boc-Glu-OBzl[45] and Z-derivatives of the ω-benzyl esters of Glu and Asp[46] have been reported. A detailed investigation of a base-labile group has been reported[47]. The 2,2-bis(4′-nitrophenyl)ethane-1-oxycarbonyl group (Bnpeoc)(**1**) is obviously a near relation of the Fmoc group but has two advantages. Bnpeoc derivatives of amino acids are more soluble than Fmoc derivatives in CH_2Cl_2 and they are less expensive to make. Whether these two factors will be sufficient to entice peptide chemists away from Fmoc chemistry remains to be seen. The Bnpeoc group is very stable to acid and hydrogenolysis leads to a mixture of products because of the presence of two nitro groups. Deprotection occurs in presence of 20% piperidine (v/v) in $CHONMe_2$ leading to (**2**) and variable amounts of (**3**). The cyclic amidines, 1,8-diaza-bicyclo[5.4.0]undec-7-ene (DBU) and 1,5-diazabicyclo[4.3.0]non-5-ene, were also effective deprotecting agents. It was found possible to hydrolyse ester



(1)



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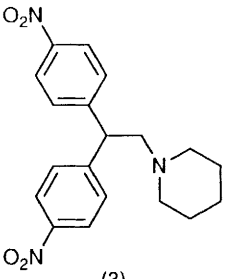

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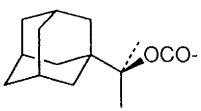

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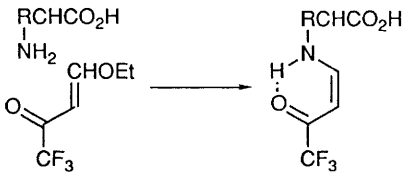
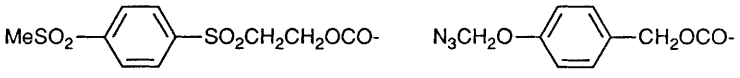
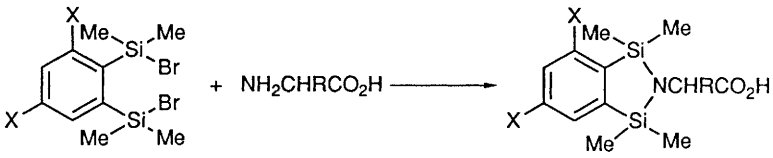


Scheme 1

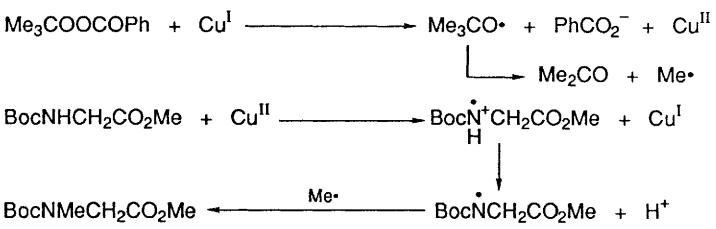


(5) (6)



(X = H, F) (7)

Scheme 2



Scheme 3

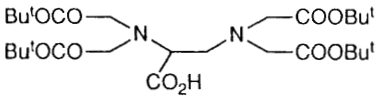
groups without affecting Bnpeoc groups using 0.1 M NaOH (1 equiv.), aqueous H_2O_2 (2 equiv.) in 20% aqueous acetone. Finally, the group was shown to have potential in the synthesis of glycopeptides. The 1-(1-adamantyl)-1-methylethoxycarbonyl (Adpoc) group (**4**) has been evaluated by its designer[48] for use in SPPS. Its extreme acid lability made possible the synthesis of LH-RH despite using an acid-labile linker and several other acid-labile protecting groups for amino-acid side chains. Unfortunately, no yield was reported although hplc indicated high purity of the product. 4-Ethoxy-1,1,1-trifluoro-3-buten-2-one is reported[49] to yield stable, crystalline derivatives of amino acids (Scheme 1) that can be coupled to amino acid esters using DCCI in CH_2Cl_2 at $-10°C$ with not more than 0.5% racemization. The protecting group is removed under mild aqueous acidic conditions. The two-stage method for introducing the protecting group and the rather slow method of deprotection, however, will probably not favour a wide application. The 2-(4′-methylsulphonylbenzene)sulphonylethoxycarbonyl group (**5**)[50] is more stable than the Fmoc group but is eliminated under mild basic conditions. The elimination product does not spontaneously polymerize and on these two counts, the group is recommended for SPPS in place of Fmoc. Protection of amines by the 4-(azidomethyleneoxy)benzyloxycarbonyl group (**6**) has been investigated[51]. The chloroformate is rather unstable so the group is more conveniently introduced using either the 4-nitrophenyl carbonate or the acyl azide. The group can be removed by $SnCl_2$ or by acid- or base-catalysed 1,6-elimination and decarboxylation. Some small peptides were synthesized using the BOP coupling reagent. Yields ranged from 63 to 85% but there is no information about concomitant racemization. 2,2,2-Trifluoro-1,1-diphenylethanesulphenyl chloride has been used to protect primary and secondary amines including amino acids[52]. The group is removed either by reduction with Na in liquid NH_3 or by HCl gas dissolved in ether. So far, no peptide syntheses have been reported using this protecting group. The 1-benzotriazolylcarbonyl group has been proposed for protecting α-amino groups[53]. It is cleaved by acids, but appears not to offer any particular advantages. Another acid-labile group[54] is illustrated in Scheme 2. Deprotection of (**7**) proceeds rapidly in CF_3CO_2H or CH_3CO_2H. Racemization was not detected when this group was deployed in peptide synthesis. *N*-Boc and *N*-Z derivatives of amino acids (particularly glycine) and *N*-Boc dipeptides containing *N*-terminal Gly give derivatives of *N*-methylamino acids when treated with *tert*-butyl perbenzoate in the presence of copper(II) octanoate in refluxing benzene under an atmosphere of N_2[55]. A free-radical mechanism was proposed (Scheme 3). This chemistry might provide a route to *N*-terminally methylated peptides that manifest useful biological properties and are

inert to aminopeptidases. The synthesis of *NNN'N'*-tetra(*tert*-butyloxycarbonylmethyl)-2-carboxyethylenediamine (**8**) permits the synthesis of peptides with an *N*-terminal group that resembles EDTA[56]. These peptides can chelate cations that are radionuclides giving potentially useful compounds for *in vivo* tumour localization and radioimmunotherapy. Specific noncovalent binding of metal-chelating peptides to proteins and nucleic acids provides a tool for cleaving the target macromolecule. Finally, ferrocene aldehyde forms a Schiff base with Gly that can be reduced to the ferrocenylmethyl (Fem) derivative[57]. The resulting secondary amine can be converted into the Boc derivative which can then be used for peptide synthesis. The Fem group alters the polarity of the peptide being synthesized and this could facilitate the purification step. The Fem group is removed with CF_3CO_2H.

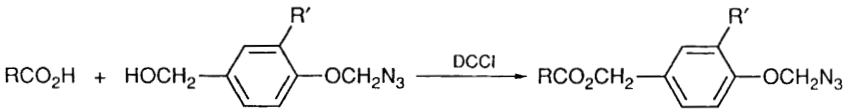
2.1.2 *Carboxyl-group protection* -n-Heptyl esters of amino acids can be prepared by azeotropic esterification using toluene-4-sulphonic acid as catalyst[58]. Coupling of the n-heptyl esters and Z-, Boc- or Alloc-amino acids is straightforward. The great attraction of the heptyl esters is their sensitivity to hydrolysis at pH 7 and room temperature using the lipase from *Rhizopus niveus* as catalyst, thereby avoiding any risk of racemization. The lipase, of course, must be free from any proteinases or peptidases.

Various 4-(azidomethoxy)benzyl esters (ABz) (Scheme 4) are readily prepared[59]. Boc and ABz groups are orthogonal since the latter withstand treatment with acid but are cleaved with $SnCl_2$ in MeOH. The ABz groups can be selectively removed when Boc groups are present by carrying out the deprotection in presence of Et_2NH. Boc-Tyr(OBzl)-Gly-Gly-Phe-Met-OH was synthesized using ABz protection for carboxyl protection; no racemization was detected.

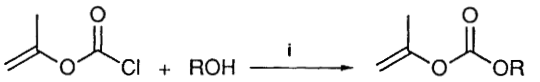
1-(Pyrenyl)benzyl esters are prepared from the stable diazo derivative[60]. The attractive properties of photolability and intrinsic fluorescence of the ester moiety are probably outweighed by the mutagenic and carcinogenic preperties of pyrene derivatives. The use of phenacyl esters is complicated when the hydroxyl group of Tyr is blocked by the *O*-(*S,S*-diphenylphosphorodithioyl) moiety[61], since deprotection of the $-CO_2H$ group using Zn-acetylacetone also removes one PhS- group from the Tyr side chain. This problem has been overcome by using ditolylmethyl esters (Dtm). Deprotection is achieved with 2% CF_3CO_2H without affecting the *O*-(*S,S*-diphenylphosphorodithioyl) or Boc groups. Several phase-transfer reagents have been used to protect carboxyl groups[62]. Finally, if the CO_2H group is protected as the phenylhydrazide, the latter group is removed using peroxidase or laccase in presence of H_2O_2[63].



(8)



Scheme 4



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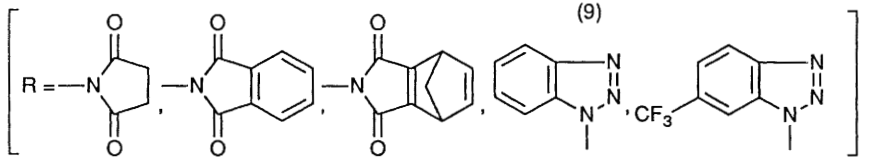
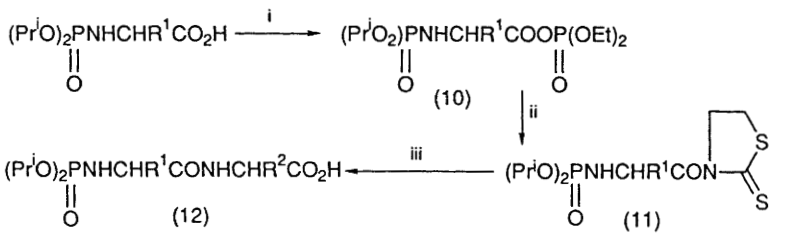


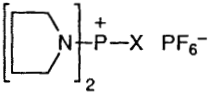
Reagents: i, Et_3N, Me_2CO at 0 °C for 4 h

Scheme 5



Reagents: i, $(EtO)_2POH$, CCl_4, Et_3N; ii, 1,3-thiazolid-2-thione;
iii, $NH_2CHR^2CO_2^-\ Na^+$ then acid

Scheme 6



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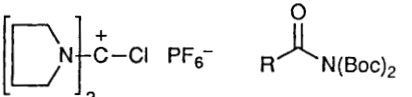
PyBOP; X = 1-oxybenzotriazole
PyBrOP; X = Br
PyClOP; X = Cl



(14)

(15)

2.1.3 *Side-chain protection* - *N*-Fmoc-*O*-Trt derivatives of Ser, Thr and Tyr have been synthesized and satisfactorily used in peptide assembly[64]. The corresponding OBu^t ethers have also been synthesized by improved methods[65]. In all cases, the carboxyl group was temporarily protected as the Me ester. For Ser and Thr, the OBu^t moiety was introduced using $Me_2C{=}CH_2$ and toluene-4-sulphonic acid in CH_2Cl_2, the ester was saponified and then the Fmoc group was introduced. For Tyr, however, the Me ester was converted into the Fmoc derivative before treatment with $Me_2C{=}CH_2$ in presence of H_2SO_4. The ester was then hydrolysed with 2% Na_2CO_3 in aqueous methanol. Safety-catch type protecting groups for the side chains of Thr and Tyr have been proposed[66]. The 4-methylsulphinylbenzyloxycarbonyl (Msz) and the 4-methylsulphinylbenzyl (Msb) groups were used respectively for Tyr and Thr. Deprotection can be effected by reduction and acidolysis. γ-Endorphin was synthesized to test this methodology. In a somewhat more specialized application, the hydroxyl groups of α-methyl-β-3,4-dihydroxyphenyl-L-alanine were protected as benzyl ethers[67].

An improved preparation of H-Lys(Z)-OMe.HCl has been described[68]. A scheme[69] to introduce a metal-chelating group on the side chain of a Lys residue resembles that described above[56]. The tribenzyl ester of EDTA was synthesized in 3 steps and coupled in several stages to Boc-Lys-OH. Although ε-amino groups in proteins have frequently been converted into guanidino groups, there has never been a thorough investigation of this potential route to synthetic peptides of arginine. This is surprising because if a synthetic peptide containing a *C*-terminal Orn residue could be guanidinated, it would constitute a substrate for the trypsin-catalysed fragment condensation to another peptide which might contain protected Arg or Lys residues. It has now been shown that a peptide chain containing an -Orn(Fmoc)-residue can be assembled by SPPS, the Fmoc group removed and the exposed δ-amino group can be guanidinated using 1-guanyl-3,5-dimethylpyrazole while the peptide is still attached to the resin[70]. It now seems appropriate to assemble a peptide containing *C*-terminal Arg as outlined above then use trypsin to detach simultaneously the peptide and couple it to the α-amino group of an acceptor peptide. Any incompletely guanidinated peptide would remain attached to the resin.

A full account has appeared of the application of the N^G-2,2,5,7,8-pentamethylchroman-6-sulphonyl group (Pmc) for the protection of the guanidino side chain of Arg[71]. Protection of the α-group is effected using either Fmoc or Bnpeoc[47]. Five analogues of the Pmc group have been examined[72]. Omission of the 2,2-dimethyl group had little effect, but acid sensitivity was significantly decreased by omission of the 8-Me group.

None of the analogues offered any marked advantages over the Pmc group. A one-pot method for preparing the Z_2 derivative of Boc-Arg-OH proceeds through a trisilylated intermediate[73].

Two protecting groups, 4-monomethoxytrityl (Mmt) and 4-monomethyltrityl (Mtt), have been used to protect the imidazole ring of His[74]. The method of introducing the group involves initial silylation with Me_2SiCl_2 followed by reaction with the appropriate trityl chloride derivative in presence of Et_2NH. The N^{τ}-atom is proposed as the site of substitution. The Mmt and Mtt groups are stable to piperidine and Et_2NH and are removed by CF_3CO_2H under much milder conditions that are required for the removal of an N^{im}-trityl group. Magainin I (1-10) was synthesized by a solid-phase method to test this methodology; deprotection was achieved by 15% CF_3CO_2H in CH_2Cl_2 in 90 min at room temperature.

The carboxamide groups of Asn and Gln can be tritylated by an acid-catalysed reaction with Ph_3COH at 50-60°C in the presence of Ac_2O to react with liberated water[75]. H-Gln(Trt)-OH is best prepared through the Z-derivative in order to obtain good yields. Amide protection by the Trt group resists strong mineral acids and organic bases; deprotection proceeds rapidly with CF_3CO_2H.

2.2 *General deprotection*

A problem referred to in last year's Report[1] concerned side reactions that can ensue when deprotecting peptides containing -His(Bom)-residues with HF. Another report[76] has appeared concerning the reaction between an *N*-terminal cysteinyl residue and the HCHO liberated on removal of a Bom group. The reader is referred to comments made in the previous Report concerning this type of side reaction. A further complication has been reported when deprotecting -Cys(Bzl)-by HF[77]; attempted simultaneous detachment of peptide from the support after SPPS of [Sar[1],Cys[8]]-angiotensin II converted the Cys into dehydroalanine.

Toluene-4-sulphonic acid is useful for removing Boc groups during SPPS on polystyrene resins[78], but is unsuitable if the Kaiser oxime resin is used. As an alternative to HF for general deprotection and provided that the protected peptide is not attached to a resin, CF_3CO_2H in the presence of Nafion-H polymer (Du Pont) can be used[79]. Me_2S is added as a scavenger for carbo-cations. A few protecting groups such as Glu(OChx), Arg(Tos), Arg(NO_2) and Cys(4-MeBzl) resisted these conditions. The deprotected peptide is left trapped in the Nafion polymer, but can easily be isolated by washing with base.

Alkyl and aralkyl ester groups can be removed without concomitant

racemization using aqueous Cs_2CO_3 with an alcohol as a cosolvent[80]. Finally, tetrabutyl ammonium fluoride is a very useful reagent for converting 4-nitrobenzyl, 2,2,2-trichloroethyl and phenacyl esters into the free acids in good yields and with insignificant risk of racemization[81].

2.3 *Peptide bond formation*

Fmoc amino acid chlorides have been prepared[82] by the action of dry HCl gas on unsymmetrical anhydrides derived from Fmoc amino acids and alkyl chloroformates in CH_2Cl_2:

$$\text{FmocNHCHR}^1\text{COOCOOR}^2 + \text{HCl} = \text{FmocNHCHR}^1\text{COCl} + \text{CO}_2 + \text{R}^2\text{OH}$$

The acyl chlorides react with the liberated R^2OH to give esters; even with $ClCO_2Pr^i$, some 5-20% of ester is formed. If anhydrides of monoisopropenyl carbonic acid are intermediates. however, acetone is liberated instead of an alcohol and the side reaction is avoided. Fmoc amino acid chlorides react sluggishly in SPPS because the presence of essential bases such as Pr^i_2EtN and *N*-methylmorpholine promote the formation of oxazolones. If a 1:1 mixture of base and HOBt is used, coupling is rapid due to the intermediate formation of esters of HOBt[83]. Two groups[84,85] have described more fully the preparation cited in last year's Report of Boc and Fmoc amino acid fluorides from the interaction of cyanuric fluoride and the corresponding acids. Most of the acyl fluorides are crystalline, stable and can be deployed in either solution syntheses or in SPPS. They could be particularly suitable for attaching the first amino acid residue to the support in SPPS.

An improved method for synthesizing reactive esters for peptide synthesis has been described (Scheme 5)[86]. Significantly, the intermediate carbonate esters (**9**) react with *N*-protected amino acids in presence of only catalytic amounts of 4-dimethylaminopyridine thereby diminishing the risk of racemization. Pentafluorophenyl diphenylphosphinate is prepared by mixing equimolar quantities of Ph_2POCl, C_6F_5OH and imidazole in CH_2Cl_2[87]. It is a useful coupling reagent, is stable in the refrigerator for several months and affords good yields of peptides without significant racemization. Blake's method of converting *S*-alkyl thioesters of partially protected peptides into the corresponding 4-nitrophenyl esters by reaction with 4-nitrophenol in presence of Ag^+ ions has been reinvestigated and applied to fragment coupling of peptides[88].

A new application[89] of *N*-acyl-1,3-thiazolidine-2-thiones to peptide synthesis extends work reported last year. *N*-Diiso-propylphosphoryl-amino acids on treatment first with dialkyl phosphite, CCl_4 and Et_3N afford the unsymmetrical anhydrides (**10**) which react with 1,3-thiazolidine-2-thione to give the rather stable *N*-acyl derivatives (**11**) (Scheme 6).

These react with amino acids in aqueous solution to give the *N*-protected peptides (**12**). Racemization can be detected by ^{31}P nmr spectroscopy and none was found in the simple cases examined. Unsymmetrical anhydrides generated from *N*-protected amino acids or peptides and 1-oxo-1-chlorophospholane cleave regiospecifically with amines to give the corresponding peptide[90]. Fragment coupling with *C*-terminal Gly or Pro gives high yields of stereochemically pure products.

The phosphonium coupling reagents have rapidly established themselves in the peptide chemist's toolbox despite the carcinogenic nature of the product, hexamethylphosphoric triamide, formed from those compounds containing the $(Me_2N)_3P^+$-moiety. Replacement of the Me_2N groups by pyrrolidino groups apparently removes the biological hazard. The notorious difficulties associated with the synthesis of peptides of α-aminoisobutyric acid (Aib) can generally be overcome[91] by using PyBOP or PyBroP (**13**). The latter reagent was required when coupling two Aib residues. The coupling of *N*-methylamino acids is another difficult exercise. PyBroP, PyCloP and the related 1,1,3,3-bis(tetramethylene)chlorouronium hexafluorophosphate (**14**) (PyClU) all gave satisfactory results with negligible racemization[92]. PyBOP has been used for coupling Fmoc amino acids in $CHCl_3$[93]. After removal of the Fmoc group, the peptide ester is isolated by a simple washing procedure. This technique might well be applicable to multistage coupling without isolation and characterization of intermediates.

Activation of *N*-protected amino acids using DCCI/HOBt in CH_2Cl_2 proceeds in <2 min despite the low solubility of HOBt[94]. Presumably, the HOBt ester is formed. It is claimed that coupling to an amino acid ester in presence of Pr^i_2EtN proceeds as rapidly as with BOP and $<0.1\%$ racemization occurred. Polar solvents should be avoided in the activation step.

Primary amides are converted into isolatable acylating reagents, *N*-acylimidodicarbonates (**15**), on reaction with di-*tert*-butyl dicarbonate[95]. Treatment of (**15**) with amines gives secondary amides and Boc-Pro-Leu-OBu^t (70%) was synthesized in a model reaction. More information about the extent of racemization would be helpful to permit an assessment of this interesting route.

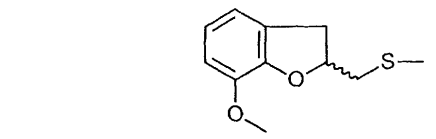
The sheer poetry of Kemp's thiol-capture method of bond formation reviewed two years ago[96] readily evoked admiration but emulation was perhaps inhibited by its rather esoteric nature. Three further papers have appeared so perhaps workers in other groups will succumb to the thiol-capture method of forming Xxx-Cys bonds. The first paper[97] reports the fragment synthesis of an analogue of a 39-residue peptide. It is a feature of Kemp's method that side chains other than those of cysteine

residues are unprotected; the present exercise was the first test with two naked His residues. Although the latter might have interfered with either the replacement of an *S*-acetamidomethyl (Acm) group by a *S*-methoxycarbonylsulphenyl (Scm) group or the *O,N*-acyl transfer that forms the peptide bond, both fears proved to be groundless. The other two papers[98,99] report the search for alternative templates to optimize the intramolecular *O,N*-acyl transfer. Most efficient rearrangement and hence peptide-bond formation was obtained with the 7-acyloxy-2-thiomethyl-2,3-dihydrobenzofuran template (**16**) which also manifested stereo-selectivity at C_2.

Stereochemical matters have prompted other studies of peptide-bond formation. The extent of racemization during DCCI coupling of *N*-benzoyl-or *N*-trifluoroacetyl-amino acids with L-amino acid esters has been determined taking account of asymmetric induction during the aminolysis of the 5(4)-oxazolid-5-one intermediate[100]. The direction of asymmetric induction depends strongly on the solvent[101]. A wider range of *N*-acyl groups has also been examined[102].

The use of Li salts to increase the solubility of peptide reactants during coupling reactions in solution has been examined[103]. Few benefits were observed and in some cases yields were decreased and the extent of racemization was increased. Nevertheless, some coupling reactions were found which allowed the use of Li salts to increase solubility of reactants without impairing the yield and purity of product. Metal complexes feature in other peptide synthetic research. The rhodium complex of H-Gly-Gly-OMe and pentamethylcyclopentadiene (**17**) reacts with α-amino acid esters in the presence of Et_3N at 20°C to give complexes of tripeptide esters (Scheme 7)[104]. α-*N*-Alloc groups can be removed by a Pd(0) compound such as $Pd(PPh_3)_4$ in presence of Bu_3SnH in CH_2Cl_2 followed by HCl gas. If a reactive ester of an *N*-protected amino acid ester is added in place of HCl, however, a dipeptide derivative is formed (Scheme 8)[105]. It is suggested that Bu_3SnH significantly enhances the nucleophilicity of nitrogen in the intermediate tin carbamate. Enol esters of *N*-protected amino acids can be generated from e.g. hex-1-yne in the presence of $RuCl_2(PPh_3)$(*p*-cymene) as catalyst[106]. The enol esters are then coupled with amino acid esters, usually in the presence of KCN, to give a fully protected dipeptide in moderate to excellent yield, with no detectable racemization.

Ozonolysis of 2-(1-phthalimido)alkyl-5-phenyloxazoles (**18**) followed by reaction with H-Gly-OMe gave phthalimidodipeptide esters in good yields[107]. Peptide-bond formation is possible by isomerization of oxaziridines (Scheme 9)[108]. In some cases, the final isomerization step occurred spontaneously during chromatography on silica gel. Finally, a



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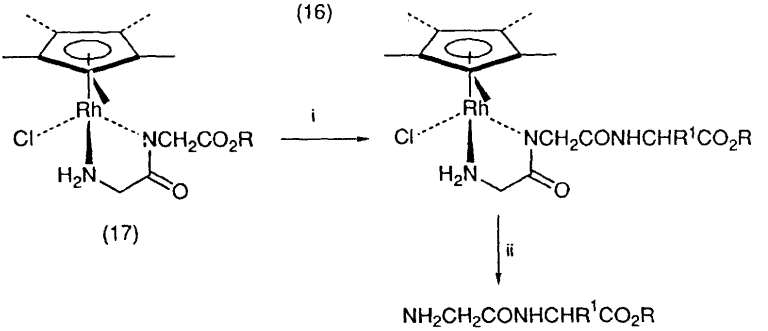
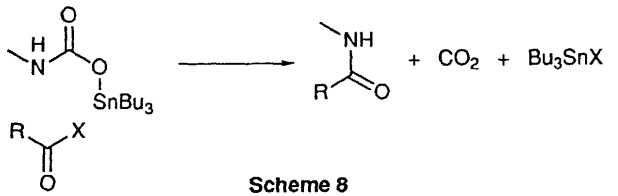


(17)

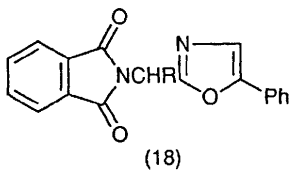
Reagents: i, $NH_2CHR^1CO_2R$, Et_3N; ii, HCl/MeOH

Scheme 7

Boc-Ala-OPfp + Alloc-Ala-OMe + $Pd(PPh_3)_4$ + Bu_3SnH ⟶ Boc-Ala-Ala-OMe



Scheme 8



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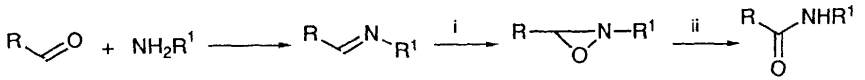


Reagents: i, *m*-ClC_6H_4COOOH; ii, UV radiation

Scheme 9

mechanistically interesting method of peptide bond formation uses Bu_3P and Ph_2Se_2 (Scheme 10)[109]. The peptide derivative can be formed directly or through an intermediate phenylseleno ester.

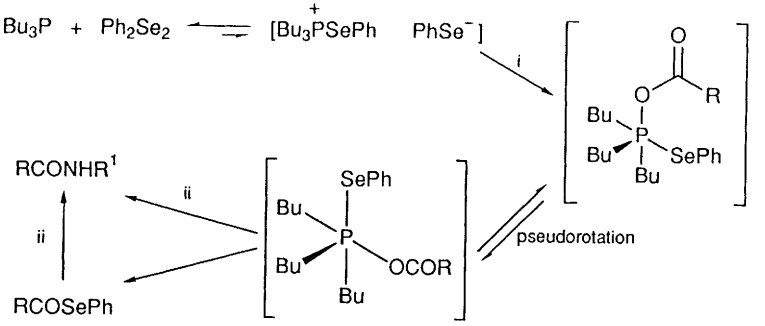
2.4 *Disulphide bond formation*

In the synthesis of analogues of α-conotoxin, which is an antagonist of acetylcholine receptors and which contains two disulphide bonds, the products formed by oxidation of the corresponding tetrathiol have been examined[110]. Sequences corresponding to calcitonin (1-10) and (Tyr^1)-somatostatin-14 were assembled[111] on a 2-chlorotrityl-substituted resin. Thiol groups were protected by Trt groups; removal of the latter, oxidation of thiol groups to -S-S-bonds and release of the peptide from the resin could be effected simultaneously. An extensive study to optimize the conditions for oxidation of cysteinyl to cystinyl residues by MeSOMe has been undertaken[112]. The miscibility of MeSOMe with water permits much higher concentrations of oxidant to be used than is feasible with aerial oxidation. The reaction can be conducted over a wide range of pH. Moreover, amino acids with nucleophilic side chains (His, Tyr, Trp and Met) are secure from oxidation under the usual conditions employed. The assembly of human defensin which contains three -S-S-moieties is an excellent recommendation for the technique. In contrast, an alternative approach[113] uses MeSOMe in CF_3CO_2H. It would be interesting to know if Met residues are sensitive to oxidation under these conditions. A sulphoxide is also the oxidant in a third method[114]. If thiol groups are protected by Acm or Bam groups, the latter can be removed with $MeSiCl_3$ or $SiCl_4$ in CF_3CO_2H in the presence of a sulphoxide which then oxidizes the liberated thiol groups to disulphide directly. An obvious but neglected approach to ensure intramolecular disulphide occurs to the exclusion of intermolecular reaction is to conduct the oxidation step while the peptide is still attached to the resin in SPPS. This technique has now been studied in depth[115] and is successful with either Fmoc or Boc protection of amino groups and with either *S*-Acm, *S*-Fm or *S*-Trt protection.

In a quite different approach, polystyrene sodium sulphinate reacts with thionitrite esters, RSNO, to give polystyrene thiolsulphonates. These insoluble thioalkylating agents were used to synthesize some simple model unsymmetrical disulphides[116]:

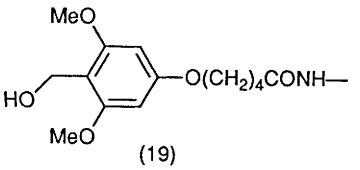
$$\text{Polystyrene-}SO_2SR + R'SH \rightarrow RSSR' + \text{Polystyrene-}SO_2H$$

(e.g. R = -$CH_2CH_2SO_3Na$, R′ = $AcNHCH(CO_2H)CH_2$-).
A disulphide originating from glutathione is the most ambitious product so far described, but the method is an interestingly novel approach.



Reagents: i, RCO_2H; ii, R^1NH_2

Scheme 10



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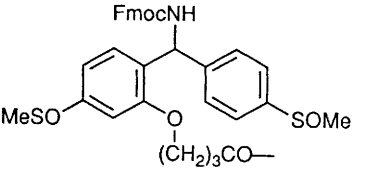

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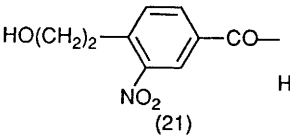

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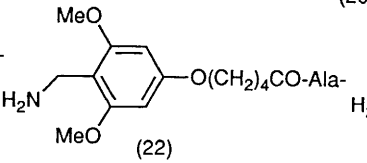

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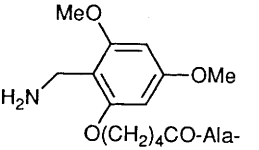

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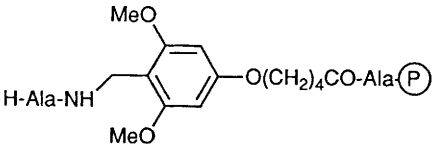

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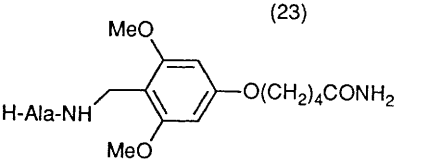

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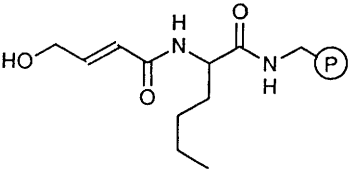

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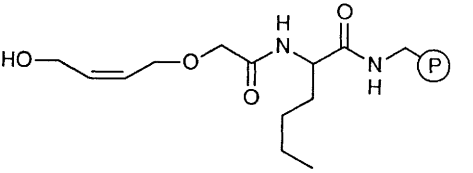


(27)

2.5 *Solid-phase peptide synthesis (SPPS)*

A linker (**19**) which gives rise to a tris(alkoxy)benzyl ester of a peptide during SPPS is extremely acid-labile. After using Fmoc protection of amino groups and *tert*-butyl ethers and esters for side-chain protection, detachment from the support is effected with 0.05-0.1% v/v aqueous CF_3CO_2H or with 10% CH_3CO_2H in CH_2Cl_2[117]. A new linker (**20**) for the synthesis of peptide amides has been synthesized and used to make a calcitonin fragment[118]. The sulphoxide group facilitates the acidolytic cleavage of the final peptide amide with $Me_3SiBr/PhSMe/CF_3CO_2H$. Moreover, the linker can be used with either Fmoc or Boc protection of amino groups. A third type of linker (**21**) has been used for the synthesis of both peptides and oligonucleotides[119]. It can be attached to a MBHA-resin via the 2,4,5-trichlorophenyl ester in presence of HOBt. This linker is claimed to have favourable properties for peptide synthesis because (*a*) it is stable to acids, (*b*) the assembled peptide can be detached by base (DBU or piperidine) and (*c*) diketopiperazine formation is low when the amino group of the second amino-acid residue is exposed.

A hydrophilic, cross-linked aminoalkyl polydimethylacrylamide resin has been prepared by the copolymerization of CH_2:$CHCONMe_2$, CH_2:$CHCONH(CH_2)_3NHCOCH$:CH_2 and a functional monomer such as CH_2:$CMeCONH(CH_2)_3NH_2$ in a reverse-phase, detergent-emulsified suspension[120]. Peptide-support conjugates for antibody production have been prepared using both Boc and Fmoc chemistry. An improved synthesis of 4-nitrobenzophenone oxime resin based on polystyrene has been reported[121]. The capacity was 0.2-0.8 mequiv./g. Cotton has been examined as a support for SPPS[122]. There appears to be no special difficulty, especially since the notorious ACP 65-74 sequence was assembled on it. Advantages claimed are (*a*) great economy in solvent use and (*b*) peptide-cotton conjugates are useful in ELISA tests. It occurs to the Reporter that cotton lint, on which a chemotactic peptide for leukocytes has been assembled, might be a useful type of wound dressing. Finally in connection with supports, a pyrazolone resin has been described[123] and has been used to synthesize dipeptides.

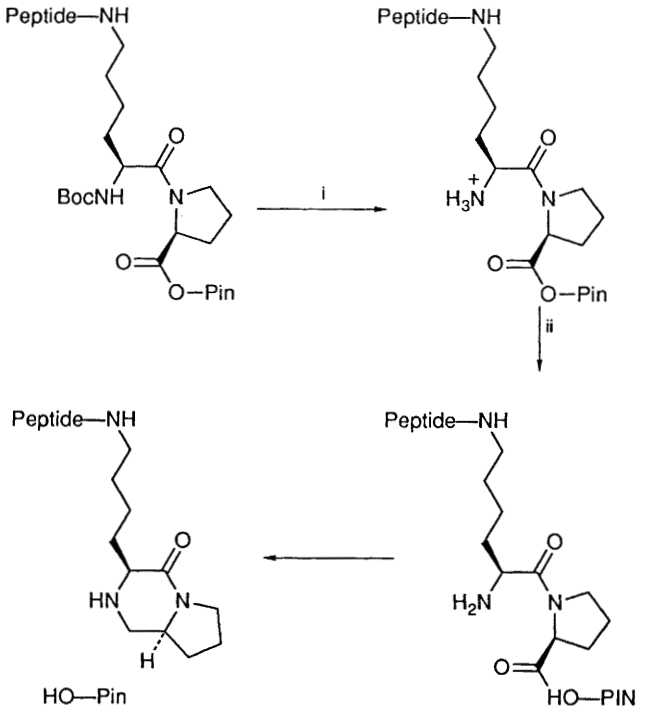
There are several papers concerned with side-chain protection during SPPS. H-Thr-Tyr-Asn-Thr-Thr-Thr-Ser-Ala-OH was synthesized with and without blocking the Thr hydroxyl group and it is claimed that protection is unnecessary[124]. Reference has been made above[48,50,64] to the use of several protecting groups in SPPS. In the synthesis of several Arg peptides related to the *C*-terminus of NPY, Mtr and Pmc groups were used to protect the guanidino group. Some byproducts containing sulphonated Arg side chains were detected following detachment of the peptide using CF_3CO_2H[125]. This presumably resulted from competing

cleavage routes involving C-S and N-S bonds. The assembly of peptides containing phosphorylated side chains of Tyr appears to be reasonably straightforward[126-128].

The attachment of Boc-Asn-OH and Boc-Gln-OH as *C*-terminal residues to the Merrifield resin is reported to proceed satisfactorily using dicyclohexylamine as base[129]. Coupling yields are sometimes improved by adding Li salts, possibly because of enhanced matrix swelling in some solvents[130]. The use of an equimolar mixture of Fmoc-amino acid chloride and HOBt has been mentioned above[83]. Excellent results were obtained in SPPS using Fmoc amino acids with 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) as the coupling reagent[131]. Reaction was very rapid in $CHO.NMe_2$ or *N*-methyl-pyrrolidone and a single coupling process only was required with the most sterically hindered amino acids. Extensive data on coupling rates are available when using Boc-amino acid anhydrides[132]. Second order kinetics were observed. An attempt has been made to determine if difficult couplings are predictable[133]. The results are promising although only a qualitative Kaiser test was used to assess if a coupling step had gone to completion. The compilation of a comprehensive data base would greatly enhance the precision with which coupling conditions could be defined and programmed. In a related fundamental approach to optimize coupling conditions and hence yield, a limited study of the effect of temperature has been made[134]. The importance of solvent in determining accessibility of the growing insolubilized peptide to coupling agent has long been recognized. It has now been demonstrated[135] that effective solvation with Merrifield-type resin can be correlated with the Hildebrand and hydrogen-bonding solubility parameters. Side-chain protecting groups also influence solvation. It may well be that accumulation of an extensive data base for coupling steps recommended above should include consideration of solvent composition as an additional important parameter. If a benzotriazol-1-yl polystyrene is used in a carbodiimide coupling with an *N*-protected amino acid or peptide, a reactive ester is produced which can be easily freed from the corresponding urea and any *N*-acylurea by a solvent wash[136]. Racemization during coupling was not detected even when using an *N*-protected peptide, so the method could be useful for fragment coupling.

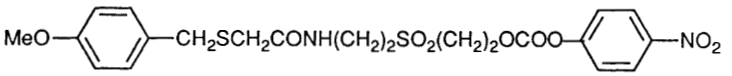
The cyclic amidine, DBU, which had been shown several years ago to remove Fmoc groups, has been shown to be very suitable for the same purpose in continuous-flow SPPS[137]. DBU is not nucleophilic yet removes Fmoc groups in less than 10 min. It does not cause cyclization of either -Asp(OBu^t)-Gly-or -Asn-Gly-to succinimido derivatives. Moreover, DBU is less likely than piperidine to cause racemization of a *C*-terminal

-Cys(Trt)-residue. It has been separately shown[138] that DBU is a highly effective catalyst for saponifying peptide esters in aqueous tetrahydrofuran at room temperature. Alternatively, in the presence of an excess of another alcohol (but not Bu^tOH), transesterification can be achieved. No racemization was detected with Boc-or Z-peptide esters. The same reagent can be used to detach peptides from PAM resins after SPPS. A substantial body of work has been reported on the detachment of peptides from supports and removal of side-chain protecting groups. A new two-step procedure has been developed when Boc chemistry is used in conjunction with a PAM resin[139]. First, protecting groups are removed while the peptide is still attached to the resin using $Me_3SiBr/PhSMe/CF_3CO_2H$ (weak hard acid). In the second stage, the peptide is detached using either $CF_3SO_3SiMe_3$ in CF_3CO_2H or HF (strong hard acid). An unexpected outcome resulted from a kinetic study of the cleavage of peptides from an aminomethylpolystyrene support bearing either of the linkers (**22,23**)[140]. Cleavage of (**24**) with CF_3CO_2H gave (**25**) as an intermediate. Hence, not only is the -NH-CHMe-bond in Ala cleaved, but this reaction proceeds faster than the expected cleavage of the *N*-terminal H-Ala-NH_2. Quantitative ninhydrin assays were used to follow the detachment of peptide using CF_3SO_3H[141]. It was concluded that the latter is a satisfactory replacement for HF. The use of a 2-chlorotrityl resin permits peptide detachment under very mild conditions using $CH_3CO_2H/CF_3CH_2OH/CH_2Cl_2$ at room temperature for 15-60 min without affecting Bu^t-protecting groups on side chains[142]. Similar selectivity and sensitivity was obtained using an allylic anchoring group[143]. Peptides can be assembled using either of the linkers (**26,27**) and then detached preferably using $Pd(PPh_3)_4$ in the presence of tetrahydrofuran/MeSOMe/0.5 M HCl (2:2:1) and with morpholine as the nucleophile. Side-chain protecting groups are unaffected so that the products can be used subsequently in fragment condensations. The opposite approach of removing side-chain protecting groups before peptide detachment has also been investigated[144]. A gastrin analogue sequence was assembled with $HOCH_2CH{=}CHCO$-β-Ala-Nle-as linker. Bu^t-groups were removed with CF_3CO_2H and the peptide was detached with $Pd(PPh_3)_4$ in the presence of HOBt. The amount of alkylation of susceptible amino acids such as Trp and Tyr was less than when deprotection was carried out while the same sequence was attached to the resin. The authors attribute the protective action of the resin to protonation of the amide groups in the resin which inhibited entry by carbocations. Gas-phase cleavage of peptide amides from solid supports is a new idea that is suited to the generation of small quantities of peptides[145]. It is particularly recommended for simultaneous multiple peptide synthesis on polyethylene pins. A further study[146] of the use of the photolabile



Reagents: i, 0.1% HCl in MeOH/H_2O then pH3 soak; ii, pH7 buffer

Scheme 11



(28)

2-nitrobenzyl linker has provided some improvements. Three methods were used for attaching the first amino acid; the preferred method, albeit the longest, involved attaching the *C*-terminal residue to the linker before conjugation to the solid support. The formation of diketopiperazines after the attachment and deprotection of the second residue can be troublesome with benzyl-type linkers. The use of BOP in presence of Pr^i_2EtN is the recommended procedure for attaching the third residue to the protonated *C*-terminal dipeptide. In contrast to this methodology, the proclivity to form diketopiperazines is harnessed to facilitate detachment of peptides from a support[147]. Boc-Lys-Pro-support is formed and the required sequence is assembled on the ε-amino group (Scheme 11). This method is very suitable for simultaneous synthesis on pins for the production of antibodies where the presence of the diketopiperazine moiety will not interfere unduly with the biological use of the peptides. A similar process has been described by another group[148].

Multiple syntheses by the teabag technique has been further studied[149]; Fmoc chemistry and coupling with HBTU were used. An important methodological development in multiple peptide synthesis has been described[150]. SPPS is conducted on glass plates that have been treated with 3-(aminopropyl)triethoxysilane. Amino groups are blocked with the photochemically scissile 4-nitroveratryloxycarbonyl (Nvoc) moiety. Using a suitable optical mask, strips or squares of the plate can be deprotected for subsequent coupling of an amino-acid residue using a HOBt ester. The size of the orifices in the mask is progressively decreased to permit increasing variability of sequence as peptide assembly proceeds. An array of 1024 peptides was assembled in ten steps using this technique. The same kind of technique can be used to build a library of oligonucleotides. It is also possible to build peptide libraries using individual resin beads for each peptide[151]. The absence of an addressing facility, however, makes this method less convenient than the foregoing. An even simpler technique is adequate if it is only required to produce a randomized mixture of peptides[152].

Several other technical developments have been described. A simple, semi-automatic machine with continuous flow facilities has been described[153]; Fmoc chemistry and a polyamide support were used. The use of an ultrasonicator is reported to accelerate coupling[154]. Conductance measurements have been used to monitor acylation by Pfp and Hbt esters with Fmoc chemistry[155]. The ninhydrin method of assaying free amino groups has been improved by using an ancillary apparatus for filtering and transferring samples[156]. Increasing the flow rate during deblocking and washing is reported to minimize the formation of diketopiperazines[157]. A thorough examination of the Fmoc-method of SPPS has

revealed examples of double additions during single cycles, incomplete removal of Fmoc group by piperidine and occasional failure of the Kaiser method to detect free amino groups on certain amino acids[158]. This should be regarded as another warning that SPPS still requires some human intelligence and skill. The results of this type of fundamental study need to be stored and called upon in appropriate software so that the operator can be forewarned about possible difficulties.

This section is closed with an ingenious idea for purifying peptides made by SPPS[159]. After attachment of the *N*-terminal residue and deprotecting the α-amino group, the immobilized peptide is treated with (**28**). The 4-methoxybenzyl group is removed and the peptide is detached from the resin using Me_3SiBr/ $CF_3SO_3SiMe_3$ in CF_3CO_2H. The peptide, which now has a free thiol group, is allowed to bind to a resin bearing a ICH_2CONH-moiety. Impurities can be washed off the column then the product is released by treatment with 5% NH_4OH.

2.6 *Enzyme-mediated synthesis and semi-synthesis*

Fundamental studies of this aspect of peptide synthesis continue to abound but the number of applications is still quite small. Perhaps the two main reasons are (*a*) the absence of computerized hardware permitting the use of enzymic methods in SPPS, and (*b*) a reluctance by chemists to use enzymic methods.

Most attention has been directed towards the use of proteinases in systems of low water content. Some kinetic studies have been reported[160,161], but generally high yields of product have been the main target[162-169]. Sometimes the stereoselectivity is affected by conditions such as solvent and apparent pH[169-170]. The effect of additives has also received attention. For example, crown ethers promote transesterification reactions involving Ac-L-Phe-OEt in the presence of α-chymotrypsin[171] Again the yield of product can vary widely if different salt hydrates are added to the system[172,173]. Conjugation or simply the addition of enzyme to soluble or insoluble macromolecules continues to find favour, especially if it facilitates product isolation and enzyme recovery[174-185]. If the stability of the proteinase conformation in the presence of high concentrations of organic solvent is a limiting factor, it may be possible to develop a more stable mutant by genetic engineering. For example, a number of mutants of subtilisin have been constructed in order to increase the stability of the enzyme in $CHONMe_2$[186]. As interest in using proteinases in high concentrations of organic solvents has waxed so the study of enzymes in reverse micelles has waned[187]. The choice of *N*-protecting groups to be used in media containing organic solvents requires a compromise[188]. While small hydrophilic groups obviously

favour solution in aqueous solution, more hydrophobic groups tend to give better yields of product. It is appropriate to sound another warning about the possible complications in enzyme-catalysed peptide synthesis due to transpeptidation. A further study has been made of this phenomenon using penicillopepsin[189].

As well as using enzymes to catalyse peptide bond formation, deprotection of esters in particular is also possible. For example, as noted above, heptyl esters of *N*-protected peptides are hydrolysed by a lipase from *Rhizopus niveus*[58]. The problems engendered by racemization during peptide bond formation may be unravelled by stereoselective hydrolysis of diastereoisomeric peptide esters. Thus, alcalase, an enzyme from *B. licheniformis* with Z-D,L-Ala-L-Phe-OBzl as substrate, specifically hydrolyses the LL-diastereoisomer to the free acid[190]. Some other applications of nonproteolytic enzymes are noteworthy. With H-Phe-Lys-OBu^t and $CH_3CO_2CH_2CF_3$ as substrates, several lipases catalysed ε-*N*-acetylation[191]. Despite the comment above about unsatisfactory results with *N*-formyl or *N*-acetyl amino acids, these are the favoured substrates for acylpeptidase used in the synthetic mode[192]. A peptide can be extended by one residue at the *N*-terminus. Although *N*-phenylacetyl groups have played no significant role in peptide synthesis hitherto, the penicillin acylase from *E. coli* catalyses the introduction and removal of this group and this observation has been used in an enzymic synthesis of Leu-enkephalin[193]. It has long been known that the first step in the biosynthesis of penicillins and cephalosporins involves the non-ribosomal synthesis of H-Aad-Cys-D-Val-OH. It has now been shown that if the enzyme responsible (ACV synthetase) is incubated with [$^{18}O_2$]-D-valine, the resultant peptide contains only one ^{18}O-atom[194]. This result is consistent with a mechanism involving the formation of a valyl-enzyme intermediate.

Some examples of enzyme-catalysed peptide synthesis are given here. Kyotorphin (H-Tyr-Arg-OH) has been made using chymotrypsin as catalyst[195,196]. A range of di- and tri-peptides containing Asn has been made using thermolysin[197]. The protected peptide, Z-Cys(Bzl)-Tyr-Ile-OBu^t, has been constructed enzymically in both directions[198]. Protected peptides that are useful as substrates for trypsin-like enzymes because they contain a *C*-terminal -Arg-pNA or -Lys-pNA moiety have been made using acylpeptide esters as substrates for a *B. subtilis* proteinase[199]. Some peptides are tricky to synthesize enzymically either because they contain more than one amino acid of a similar type and are thus prone to hydrolysis at one site while while another bond is being synthesized or they have an unfavourable amino acid at the site of peptide-bond formation. They can be assembled using trypsin as catalyst and an inverse

substrate[200]. Z-Ala-Leu-NH_2 and Z-Ala-Val-NH_2 were synthesized from the 4-guanidinophenyl ester of Z-Ala-OH and either H-Leu-NH_2 or H-Val-NH_2. Because the product is stable to trypsin, the reaction is irreversible and can be carried out in solvents of high aqueous content and hence can be controlled and monitored by a pH-stat. This ingenious technique will surely be exploited with more valuable products as targets.

There have been a few examples of the enzyme-catalysed semi-synthesis of large peptides. Thus, GRF(1-29) ends at the *C*-terminus with the sequence -Met-Ser-Arg-NH_2. In model experiments, it was shown[201] that Bz-Met-Ser-X-OH (X = Ala, Leu, Arg) and an excess of H-Arg-NH_2 gave Bz-Met-Ser-Arg-NH_2 using carboxypeptidase Y as catalyst. In the best case (X = Ala), the yield was nearly quantitative. Accordingly, the peptide GRF(1-28)-Ala-OH was then assembled by continuous-flow SPPS. When this was incubated with H-Arg-NH_2 and carboxypeptidase Y, the yield of GRF(1-29)-NH_2 was 87%. The proteinase inhibitor, aprotinin, with the Tyr^{15}-Ala^{16} bond cleaved, was treated with amino-peptidase K to remove Ala^{16} and Arg^{17}. Exposure of the modified protein to various dipeptides containing Arg or Lys at the *C*-terminus in the presence of trypsin[202] effected a semi-synthesis with new amino acids at position 16 and with Arg^{17} or Lys^{17}. In the trypsin-catalysed reaction:

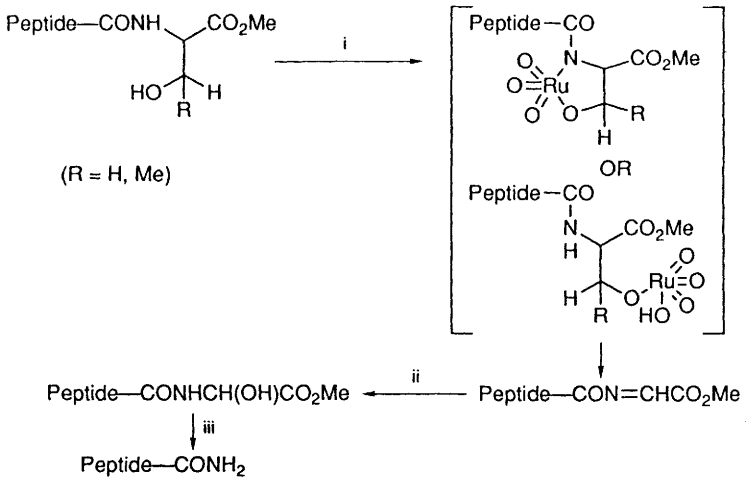
Porcine insulin + H-Thr-OMe human insulin-OMe + H-Ala-OH

^{18}O is rapidly incorporated from the medium into the terminal peptide bond[203], a result that is consistent with a mechanism involving hydrolysis. A small proportion of molecules, however, undergo aminolysis directly. In a similar reaction using lysyl endopeptidase and H-Thr-OBu^t, up to 90% of the human insulin ester could be obtained[204]. Finally, we take a peep into a possible future scenario. The strength of genetic engineering is the potential to synthesize long proteins faithfully with freedom from racemization. The weakness has been the limitation to the 20 coded amino acids and even post-translational modifications are not always accessible. A few attempts have been made to overcome this constraint by using an artificially acylated tRNA. A new technique is now being developed which involves site-specific mutagenesis to incorporate a termination codon at the desired position in the gene and to provide the translation system with the corresponding semi-synthetic suppressor tRNA charged with the noncoded amino acid to be incorporated. A rapid assay has been developed[205] which permits the determination of the efficiency of suppression and translation, defined as the percentage of suppression product relative to the total of suppression plus termination products. The results cover a wide spectrum. Thus, *N*-methylphenylalanine, cyclohexylalanine, 2-amino-4-phosphonobutyric acid and 3′-iodotyro-

sine gave very satisfactory incorporation, the results with 5-aminovaleric acid and 2-amino-3,3-dimethyl butyric acid were poor and D-phenylalanine was not incorporated at all. These results are encouraging and offer the prospect of synthesizing proteins with certain bonds stable to the action of proteinases, for example, by incorporating *N*-methylamino acids. The authors cite some difficulties that are being addressed, including the simplification of the synthesis of required acylated tRNAs and the scaling up of the operation to fermentator-scale processes. There are other potential shortcomings. The incorporation of surrogate peptide bonds such as -CSNH-is not yet possible and the complete failure of the method to permit the incorporation of -Gly-Gly-means that dipeptide surrogates such as statine are not yet candidates for this process. Nevertheless, the positive results obtained so far offer an exciting prospect for the future, but maximum progress is going to require the closest collaboration between organic chemists, biochemists and molecular biologists. This, rather than the scientific difficulties, may prove to be the rate-limiting step.

2.7 *Miscellaneous reactions related to peptide synthesis*

Hydrogenolysis of Fmoc or Z derivatives of amino acids in MeOH/CH_3CO_2H (1:1) produced small quantities of *N*-methylamino acids[206]. This side reaction was insignificant with peptide derivatives, presumably because these are deprotected faster than the amino acid derivatives. Synthetic eledoisin was found to be mutagenic towards *Salmonella typhimurum*[207]; this activity was traced to the presence of small quantities of salts of HN_3 formed during a final azide coupling step. Peptides with *C*-terminal -Ser-OR or -Thr-OR can be oxidatively degraded (Scheme 12) to peptide amides. This is regarded as a possible model for the biosynthesis of amides[208]. The synthesis of peptides of Asp, Glu, Asn and Gln continues to offer technical and mechanistic challenges. The formation of aminosuccinyl intermediates from -Asp(OR)-peptides and the resultant epimerization in presence of bases has been further studied[209]. At 37° C and pH7.4, Gln peptides spontaneously cyclize to glutarimide albeit more slowly than Asn peptides cyclize. Ring-opening then occurs to give a mixture of α-Glu and γ-Glu peptides[210]. These reactions may not be a serious complication during conventional syntheses but may be important in the storage or solutions of peptides and proteins. A method for the radioiodination of Phe peptides has been described[211]. The analogue containing 4′-aminophenylalanine is synthesized then subjected to the Sandmeyer process to give the 4′-iodo-derivative. Yields were improved by incorporating 18-crown-6 during the reaction of the diazonium salt with CuCN. The *N*-nitroacetyl group is a peptide synthon[212,213] since



Reagents: i, RuO_4; ii, H_2O; iii, MeOH/HCl

Scheme 12

alkylation of e.g. NO_2CH_2CO-Pro-OR can be effected on the nitroacetyl moiety.

3 Selected examples of peptide syntheses

The need for this section is perhaps diminishing in view of the successful synthesis of long sequences particularly by SPPS. Accordingly, only a few references are cited here. Using a 2-chlorotriphenyl linker, prothymosin α (109 residues) has been assembled by SPPS using Fmoc chemistry and carbodiimide coupling in presence of HOBt[214]. As a counterbalance, eglin c (70 residues) has been synthesized by classical solution methods using the azide method to couple fragments and HF to deprotect the completed sequence[215,216]. As an example of the assembly of a 3-chain molecule containing two disulphide bonds, a fragment of laminin (95 residues in total) was made by assembling the three chains by SPPS then forming the disulphide bridges by aerial oxidation and thallium (III) trifluoroacetate in a stepwise fashion[217]. Finally, genetic engineering and semisynthesis have been used in conjunction in a preliminary exercise[218]. An engineered mutant (S65M) of yeast cytochrome c was cleaved with CNBr at Met^{65}. The fragments obtained by cleavage refolded co-operatively bringing together the breakpoint termini, enabling autocatalytic peptide bond synthesis to take place. One can now visualize the possibility of genetically engineering a regulatory domain of one enzyme and the binding site of another and joining these molecules covalently by a mild semisynthetic technique.

4 Appendix. A list of Syntheses Reported in 1991

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

Peptide/protein	*Ref.*
4.1 **Natural peptides, proteins and partial sequences**	
Acetylcholine receptor	
Fragments related to rat α subunit	219
Fragment (172-227) of *Torpedo* receptor	220
Actin	
Synthesis of actin (1-28)	221
Acyl carrier peptide	
SPPS of fragment (65-74) on cotton	122
Albomycin	
Synthesis of albomycin-like peptides	222

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3

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

BY C. M. BLADON

1 Introduction

The format of this chapter is similar to that used in recent years. Minor tinkering with the sub-divisions, most notably by including HIV inhibitors separately, is a reflection in the interest within these areas. Section 7 has not been sub-divided as many conformation studies now use combinations of n.m.r., c.d., X-ray and computational methods.

Material for this chapter was obtained by scanning the major bio-organic journals and Chem. Abs. Selects: Amino Acids, Peptides, and Proteins up to May 18th 1992 (issue 10). Individual contributions from the proceedings of the 12th American Peptide Symposium[1] have not been included and the patent literature has not been reviewed. Academic institutes contributed approximately 75% of the papers with the other 25% coming from industrial organisations.

2 Peptide-backbone modifications

2.1 *ψ[CSNH]-Analogues*

Lawesson's thionylation procedure converted Boc-Met-NH_2 into the thioamide and this was then incorporated[2] into the Substance P analogue pGlu-Phe-Phe-Gly-Leu-Metψ[$CSNH_2$]. The potency of this compound as assessed by the guinea pig ileum test was considerably lower than that of [pGlu^6]$SP_{6\ 11}$. In the crystal structure[3] of cyclo[-Pro-D-Pheψ[CSNH]Ile-D-Thp-Thp-D-MePhe-] (oxytocin-receptor ligand) the backbone was relatively flat except at the thioamide bond which, as a result of the larger steric requirement of the sulphur atom, was pushed out of the plane of the rest of the molecule.

2.2 *ψ[NHCO] - Retro-inverso Analogues*

A theoretical approach[4] to analysing the conformational effects of reduced and retroamide links in model peptides concluded that incorporation of a ψ[CH_2NH] link generally favoured formation of a β-turn while

very stable β-sheets were formed by reversing every second amide bond of each strand. Both types of linkage caused a reduction in the stability of α-helices and the effect was more pronounced in peptides with a retro-amide bond. Incorporation of the retro-amide bond in the tuftsin analogue H-Thrψ[NHCO](*R,S*)Lys-Pro-Arg-OH was achieved in high yield by coupling Meldrum's acid derivative (1) to O-*t*-butyl-D-threonine amide.[5] The analogue was completely resistant to enzymatic degradation *in vitro*. Cyclic retro-inverso dipeptides with two aromatic side chains were prepared as part of a study to define the mimimum structural requirements for binding affinity with opiate receptors.[6] Conformational studies on these compounds (2) were reported in a second paper.[7]

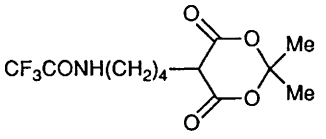
2.3 *ψ[CH$_2$NH] - Amino Methylene Analogues*

The Pheψ[CH$_2$N]Pro linkage has been incorporated into a number of potential HIV-1 protease inhibitors.[8] The ψ[CH$_2$N] bond was obtained by reduction of the amide bond with B_2H_6 and the most potent of the inhibitors was H-Thr-Leu-Asn-Pheψ[CH$_2$N]Pro-Ile-Ser-OH with an IC_{50} of 1.1μg/ml. Reductive amination of Boc-Val-Val with resin-bound peptide using $NaBH_3CN$ was the route[9] used to prepare the neurokinin analogue H-Asp-Ser-Phe-Valψ[CH$_2$NH]Gly-Leu-Nle-NH$_2$. Both this compound and Ac-Arg-Phe-Phe-Sarψ[CH$_2$NH]Leu-Met(O$_2$)-NH$_2$ were less potent than their parent compounds. The first active pseudopeptide analogue of an insect neuropeptide has been reported.[10] Unlike its amide-bond containing counterpart the activity of Pheψ[CH$_2$NH]Phe-Ser-Trp-Gly-NH$_2$ was not destroyed upon exposure to aminopeptidase M.

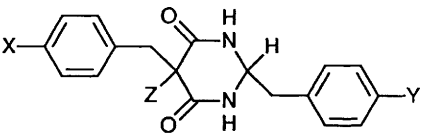
From an electronic point of view the ψ[CH(CN)NH] group is a better mimic for the amide bond than ψ[CH$_2$NH]. This new type of peptide bond isostere was formed by a Lewis acid-catalysed reaction of an N-protected α-amino aldehyde with a C-protected amino acid in the presence of trimethylsilylcyanide.[11] Extension at the N-terminus gave peptides such as Boc-Phe-Pheψ[CH(CN)NH]Leu-OMe.

2.4 *ψ[CH=CH] and ψ[CF=CH] - Ethylenic Isosteres*

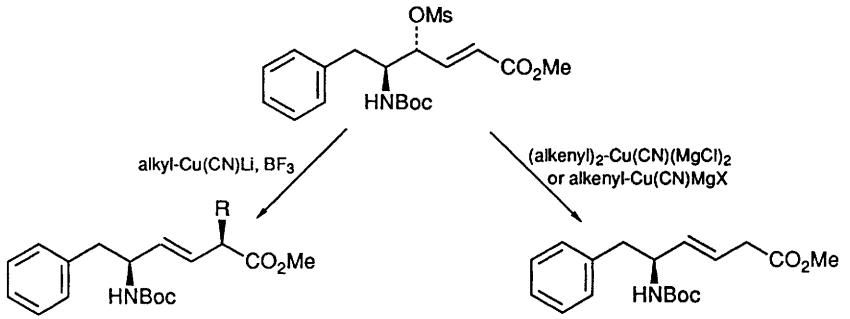
Several stereocontrolled syntheses of (*E*)-alkene dipeptide isosteres have been reported. Reaction of the mesylate intermediates, available in both stereoisomeric forms, with alkylcyanocopper-boron trifluoride reagents[12] (Scheme 1) enabled sterically hindered Pr^i or Bu^t groups to be introduced α to the ester group. The corresponding reaction with alkenyl-copper reagents[13] gave a high yield of the reductive elimination product. The key step in a second method[14] involved the stereospecific alkylation of (*R*) and (*S*) mesylates with Grignard reagents (Scheme 2). No isomer crossover was observed in the examples cited and the unoptimised yields



(1)



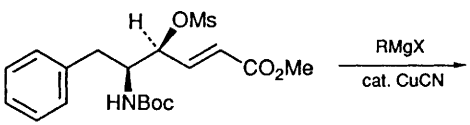
(2) X = H, Y = OH, Z = H
X = OH, Y = H, Z = H
X = OH, Y = H, Z = NH_2



exclusive anti-S_N2'

exclusive reductive elimination

Scheme 1



R = CH_2Ph, Ph, cyclohexyl, CH_2CHMe_2
similarly for other mesylate isomer

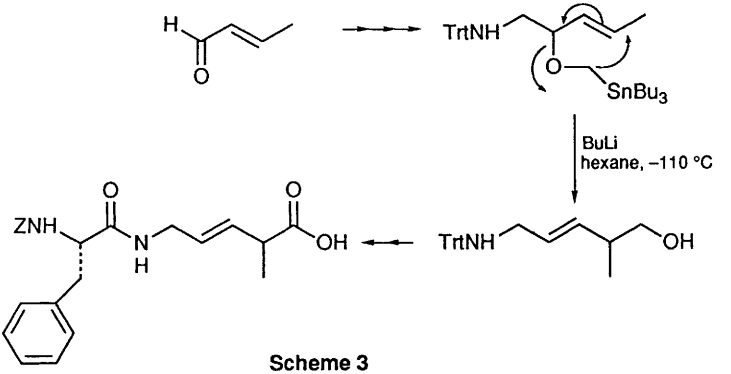
Scheme 2

were >70%. A [2,3]-Wittig-Still rearrangement,[15] illustrated by the synthesis of Z-Phe-Glyψ[E-CH=CH]D,L-Ala-OH (Scheme 3), offers a new approach to the synthesis of alkene dipeptide isosteres. Treatment of the Wittig precursor in hexane with n-BuLi at low temperatures gave largely the [2,3] rearranged product with a preference for the *trans* double bond (*cis*/*trans* ratio 1:1.5).

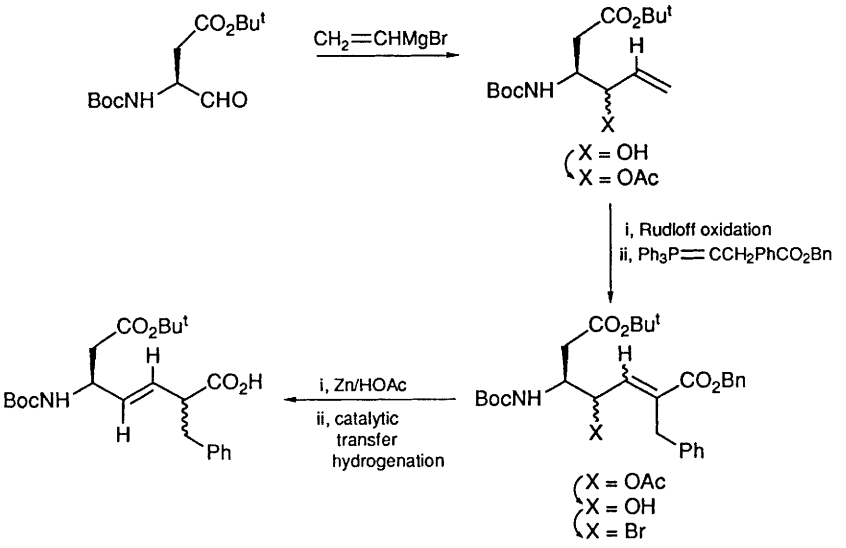
Protected double bond isosteres Boc-Asp(OBu^t)ψ[*E*-CH=CH]D,L-Phe-OH and Boc-Glyψ[*E*-CH=CH]D,L-Trp-OH, suitable for incorporation into peptides have been prepared (Scheme 4).[16] A shortcoming of the reaction sequence is the lack of stereocontrol at the α-centre of the second amino acid residue. Mixtures of diastereoisomers were also obtained *via* α-alkylation of β,γ-unsaturated acids. This route was used in the synthesis of bradykinin analogues[17] with ψ[*E*-CH=CH] and/or ψ[CH_2NH]/ψ[CH_2NCH_3] isosteres replacing the Gly^4-Phe^5, Phe^5-Ser^6 or Pro^7-Phe^8 amide bonds. The pseudodipeptides were incorporated into the bradykinin peptides using standard Merrifield protocols. The prolonged activity of the Pro^7-Phe^8 analogues correlated well with the cleavage of the Pro^7-Phe^8 bond being a main degradative pathway. Multiple replacements of the 4-5 or 5-6 and the 7-8 amide bonds resulted in analogues retaining considerable potency and having prolonged activity. For example, the diastereoisomeric mixture Arg-Pro-Pro-Glyψ[*E*-CH=CH]D,L-Phe-Ser-Proψ[*E*-CH=CH]D,L-Phe-Arg still lowered blood pressure by 20% after 1h. The optical purity of isosteres (*E*)-Boc-L-NH-CHRCH=$CHCH_2CO_2H$ (R=CH_2Ph, Me, $CH_2C_6H_4F$-4), was monitored by h.p.l.c.[18] Reaction of the Boc-dipeptide and a chiral glucopyransoyl isothiocyanate reagent revealed that the low optical purity observed for phenylalanine peptides was due to racemisation during the Wittig reaction. Enantiomeric impurities in the 1% range were reported.

2.5 *Phosphono-Peptides*

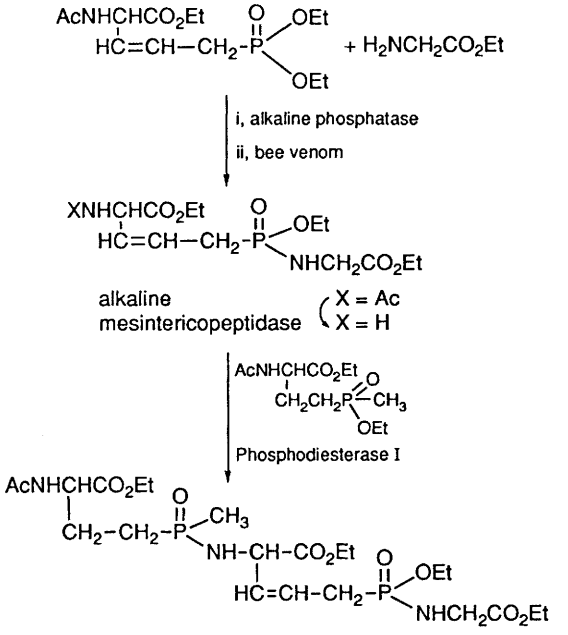
Phospha-C-di- and tripeptides have been prepared[19] using enzymatic methods for the condensation and subsequent hydrolysis of protecting groups (Scheme 5). The introduction of a phosphonoamide bond using BOP activation has been explored.[20] The reaction was monitored by ^{31}P n.m.r. and formation of the phosphonamidyl dipeptide (3) involved at least two distinct pathways, through an intermediate pyrophosphonate [(PhthNCH$_2$P(O)(OEt)]$_2$O and an intermediate HOBt active ester. C-Alkylation of (decarboxysarcosinyl) phosphonates gave derivatives with a variety of side chain functionality.[21] The reaction (LDA/alkyl halide) was essentially unselective and yielded mixtures of diastereoisomers. The phosphonoisostere of histidine[22] was synthesised from 4-imidazolylmethanol.



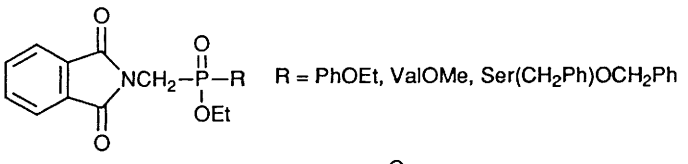
Scheme 3



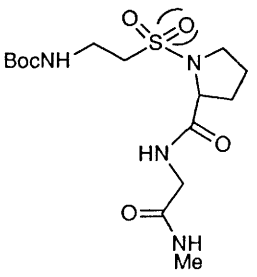
Scheme 4



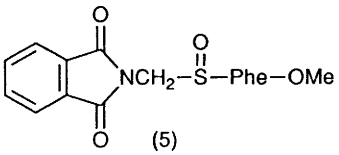
Scheme 5



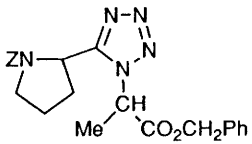
(3)



(4)



(5)



(6)

2.6 *ψ[CH$_2$O] - Methyleneoxy Analogues*

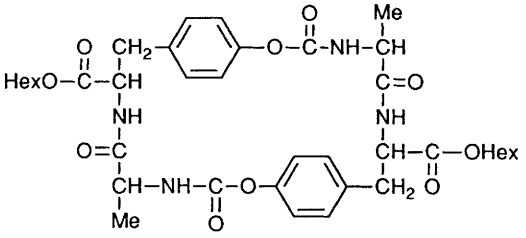
The ψ[CH_2O] isostere has been incorporated into substance P and enkephalin neuropeptides using protected pseudodipeptide units.[23] In a number of pharmacological assays [pGlu^6, Phe^{8}ψ[CH_2O]Gly^9]SP_{6-11} and [Tyr^{1}ψ[CH_2O]Gly^2, Leu^5]enkephalinamide were shown to be potent agonists whereas a second enkephalin analogue, H-Tyr-Glyψ[CH_2O]Gly-Phe-Leu-NH_2, had low biological activity for the m and d binding sites. The Gly^2-Gly^3 bond is therefore crucial for effective interaction with the opiate receptor.

2.7 *Miscellaneous Modifications*

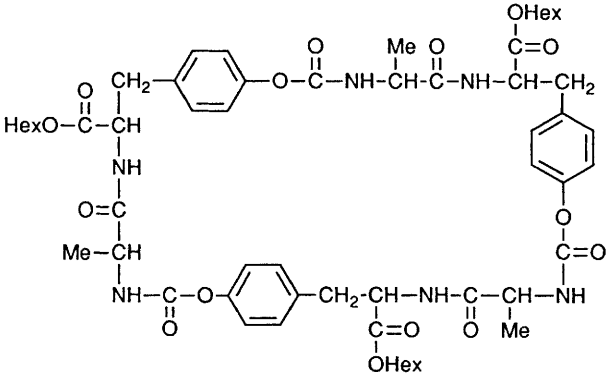
An n.m.r. study in $CDCl_3$ of cyclo[-Gly-Proψ[CH_2(*R,S*)SO]Gly-D-Phe-Pro-] indicated that both sulphoxides adopted a γ-turn conformation[24] stabilised by a hydrogen bond between the CO of Phe^3 and the NH of Gly^1. A *cis* Gly^1-Pro^2 bond and a type II′ β-turn involving the Gly-D-Phe-Pro-Gly residues was observed in the crystal structure and this conformation was also seen to a limited extent in DMSO solution. These structure differed from those of the ψ[CH_2S] and amide parent compounds due to the fact that the sulphoxide moiety did not participate in hydrogen bonding. Lack of hydrogen bonding resulting in a conformation difference from the all amide structure was also observed in Boc-Pro-Leuψ[$NHSO_2$]Gly-NH_2 (MIF-1).[25] The ψ[$NHSO_2$] induced a cisoidal conformation around the N-S bond and prevented the hydrogen bonded β-turn conformation found in the parent peptide.

Sulphinamides and sulphonamides are of interest as replacements for amide bonds in the development of protease inhibitors. Tripeptide analogues (4) of the Gly^{312}-Pro-Gly^{314} sequence in HIV gp120 incorporating these isosteric units were prepared[26] by reaction of the appropriate sulphinyl chloride and amino moieties. Sulphinamides were oxidised to sulphonamides by $RuCl_3/NaIO_4$. In the crystal structure of the *R*-sulphinamide (5)[27] the substituents around the sulphur atom were arranged tetrahedrally and the adjacent nitrogen atom adopted an almost, but not quite, planar conformation.

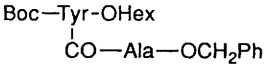
The use of a 1,5-disubstituted tetrazole ring as a surrogate for a *cis* peptide bond has been reviewed.[28] The crystal structure of Z-Proψ[CN_4]Ala-OBzl (6) revealed that the tetrazole ring system locked the geometry into a conformation corresponding to the *cis* isomer.[29] A *cis* conformation was induced by the urethane bond in the macrocycles (7) and (8) which were formed by cyclisation of the Tyr-Ala pseudopeptide (9).[30] The 24-membered macrocycle solubilised up to 0.6 molar equivalents of Li^+ ions in $CHCl_3$, and thus these compounds may find applications as cryptands.



(7)



(8)



(9)

Optically active monofluoro ketone isosteres (10)[31] with the ideal peptide backbone spacing have been prepared as potential inhibitors for the serine proteases. The latter stages of the synthesis are outlined in Scheme 6 and a key step of selectively deprotonating the α′ position was achieved using potassium hexamethyldisilazide.

Dipeptide units in which a 2,5,5-trisubstituted imidazoline ring replaced the amide bond have been prepared. To test the synthetic utility of these isosteric replacements the Trp-Nle unit was incorporated into the CCK-4 (11) and pentagastrin (12) derivatives.[32]

2.8 *C-Terminal Modifications*

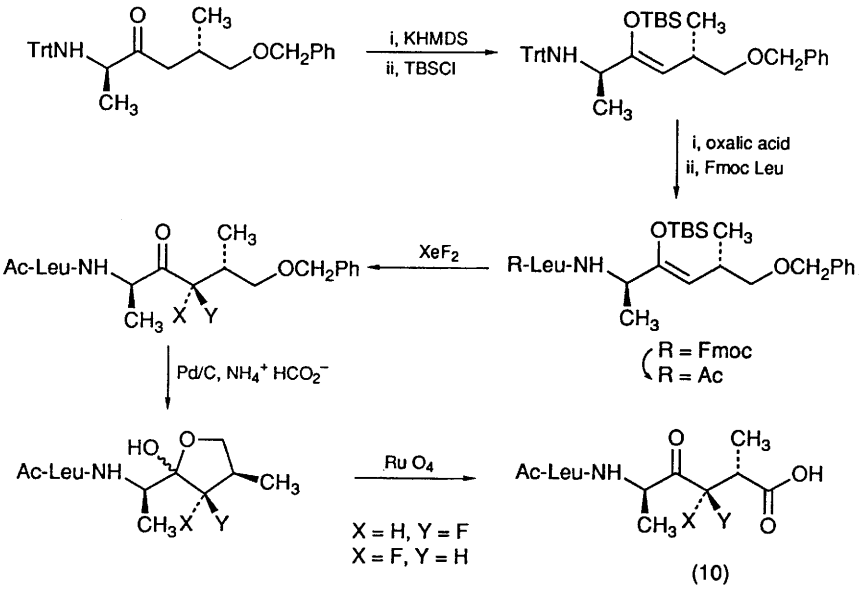
Structural elements necessary for recognition between enkephalins and their receptors have been studied using enkephalins with a C-terminal mercaptomethyl (CH_2SH) and hydroxymethyl (CH_2OH) groups.[33] The carboxyl group at the C-terminus was found to be important, although not electrostatically, for interaction with the δ-receptor. In the μ-receptor the reactive thiol group became covalently bound *via* disulphide formation to leucinthiolenkephalin after prolonged incubation.

Two series of gastrin releasing peptide (GRP) antagonists were developed in which the C-terminal Leu^{26}-Met^{27} region was replaced with either an alkyl chain or alkyl ether moiety.[34] One member of the alkyl ether series (13) specifically blocked radiolabelled GRP binding with an IC_{50} of 6nM. The corresponding alkylamide isostere (14) had four times the affinity for the GRP receptor - equipotent to native GRP in receptor binding assays - and both compounds were resistant to proteolytic degradation *in vitro*.

An analysis of the crystal structure of a number of simple peptides containing the hydrazide ψ[CONHN] or Z-aminoamide ψ[CON(NHZ)] link concluded[35] that the N-N bond in the hydrazide was more flexible and was involved in different conformational units including the β-turn structure. A β-turn was also evident in the crystal structure of the azaalanine dipeptide $Bu^tCOProNHNMeCONHCHMe_2$.[36]

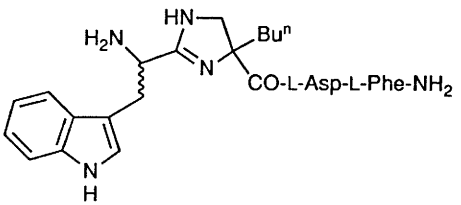
2.9 *α,α-Dialkylated Glycine Analogues*

Several Fmoc protected dipeptide building blocks containing C-terminal α,α-dialkyl amino acids have been prepared.[37] The one-pot synthesis involved coupling the dialkylated amino acid, temporarily protected with trimethyl silyl groups, with an appropriate Fmoc amino acid active ester (Scheme 7). Enolate alkylation of oxazinones gave α,α-disubstituted amino acids in essentially optically pure forms (Scheme 8).[38] Shielding of the C3 position by the bulky phenyl groups directed the alkylation to the less hindered face. The very sterically hindered amino

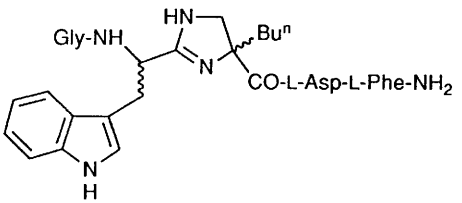


(10)

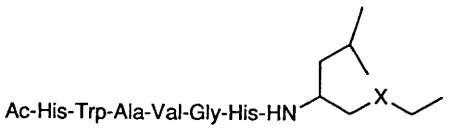
Scheme 6



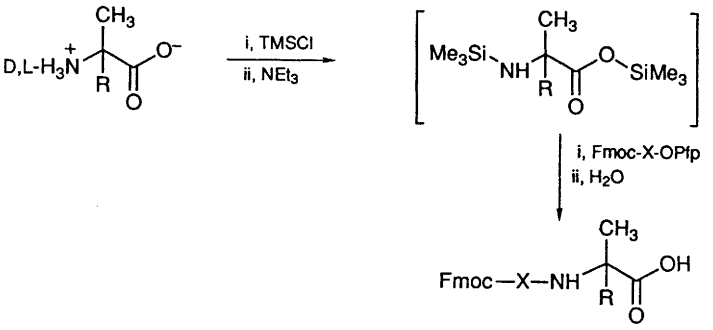
(11) CCK-4-analogue



(12) pentagastrin analogue

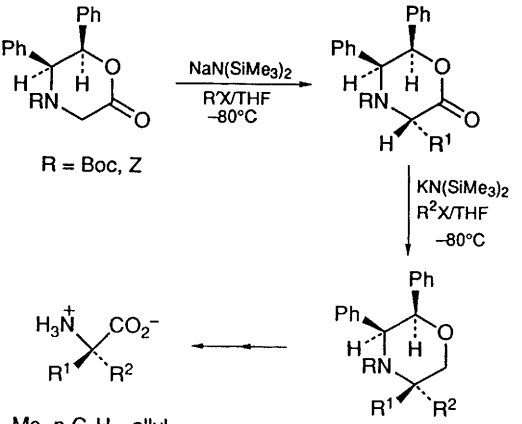


(13) X = O
(14) X = CH_2



R = CH_3 or CH_2OH
X = Ala, Val, Leu, Ser(Bu^t), Thr(Bu^t)

Scheme 7



R = Boc, Z

R^1 = Me, n-C_3H_7, allyl
R^2 = CH_2Ph, $CH_2CH{=}CHPh$
reverse stereochemistry starting from 5*R*, 6*S*-oxazinone

Scheme 8

acid, α,α-diisopropylglycine was prepared *via* a high pressure (9kbar) Ugi reaction which lasted 14 days.[39]

Pd(0) catalysed bisallylation of N-(nitroacetyl)amino acid esters followed by reduction of the nitro group was a convenient route to the dipeptide unit with identical α-allyl substituents at the N-terminal residue.[40] The dipeptide esters Boc-NHCRR′CO-Gly-OEt (R, R′ = various alkyl groups) were prepared by DCC mediated coupling of the Boc α,α-disubstituted amino acids with H-Gly-OEt.[41] The tripeptides Boc-X-Glu-Val (X = αMeGlu or the five and six membered cyclic analogues of glutamic acid) were found to be effective substrates for the vitamin K-dependent carboxylation reaction.[42]

A comprehensive review[43] on the structure of polypeptides incorporating several α,α-disubstituted residues has been reported. The bulk and nature (linear or cyclic) of the side chains affects whether long sequences of the disubstituted residues adopt a 3_{10}-helix or extended (C_5) conformation. The same group of workers also reported that the minimum energy conformations of bulky derivatives of $C^{\alpha,\alpha}$-symmetrically disubstituted glycines[44] fall in the fully extended (C_5) regions. Furthermore, in a series of studies with chiral disubstituted glycines, L-αMeVal was found to be a strong β-turn and right-handed helix former. Conformational analysis showed that, for example, Z-L-αMeVal-Ala-Ala-OMe adopted a type I β-turn conformation which was stabilised by a 1-4 CO-NH hydrogen bond.[45] Tetra- and pentapeptides in which the αMeVal residue was incorporated into sequences of Aib (α-aminoisobutyric acid) residues folded into right-handed 3_{10} helices.[46]

In studies with Aib homopolymers, Z-$(Aib)_7$-OMe[47] and *p*BrPhCO-$(Aib)_{10}$-OBu^t,[48] both adopted regular 3_{10} helices whereas the preferred conformation for peptides with a smaller fraction of Aib residues, for example, Boc-Leu_4-Aib-Leu_4-OBzl[49] and Boc-Aib-Ala-Leu-Ala-Aib-Aib-Leu-Ala-Leu-Aib-OMe,[50] was an α-helix. A mixed 3_{10}/α-helical conformation was detected in the crystal structure of Z-Aib-Gly-Aib-Leu-Aib-$OCMe_3$,[51] the N-terminal pentapeptide of the antibiotic trichotoxin. The conformational preferences of sequence permutation isomers of octapeptides containing 75% Aib have also been investigated.[52] A type II β-turn conformation, stabilised by a very weak 4-1 hydrogen bond, was observed in the crystal structure of Z-Ile-Ala-Aib-Aib-OMe.[53] Slight differences in the β-bend conformations were observed in the crystal structures of urethane protected dipeptides containing an Aib residue and either a Hyp or a Hib ($OCMe_2CO$)moiety.[54] The chiral isovaline[55] and Ac_nc (aminocycloalkyl-1-carboxylic acid, n = no. of carbon atoms in ring)[56] residues also have a similar β-bend inducing effect to Aib.

Stabilising the helical folding of the chemo-attractant OHC-Met-

Leu-Phe-OH by replacing the phenylalanine with αMePhe[57] or Ain (2-aminoindane-2-carboxylic acid)[58] and/or the methionine with Thp (4-aminotetrahydrothiopyran-4-carboxylic acid)[59] gave analogues which retained the activity of the parent peptide.

Incorporation of $C^{\alpha,\alpha}$-dialkyl, -NMeAla, and N^{α}-C^{α} cyclic amino acids into angiotensin II octapeptide agonists and antagonists were designed to impose a conformational constraint to the molecule. Compounds with both single and multiple substitutions were prepared.[60] A range of biological results were obtained with some compounds retaining their agonist or antagonist properties whereas reduced levels of activity were found for other analogues. However, no highly constrained biologically active analogues were discovered.

3 Conformationally Restricted Cyclic and Bridged Analogues

3.1 *Rings and Bridges formed* via *Amide Bonds*

Side-chain to side-chain cyclisation to yield a lactam has been a feature of several papers this year.

High dilution and the use of diphenylphosphoryl azide gave a high yield of the cyclic peptide analogue of neuropeptide Y (NPY) (15)[61] which had a receptor binding constant comparable with that of NPY. The cyclic physalemin analogue pGlu-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2 (16)[62] and the shorter tetrapeptide unit Boc-Asp-Pro-Asn-Lys-OH[63] both adopted β-turn conformations stabilised by hydrogen bonds. The cyclic undecapeptide also largely retained the tachykinin receptor activity of the parent linear molecule. Thus the salt-bridge which stabilised the β-turn conformation of physalemin was successfully mimicked by a covalent lactam bond. The conformation of the Glu^3 analogues were, in comparison, rather flat and extended and a marked drop in biological activity, particularily at the NK-2 receptor, was observed with Glu^3-(16). The small cyclic thymopoietin analogue Asp-Lys-Asp-Glu-Tyr designed to closely mimic the proposed active site of thymopoietin was inactive. Three other lactam-bridged analogues, based on Arg-Lys-Asp-Val-Tyr were also prepared and these were biologically active although they could not mimic the thymopoietin conformation predicted from solution n.m.r. and theoretical energy minimisation studies.[64]

A range of structure/activity data was obtained for the cyclic opioid peptide analogues (17).[65] Futher restricting the conformation by incorporating Aic (a constrained phenylalanine analogue) at position three resulted in a potent agonist with a high preference for the μ receptor compared to the δ receptor. In contrast, the very low potency observed with *inter alia* the MePhe and Tic derivatives indicated that N^{α}-alkylation

at the three-position was detrimental to activity. The 14-membered ring analogue (18) showed high affinity at both the μ and δ receptors when the L-amino acid was incorporated at the fourth residue but only μ receptor activity with the D-isomer.[66] The overall structure and thus biological profile was thought to be strongly dependent on the conformation of the fourth residue. Folded conformations, with nearly parallel orientations and a close proximity (< 10 Å), of the aromatic rings of the tyrosine and naphthylalanine residues were necessary for activity at the δ receptor whereas interaction with the μ receptor required a relatively extended conformation in which the two aromatic side chains were orientated in opposite directions.

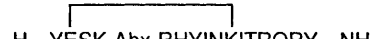
A new constrained γ-lactam moiety has been synthesised (Scheme 9)[67] and incorporated[68] into the N-terminal fragment of human growth hormone. Compound (19, $X = H_2N$-Leu-Ser-Arg-Leu-Phe, $R^2 = $Ala-$NH_2$) showed diminished hypoglycaemic activity in the *i.v.* insulin tolerance test compared with the isomeric γ-lactam (carbonyl at position 2) and aspartimide analogues.

3.2 *Bridges formed by Disulphide Bonds*

Conformationally restricting enkephalin analogues with S-S bonds has led to a series of DPDPE derivatives (20) with a range of binding and bioassay results.[69] Compounds with *S*-stereochemistry at the two chiral centres of the phenylalanine derivative showed high selectivity for the δ *vs* the μ receptor. Virtually no receptor selectivity and very low potency was observed for analogues with the other combinations of stereochemistry. All substitutions of D- and L-Cys and D- and L-Pen (Pen + penicillamine) at residues two and four in the δ-receptor selective tetrapeptide H-Tyr-D-Cys-Phe-D-Pen-OH reduced the affinity of the peptide for the δ-site.[70] Radiolabelled DPDPE,[71] prepared *via* a Sandmeyer reaction on the 4′-NH_2Phe-substitutent precursor, was also a very potent and selective ligand for the δ-opioid receptor.

Several low energy conformers of DPDPE were identified in a molecular mechanics (SYBYL) study[72] but additional conformational constraints may need to be incorporated in order to elucidate the receptor-bound conformation of this enkephalin derivative. A molecular dynamics study of the DPDPE zwitterion also showed that the aromatic side chains were very flexible and that the orientation of these residues in the bound conformation is probably largely influenced by the receptor environment.[73] The effects of salt on the structure and dynamics of the DPDPE zwitterion have also been investigated.[74]

The importance of side chain conformation for receptor interaction has been studied by utilising the constrained amino acid Tic as a



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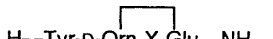

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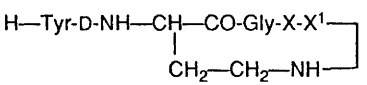
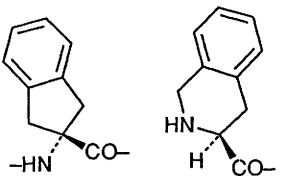
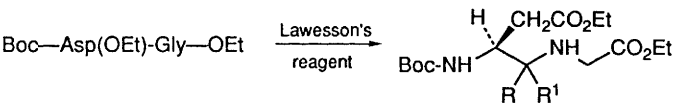
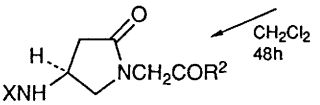


(18) X = L- or D-β-(1-naphthyl)alanine
X^1 = L- or D-Leu



Aic Tic

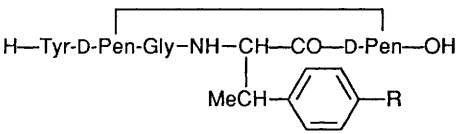




(19)

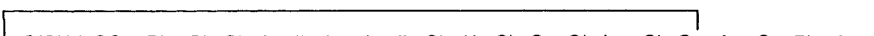
X = Boc, R^2 = OEt $\xrightarrow{NaOH}$ R^2 = OH

Scheme 9



(20) R = H, NO_2

(21)



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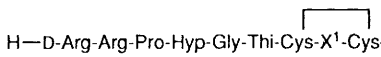
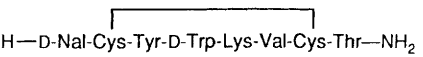


(23) X^1 = D-Tic-Phe-Arg, D-Phe-Phe



(24)

replacement for Phe and Tyr in the μ opioid specific octapeptide CTP, D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2.[75] An N-terminal Tic residue favours a gauche (−) side chain conformation ($\chi_i = +60°$) but due to pseudoallylic strain a Tic residue in an internal position has a gauche (+) side chain conformation ($\chi_i = -60°$). The D-Tic[1] analogue was found to be a better selective ligand for the μ receptor. N.m.r. and molecular dynamics calculations showed that the separation of the Tic and Thr aromatic rings was better in this compound than in CTP. As the backbone conformation of each peptide which was studied was similar the relative spatial orientation of the aromatic rings at positions one and three may be critical for interaction with the μ opioid receptor. Further work to stabilise the pipecolic ring conformation has resulted in the preparation, in high optical purity, of the four isomers of α,β-dimethyl-Tic.[76]

The cyclic disulphide (21) was found to be a highly potent antagonist of the fibrinogen receptor and the compound was developed by modifying the receptor binding sequence (-Arg-Gly-Asp-Ser-).[77] Classical methods of synthesis were adopted for the preparation of atrial natriuretic peptide analogues in which each of the residues in the C-terminal tripeptide sequence of (22) was systematically replaced by the D-amino acid.[78]

The hypothesis that bradykinin antagonists require a β-turn geometry at their C-terminus was explored with a range of analogues including the S-S bridged derivatives (23).[79] These compounds were unexpectedly inactive in the receptor binding assays and conformational analysis using energy calculations suggested that the *cis*-geometry between the Tic and Phe residues prevented formation of a bioactive conformation. More success with β-turns in cyclic disulphides was found with the biologically active somatostatin analogue (24) which adopted a β-II′turn/β-sheet structure in solution.[80]

Model cyclic tetrapeptides Ac-L-X-L-Pro-D-Val-L-Cys-NH_2, (X = Pen, Cys) also assumed type II β-turns in solution[81] although the dihedral angle of the disulphide bridge was different in the two peptides. Short α-helical model peptides have been stabilised by forming a disulphide bridge between the $(CH_2)_4SH$ side chains of 2-amino-6-mercaptohexanoic acid at positions i and i + 7.[82] The peptide was only locked into the helical conformation if the δ-amino acid was at position i and the L-amino acid was at the i + 7 position.

3.3 *Miscellaneous Bridges and β-turn Mimetics*

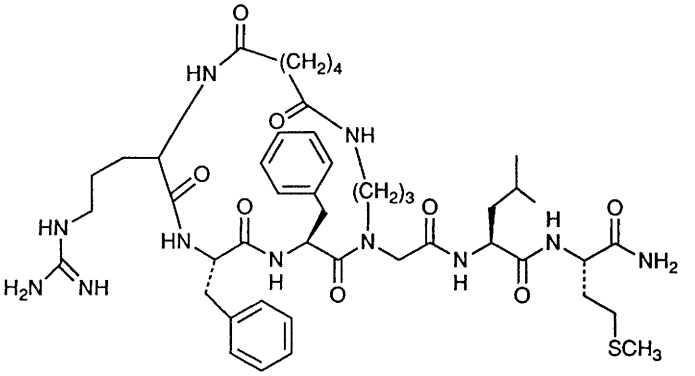
A table of the most important peptide hormone conformations and their secondary structures has been included in a two-part review on peptide conformation mimetics.[83,84]

A new concept of N- and C-backbone cyclisations involved linking the ω-substituted alkylidene chains which replaced the N^{α} or C^{α} hydrogens in a peptide backbone.[85] The method was applied to the active region of substance P and the 20-membered ring compound (25) was active, selective for the NK-1 receptor, and metabolically stable. Substance P_{6-11} derivatives in which the side chain of Glu^6 and the C-terminal carboxylic acid were linked *via* an oligomethylene diamine bridge[86] showed $\leqslant 0.1\%$ of the substance P activity in the guinea pig ileum assay.

Elaboration of the imine group of a 2,3 methanoproline derivative with aspartic acid gave the ethylene-bridged analogue (26) of H-L-Asp-NH-△-CO_2Pr.[87] However the bridged compound had a bitter taste with no indication of the sweetness of the cyclopropane-containing peptide. The absolute configuration of the 1,2,3-trisubstituted cyclopropane (27)[88] was determined from an X-ray crystal structure of the (-) menthol derivative. Conformational studies on *N,N'*-ethylene-bridged dipeptides (28) established that the piperazinone ring adopted a pseudo-chair conformation and the side chain on the ring was predominantly quasi-equatorial.[89]

As in linear and cyclic peptides containing a sulphonamide junction, the S-N bond of (29) held the C^{α} atoms of taurine and proline in a cisoidal arrangement. The crystal structure also showed that the seven-membered ring adopted a twist-chair conformation while the pyrrolidine and oxazolidinone rings showed an envelope conformation.[90] The tricyclic compound (30) was designed and synthesised (see Scheme 10 for outline of synthetic route) as a potential helical template.[91] X-ray analysis and n.m.r. studies in $CDCl_3$ showed that (30) adopted a conformation unsuitable for helix nucleation. In more polar solvents an equilibrium mixture of several conformational states was detected and one of these states is a potent helix nucleator. The compound has been linked (*via* the 5-CO_2H group) to alanine oligomers.[92] The stereochemistry at the α-carbon of Boc-Orn(Z)-CH_2-$CR(CO_2Me)_2$ was retained during the three-step/one-pot conversion of the pseudodipeptide derivative to the 8-amino-3-oxoindolizidines (31).[93] Although a *trans* disposition of the C-3 and C-6 substitutents in the diketopiperidine (32) was prefered, the level of stereoselectivity obtained at the C-3 position was dependent on the starting amino acid derivative (Z-X-CH_2-$CH(CO_2Me)_2$, X + Phe, Trp).[94]

A simple substitution of proline by the α-methyl derivative stabilised the β-turn conformation at the N-terminus of a peptide antigen resulting in a compound (33) with a higher affinity for two antibodies raised against the native sequence.[95] The spiro γ-lactam analogue (34), predicted to adopt a similar reverse turn conformation, was not recognised by either antibody. Loss of the backbone Tyr^3 NH and steric problems associated with the extra bridging CH_2 group probably prevent-



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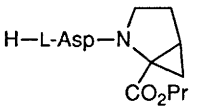

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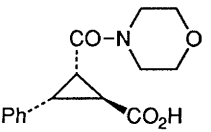

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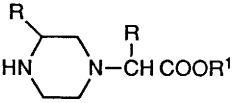

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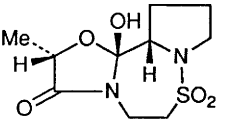

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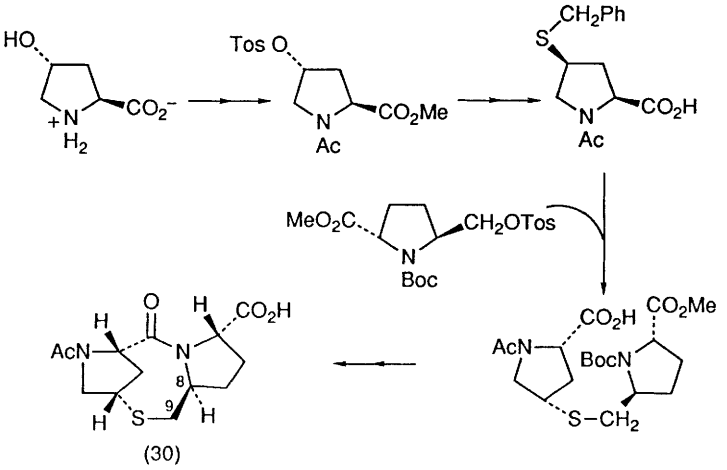


(30)

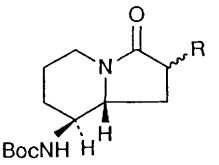
Scheme 10

ed the latter compound from making the required contacts with the antibody.

The bicyclic β-turn dipeptide (35) has been incorporated into the N-terminal 29-residue fragment of human growth hormone-releasing factor (hGHRF)[96] and gramicidin S[97] using solid phase methodology. The hGHRF analogues exhibited a similar type of *in vitro* activity to the parent molecule although the potencies were very low. The bicyclic moiety was shown in an n.m.r. study of the gramicidin derivative (36) to adopt the expected type II′ β-turn. The dipeptide sequence X-Y at the C-terminus of bradykinin analogues D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-X-Y-Arg has been replaced by the model peptides (37a,b) which were designed to favour β-turn conformations.[98] The enhanced potencies of the *trans* (to the carbonyl) alkyl derivatives in both series compared to the reference antagonist (X-Y + D-Phe-Phe) was attributed, in part, to a region of the receptor binding site able to accommodate steric bulk. To simulate the C-terminal β-turn of MDP the D-isoglutamine residue was substituted by a branched δ-prolyl moiety.[99]

4 Dehydroamino Acid Analogues

A Wittig reaction was responsible for formation of the double bond in Z-Phe-Δ^Z Phe(4Me)-CO_2Me.[100] However the key to the synthesis was the oxidative fragmentation of Ser/Thr residues with $Pb(OAc)_4$ to α-acetoxyglycine derivatives (Scheme 11). Stereoselective *anti*-elimination of HBr from the (2*RS*, 3*RS*) enantiomeric pair of PhthNCH(CHBrPh) $CONHCMe_3$ gave the (Z)-α,β double bond while the corresponding reaction on the other pair of enantiomers resulted in the (*E*)-isomer.[101] The (*E*)-isomer was found to be unusually stable and did not isomerise to the (*Z*)-isomer. It is interesting to note that the enantiomeric pairs of the β-bromophenylalanine derivatives were separated by sorting the small colourless needles (2*RS*, 3*RS*) from the larger yellow granules (2*SR*, 3*RS*). An enzymic method was favoured for the separation of the (*Z*) and (*E*) isomers of Bz-ΔPhe-OH.[102] α-Chymotrypsin hydrolysed only the (*Z*)-methyl ester and Bz-Δ^EPhe-OMe was isolated unchanged after work-up. Acid catalysed condensation of Ac-Pro-NH_2 with α-keto butyric or α-keto isocaproic acid followed by coupling with methylamine gave Ac-Pro-Δ^ZX-CONHMe (X = Abu, Leu).[103] Coupling of H-L-Leu-ΔVal-L-Ser-OMe with the hydrazinocarboxylic acid (38) led to the synthesis of the C-terminal dehydrotetrapeptide of the antimycins (39).[104] X-ray studies on For-Met-Δ^ZLeu-Phe-OMe[105] and Boc-L-Pro-Δ^ZPhe-Gly-NH_2[106] in which the Δ residue occupied the central position showed the presence of a β-turn. A significant population of two



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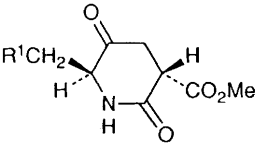

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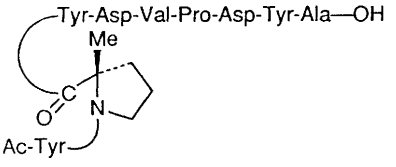

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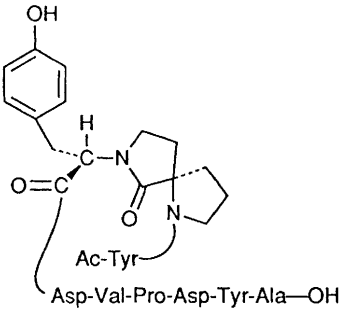

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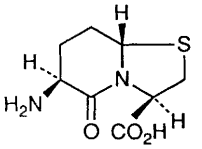

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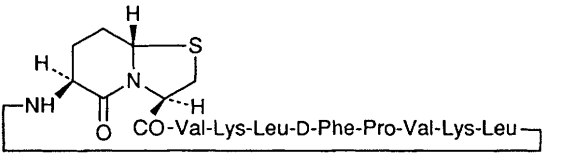
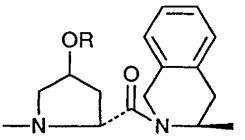
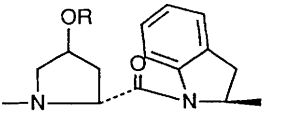


(36)

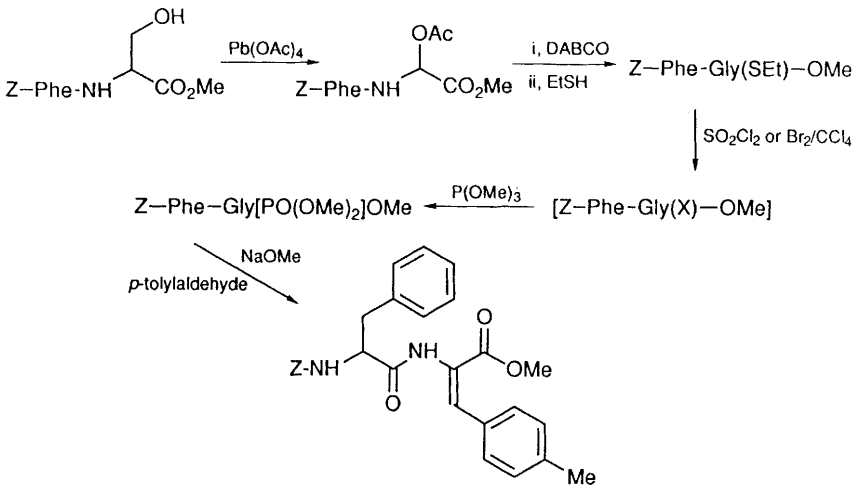
X-Y ≡



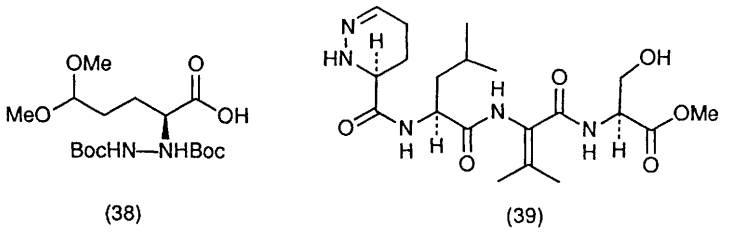
(37a) OR = *R* or *S*-CH_3 or $CH_2CH_2CH_3$

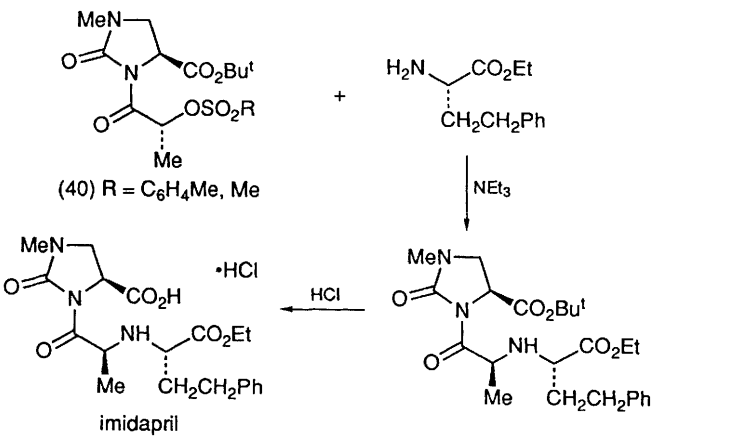


(37b) OR = *R* or *S*-$CH_2CH_2CH_3$



Scheme 11





Scheme 12

consecutive hydrogen-bonded (type II-III′) β-bends was observed in the n.m.r. of the latter compound. A type II β-turn was the predominant conformer observed in the n.m.r. spectrum of ^{t}BuCO-D,L-Ala-Δ^{Z}Phe-NH^{i}Pr.[107] The crystal structures of N-Ac-ΔPhe-L-Val-OH[108] and N-Ac-ΔPhe-L-Val-L-Val-OMe[109] showed that the backbones adopted alternate right and left handed helical conformations. The two Δ Phe residues in Boc-Ala-ΔPhe-Gly-ΔPhe-L-Ala-OMe caused the backbone to adopt a 3_{10} helical conformation which was present in solution as a solvent dependent equilibrium between the left and right handed enantiomers.[110] The crystal structure of Ac-ΔPhe-L-Val-ΔPhe-NHMe showed a right handed 3_{10} helix.[111]

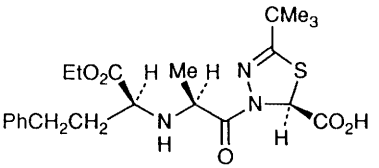
A mass spectral method has been described for the structural characterisation of isomeric didehydroamino acids.[112] The technique relies on ionisation differences between the isomers being reflected in the EI/CI spectra.

5 Enzyme Inhibitors

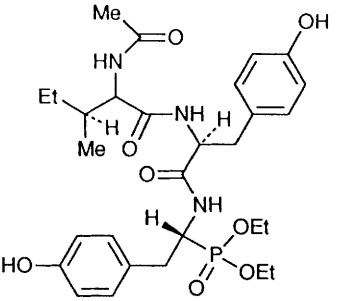
The upsurge of interest in renin inhibitors reported last year has continued into the 1991 literature. Not surprisingly the design of HIV-1 protease inhibitors has proved popular and these are now included under a separate heading. The 'everything else' section has been modified slightly and now only includes (with one exception) inhibitors of other proteases.

5.1 *Angiotensin Converting Enzyme (ACE) Inhibitors*

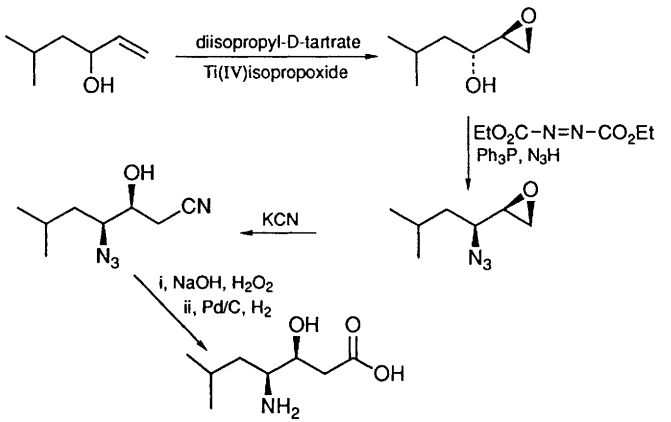
A review on the synthesis of ACE inhibitors has been published.[113] The solid state conformation of perindoprilat has been determined and compared with the crystal structures of several other structurally related ACE inhibitors.[114] In a new synthesis of imidapril, curently undergoing clinical study as an antihypertensive drug, the sulphonates (40) were less sensitive to racemisation under weakly basic conditions than the bromo compounds previously used. Coupling to the butyric acid component (Scheme 12) proceeded in an S_N2 fashion to give the diester precursor of imidapril.[115] Another ACE inhibitor (41)[116] is also undergoing clinical trials. This derivative was the most promising candidate from two series of dihydrothiadiazole ring containing compounds, and in animal models produced a long lasting antihypertensive effect after oral dosing. Analysis of the crystal structure of (42) and captopril suggested that the potency of the phospho tripeptide (equivalent to captopril) was due to similarity in disposition of heteroatoms in the two compounds (oxygens of Ile C=O



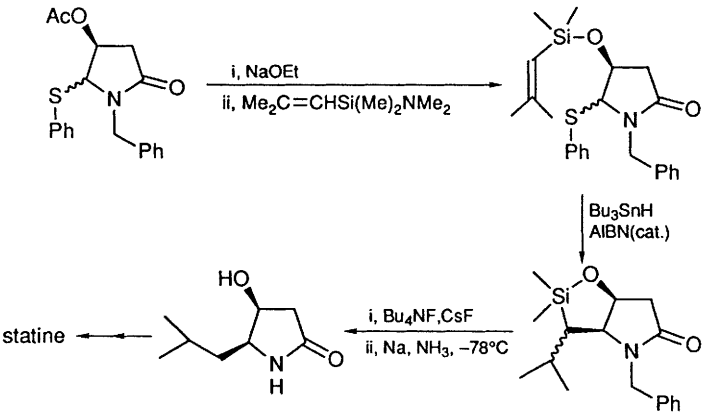
(41)



(42)



Scheme 13



Scheme 14

and phosphonate in (42) with sulphur and carboxylic acid oxygen in captopril).[117]

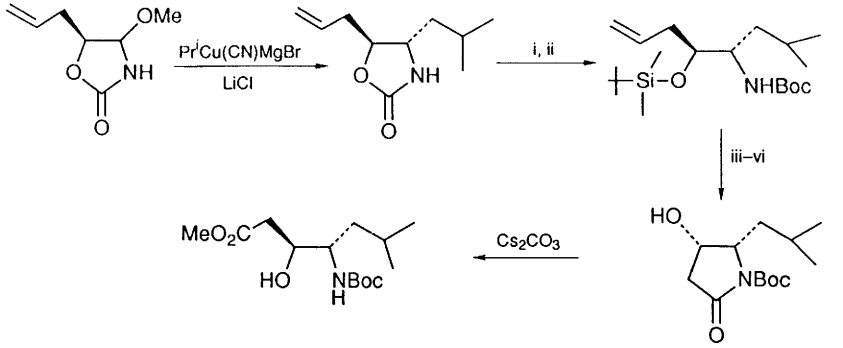
Inhibitors have also been prepared by substituting the N- and C-terminal amino acid residues of Bz-Phe-Ala-Pro-OH with quinoline derivatives.[118] Several tripeptides (Leu-X-Pro, X = Arg, Ser, Gln), isolated from a hydrolysate of zein (maize endosperm protein) were found to be weak ACE inhibitors (IC_{50}s μmolar range).[119,120]

5.2 *Renin Inhibitors*

The principles involved in the design of renin inhibitors are described in a review.[121] The use of the hydroxyethylene isostere as a building block in the synthesis of inhibitors has again proved popular.

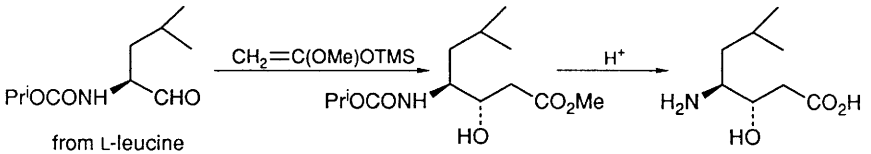
Several enantiospecific syntheses of statine have been reported and key steps are outlined in Schemes 13-16 (references 122-125). Cycloaddition of chloronitrile oxide to *N*-allyltrichloroacetamides showed low stereospecificity although the *threo*-isoxazoline was the preferred product (*threo:erythro* ratio 1.4:1) in a synthesis leading to statine (Scheme 17).[126] The presence of two additional carbon atoms in the main chain of statine containing peptides enhances the conformational degree of freedom of the molecule and thus these peptides have been observed to adopt a variety of different conformations.[127]

Hydroxyethylene dipeptide homologues of statine (43) and the lactone precursor (44) have also been the subject of much synthetic interest. Abbott laboratories have used the aldehyde (45), reported last year, as the P_1' component in a number of aldol reactions leading to (43, 44, $R^1 = CHMe_2$, $R^2 = H$)[128,129] and also to the *erythro*-dihydroxyethylene dipeptide derivative (46).[129] Use of titanium homoenolates[130] and allylic metal additions[131] were the basis of the stereocontrol in the synthesis of the amide (47, Scheme 18) and the cyclic P_1/P_1' isostere (48, Scheme 19) respectively. Sharpless epoxidation of divinylcarbinol gave the 2*R*, 3*S*-monoepoxy alcohol (97% ee) which was converted in high yield to the dihydroxyethylene isostere (49, Scheme 20).[132] Oxidative cleavage of a 1,2-diol moiety, epoxide formation with inversion of configuration, and epoxide opening with a nucleophile featured in the latter stages of another synthesis of (49). The key reaction, however, was the stereospecific addition of the imine prepared from the (2*S*,3*S*)-tartaric acid with cyclohexylmethylmagnesium bromide in the presence of cerium (III) chloride. Conversion to the oxazolidinone and then futher elaboration led to either cyclohexylnorstatine or the dihydroxyethylene isostere (49) (Scheme 21).[133] The lactone precursor (44) with a variety of substituents in the ring has been prepared starting from sugars[134,135] as well as the more common L-amino acids[136,137] (Schemes 22-25).

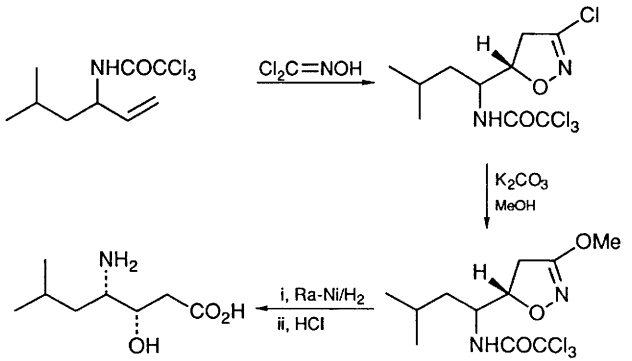


Reagents: i, $(Boc)_2O$; ii, (a) Cs_2CO_3, (b) TBDMSCl, imidazole; iii, O_3; iv, $NaBH_4$; v, PDC; vi, TBAF

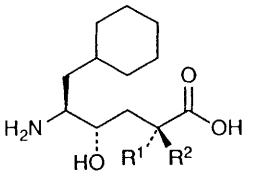
Scheme 15



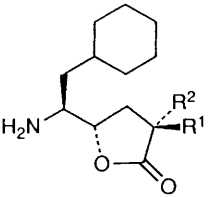
Scheme 16



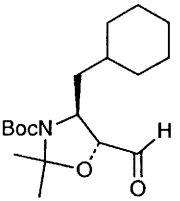
Scheme 17



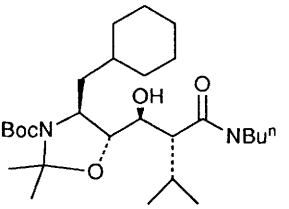
(43)



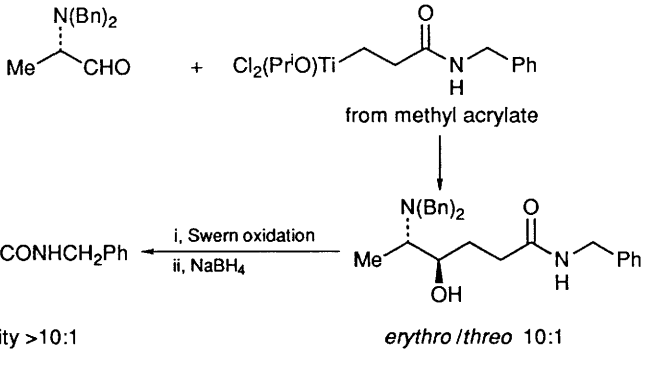
(44)



(45)

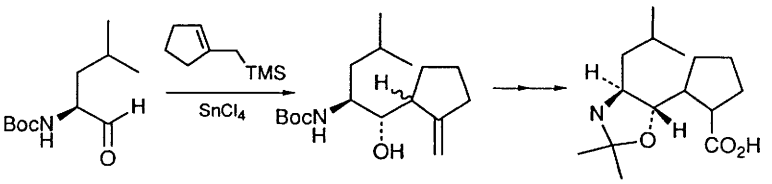


(46)



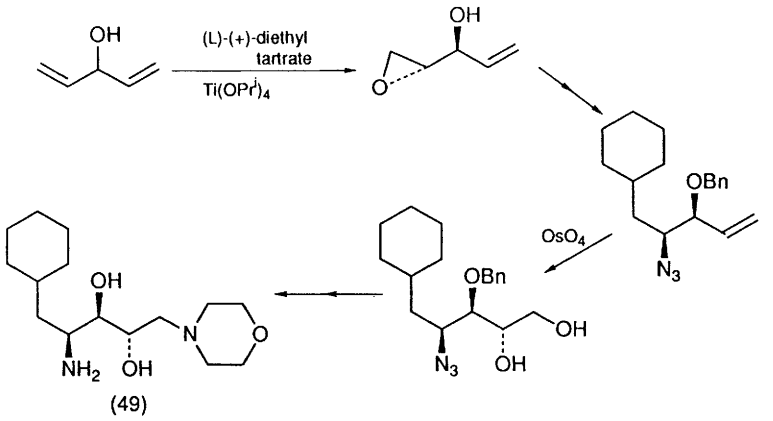
(47)

Scheme 18

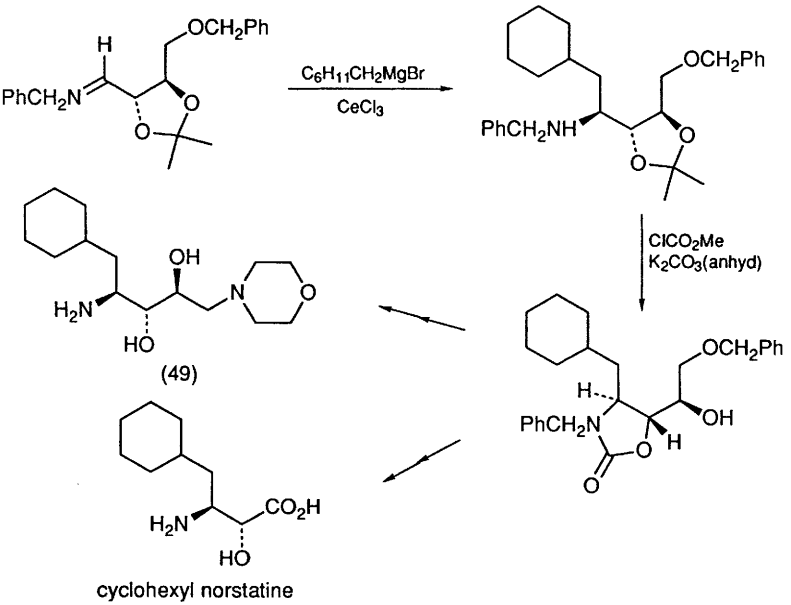


(*S*)-alcohol (48)

Scheme 19



Scheme 20



Scheme 21

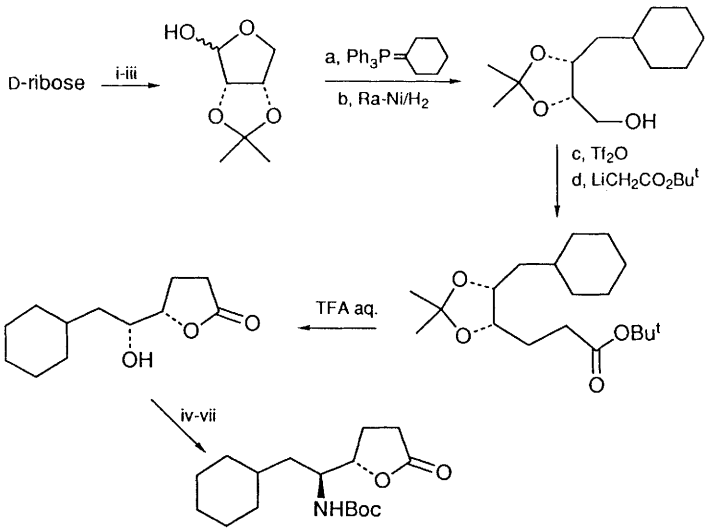
Several compounds have been prepared which combined the hydroxyethylene unit and an inverted amide bond at the C-terminus; for example, (50) inhibited human plasma renin ($IC_{50} = 1.3nM$).[138]

In an asymmetric synthesis of a cyclic derivative of ACHPA (Scheme 26)[139] the optical purity (approx. 60% ee) of the γ-hydroxy-α,β-unsaturated methyl ester formed from the reaction between optically active sulphinylacetates with $C_6H_{11}CH_2CH_2CHO$ was improved by resolution with the lipase *Pseudomonas* K-10. ACHPA was a component in the synthesis of the azapeptide inhibitor (51).[140]

The hydroxyethylene transition state isostere has also been incorporated into a macrocyclic ring in the hope of improving stability towards enzymes. One of the most active of the 13-membered ring series (52, X = Boc, n = 0) was modified further at the P_4 site to give (52, $X = CO_2(CH_2)_2NHCOCMe_3$, n = 0) with an IC_{50} of 56nM.[141] P_2-P_1' macrocycles (53, X = Boc, n = 0,1))[142] were constructed from a (2*S*)-4-butenyl analogue of ACHPA. The stereoselective synthesis of the ACHPA derivative (53, Scheme 27) utilised Evans' erythro-selective chiral oxazolidinone aldol protocol. Both the 13- and 14-membered macrocycles (53, X = BocPhe, n = 0,1) were potent *in vitro* inhibitors of human plasma renin. Lower activity was found for the P_2-P_1' 10-membered macrocycles (54)[143] and the conformational constraints imposed by the smaller ring probably prevented formation of an optimum geometry at the active site.

Conformationally restricted P_1-P_1'-dipeptide mimetics based on ACHPA have been prepared *via* aldol reactions of various lactams with N^{α}-Boc-L-cyclohexylalaninal. The best inhibitor (55, $IC_{50} = 1.3nM$) had *S* configurations at the two new chiral centres and methyl groups at the 5-position of the γ-lactam.[144] Docking experiments with a model of human renin suggested that the hydrophobic part of the lactam ring of (55) can mimic the P_1' side chains of angiotensinogen. The X-ray structure of the P_4-P_1' pentapeptide inhibitor (56, KRI-1314)[145] revealed that the backbone chain assumed an L-shape and was largely twisted at the cyclohexylnorstatine residue. This compound was crystallised as a cinnamic acid salt and the hydrogen bond between the imidazole ring of the histidine residue and the carboxyl group of cinnamic acid may mimic the probable interaction between the histidine residue of angiotensinogen and the hydroxyl group of serine-233 in renin. The C-terminal cyclohexylnorstatine moiety of KRI-1314 has been synthesised in approx. 10% overall yield from δ-glucose.[146]

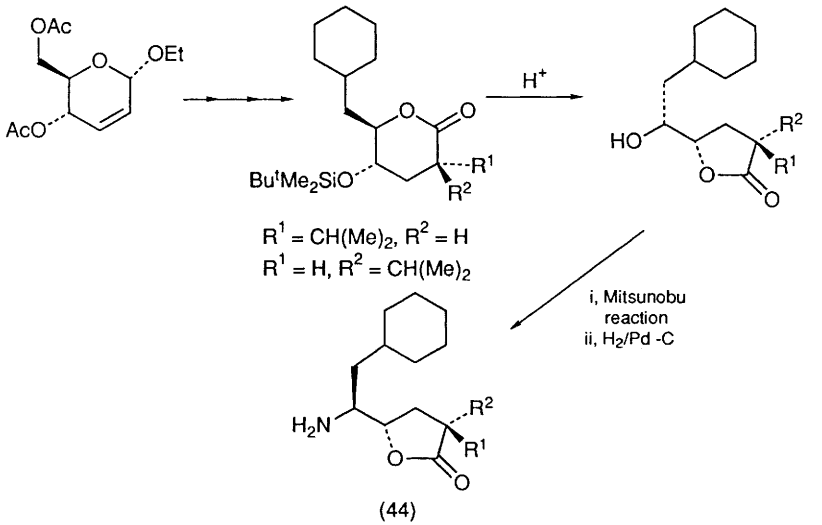
Modelling studies with the potent sulphone inhibitor (57), prepared by coupling the morpholine acid with the dihydroxyethylene derivative, suggested that replacement of the P_3-P_2 amide bond by a sulphone-



(44)

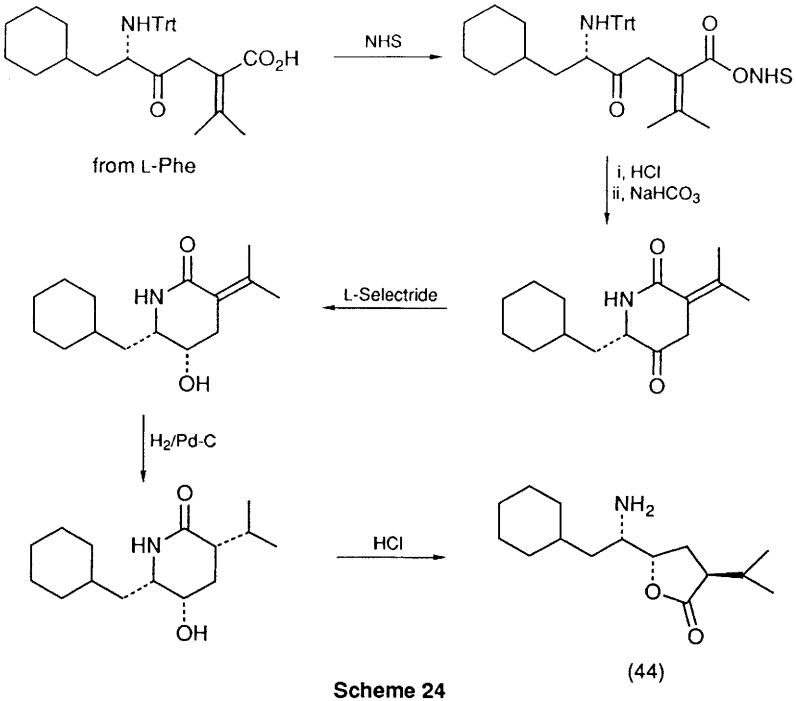
Reagents: i, conc H_2SO_4; ii, $NaBH_4$; iii, $NaIO_4$; iv, MsCl; v, NaN_3; vi, H_2/Pd-C; vii, Boc_2O

Scheme 22

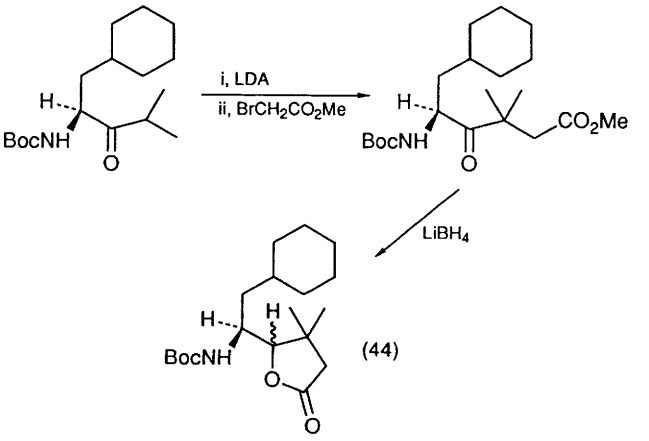


(44)

Scheme 23

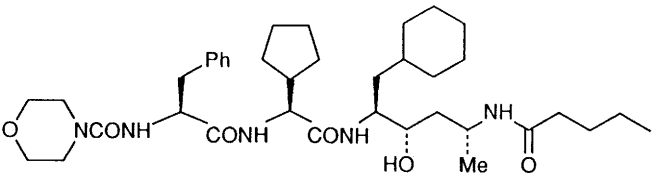


Scheme 24

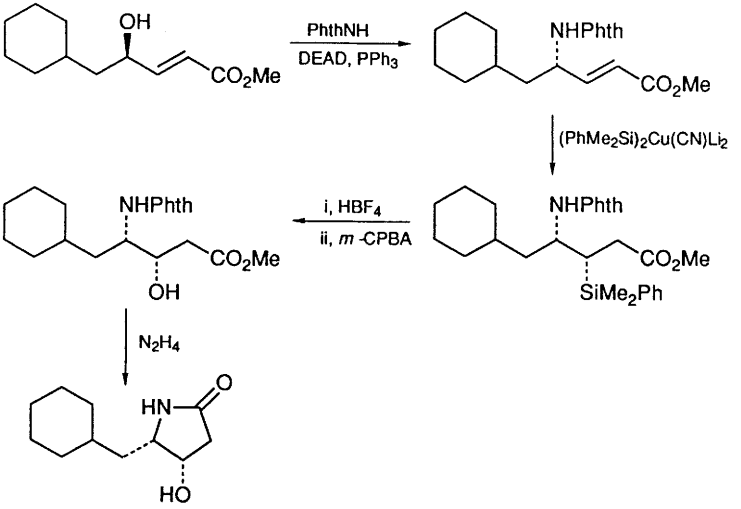


diastereoisomers separated by chromatography

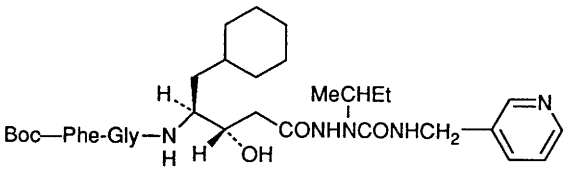
Scheme 25



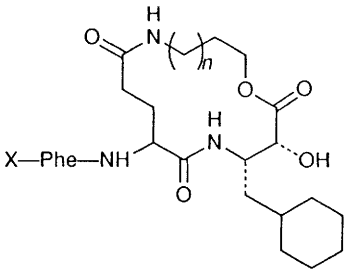
(50)



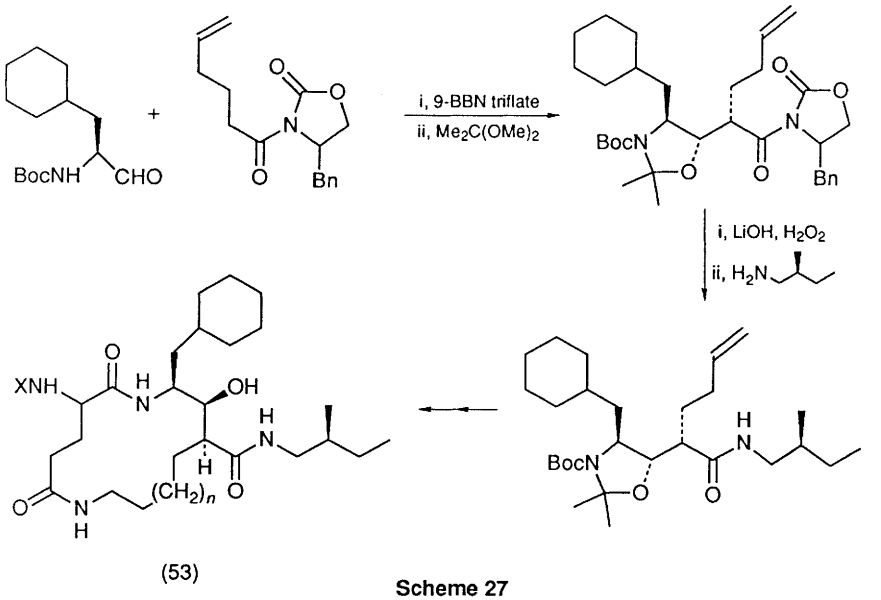
Scheme 26



(51)

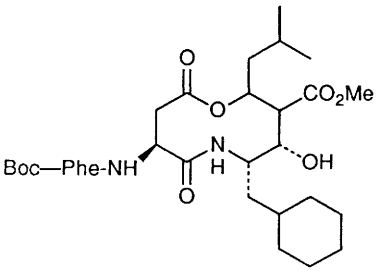


(52) X = Boc, n = 0, 1

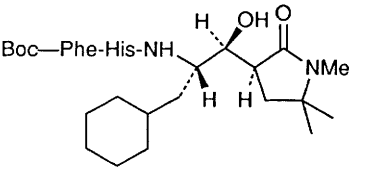


(53)

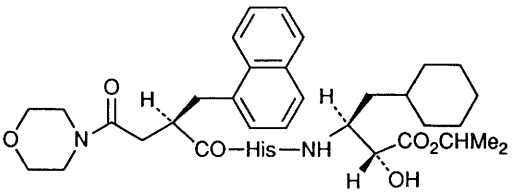
Scheme 27



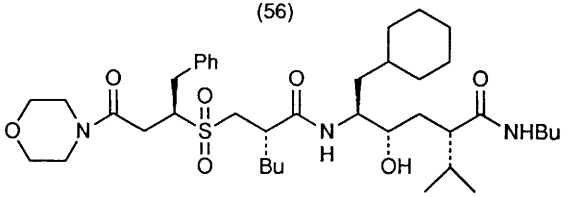
(54)



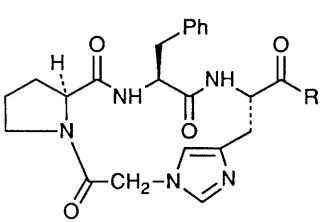
(55)



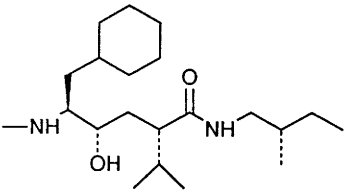
(56)



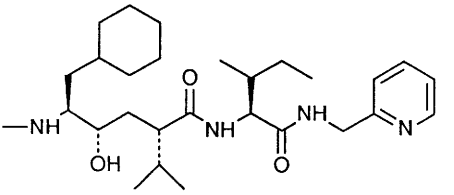
(57)

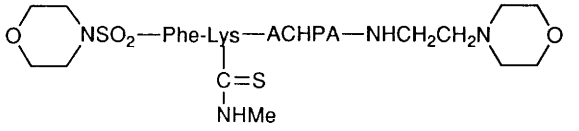


58 (a) R =



(b) R =





(59)

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

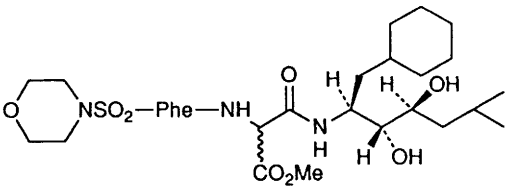
methylene did not perturb the overall geometry of the backbone and that one of the sulphone oxygens was aligned to accept the hydrogen bond from the amide nitrogen of Ser-230 in the enzyme backbone.[147] A conformationally restricted analogue of the P_4-P_2 region was prepared by linking the proline at the P_4 site to the imidazole ring of histidine at the P_2 site *via* a carboxymethylene fragment. Incorporation of this 14-membered macrocycle into renin inhibitory peptides gave (58a,b) which had high binding affinities and IC_{50} values in the nanomolar and subnanomolar range.[148]

Structure-activity relationships have led to the conclusion that, for a particular P_2 fragment, the *in vitro* potency is highly dependent on the P_2' portion in addition to the P_1-P_1' group. The thiourea analogue (59)[149] with an IC_{50} of 3.70nM (monkey plasma) and a high specificity for renin had a half-life of 27min in simulated intestinal juices and was unaffected after 4h in simulated gastric juices. A combination of the diol isostere at P_1-P_1' and an ester side chain at P_2 gave (60) which was a potent inhibitor of primate renin although very susceptible to degradation by digestive enzymes.[150] This compound has also been prepared with a ^{14}C-label at the carboxyl group of phenylalanine.[151] High portal drug levels were observed by replacement of the imidazol-4-yl ring of histidine in (61) with the less reactive and less basic thiazol-4-yl and thiophenyl-2-yl analogues.[152] Addition of polar and hydrophilic moieties at the N-terminus (P_4) of inhibitors with β-alanine at the P_2 position gave orally active compounds, for example (62), which were longer lasting than their counterparts with α-amino acids at the P_2 position.[153] Renin inhibitory activity was observed in a series of compounds which combined an aminodeoxystatine or difluorostatone residue at P_1-P_1' and a pyrazine derivative at the P_2-P_4 positions.[154] A lowering of the blood pressure was observed by *i.v.* but not oral administration of (63,64) to marmosets.

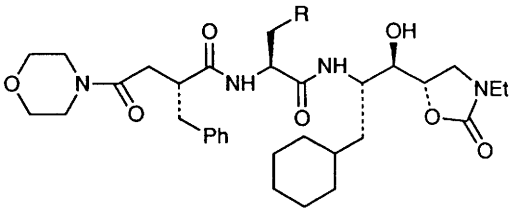
Oral efficacy and a high level of *in vitro* enzyme inhibitory activity were retained by incorporating hydrophilic end groups into the lipophilic renin inhibitor Boc-Pro-Phe-N-MeHis-Leuψ[$CH(OH)CH_2$]Ile-Amp.[155] The total structure of cyclothiazomycin, a novel renin inhibitor isolated from the broth of *Streptomyces* NRO516, has been elucidated by extensive 2D n.m.r. experiments.[156,157]

5.3 *HIV-1 Protease Inhibitors*

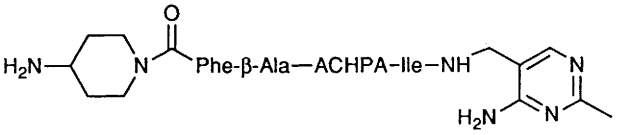
The synthesis of HIV-1 protease inhibitors, as with the renin inhibitors, tends to be based on the transition-state analogue concept. HIV-1 protease recognizes the Phe-Pro and Tyr-Pro sequences as the cleavage sites and design of inhibitors has therefore focused on isosteric


O
NSO2—Phe—NH
O
N
H
CO2Me
H
H
OH
H
OH

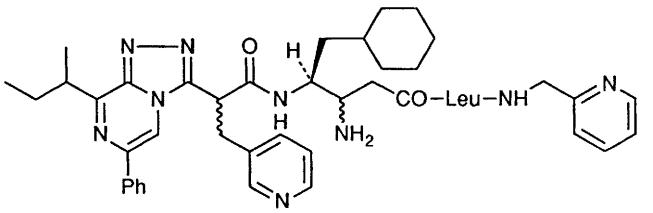
(60)


O
N
O
NH
R
NH
OH
O
Ph
NEt
O
O

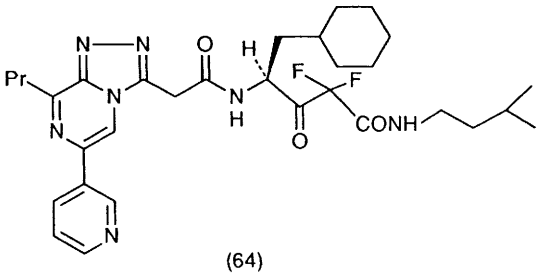
(61)


H2N
N
O
Phe-β-Ala—ACHPA-Ile—NH
N
H2N
N

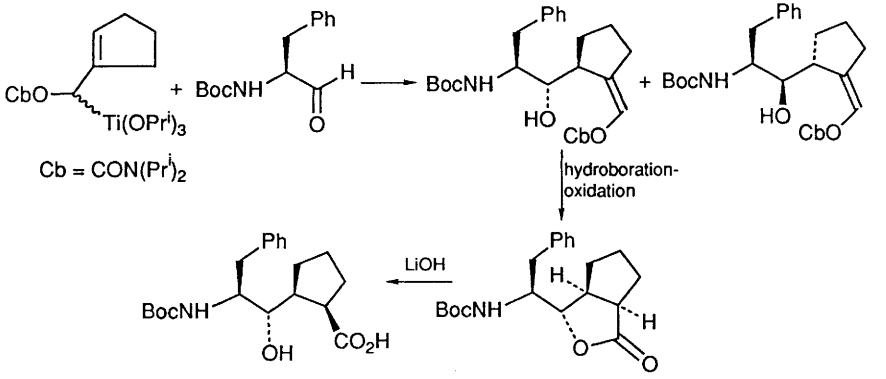
(62)


N
N
N
N
Ph
O
N
H
H
NH2
CO—Leu—NH
N
N

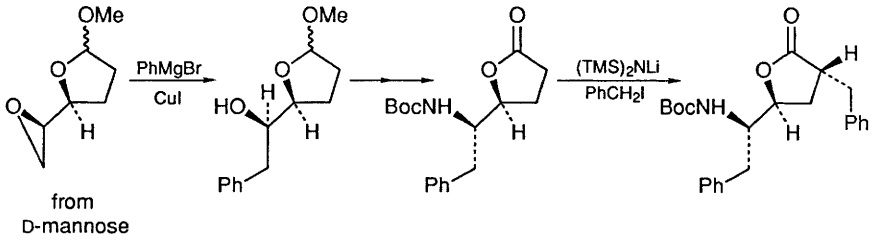
(63)


N
N
Pr
N
N
O
N
H
H
F
F
O
CONH
N

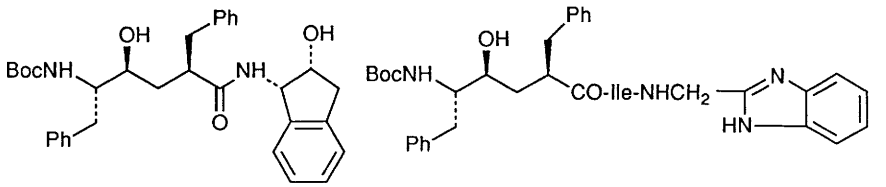
(64)



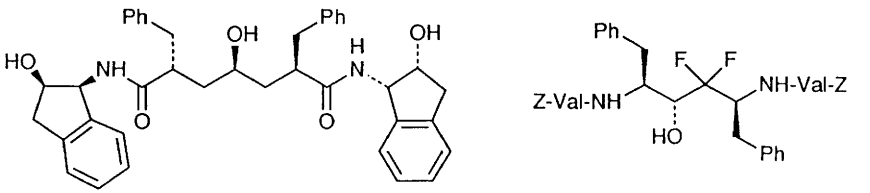
Scheme 27



Scheme 28



(65) (66)



(67) (68)

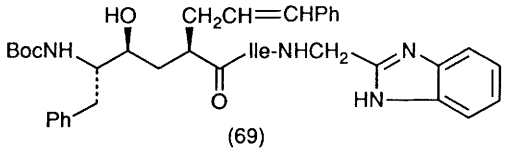
replacement of these dipeptide units. Progress in this area is included in a review on HIV-1 protease.[158]

The synthesis of the Pheψ[CH(OH)CH]Pro isostere is outlined in Scheme 27.[159] As in the synthesis of (47), the initial step involves stereospecific addition of a titanium homoenolate to an amino aldehyde. Futher stereospecific oxidation and reduction procedures generated the *syn* adducts. The Phe-Phe hydroxyethylene dipeptide isostere has been synthesised from δ-mannose.[160] As the aromatic substitutents at C-2 and C-5 were introduced stereoselectively (see Scheme 28) this route is potentially useful for the synthesis of other dipeptide isosteres with a variety of substitutents. Tetrapeptide analogues (65)[161] and (66)[162] in which the Phe-Phe isostere formed the N-terminal component and the carboxy terminus was modified with a heterocyclic moiety were both effective in inhibiting the viral spread in infected cells. Rotation of the C-terminal half of (65) around the central hydroxy-bearing carbon led to the design and synthesis of (67) which also proved effective in halting the spread of HIV infection in cell cultures.[163]

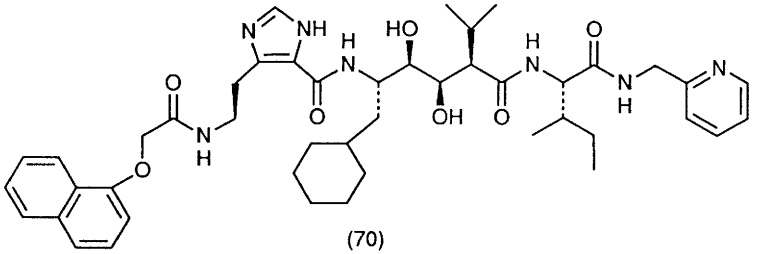
Both the N- and C-terminii of the hydroxy difluoromethylene Phe-Phe isostere were coupled to valine residues and the resulting compound (68) was a potent inhibitor of the enzyme ($K_i = 1.0$nmol dm^{-3}).[164] However oxidation to the difluoroketone gave an even better inhibitor ($K_i = 0.1$nmol dm^{-3}).

Screening of renin inhibitors for their effectiveness against HIV-1 protease gave a lead compound, which by further modification led to (69).[165] The peptidomimetic and dihydroxyethylene isostere strategies of renin inhibitor design were applied to HIV protease and resulted in (70)[166] which was highly effective in blocking polypeptide processing in *in vitro* cell culture assays. Hydroxymethylcarbonyl and hydroxyethylamine isosteric replacements of the Phe-Pro bond have also led to potent inhibitors (Table). The configuration of the hydroxyl group required for maximum inhibitory activity appears to be dependent on the peptide framework.

A stereoselective synthesis of a protected derivative of the Phe-Gly hydroxyethylene isostere has been reported.[171] The authors claim that this route, starting from a carbohydrate precursor, is suitable for the preparation of multigram quantities of peptidomimetic analogues. Initial biological results with [Leu^5]enkephalin-related glycoconjugates suggest that these compounds are suitable candidates for futher development.[172] The tetrapeptide Ac-Phe-Ile-Sta-D-Leu-NH_2 was identified as an HIV inhibitor by screening a library of peptides prepared by the couple, divide, and recombine procedure.[173]

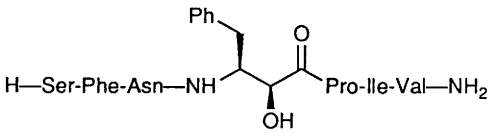
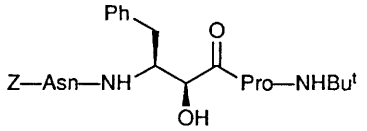
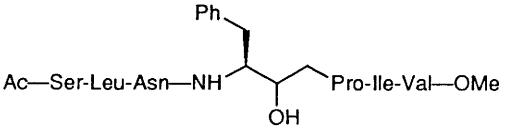
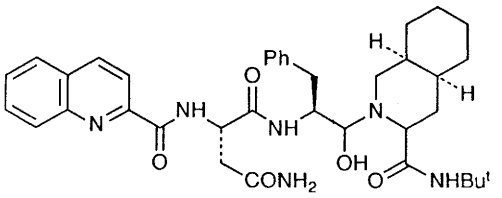


(69)



(70)

Table

	Inhibition of HIV-1 Protease, IC_{50} (nM)
	5^{167}
	89^{168}
	(*S*)-OH 3.4^{169} (*R*)-OH 65^{169}
	(*S*)-OH $>>100^{170}$ (*R*)-OH $< 0.4^{170}$

5.4 *Inhibitors of Other Proteases*

The synthesis of sulphinamide and sulphonamide moieties as transition state analogues of protease inhibitors has been noted in section **2.7**.

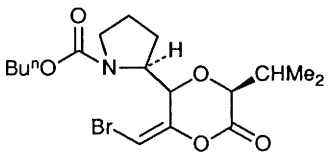
The bromo enol lactone pseudodipeptide (71)[174] was an effective inhibitor of both chymotrypsin and human leukocyte elastase.[175] The diphenyl ester phosphonate Suc-Val-Pro-Phe^{P}(OPh)$_2$ was found to phosphorylate serine proteases and the half-life for dephosphorylation of chymotrypsin was 7.5-26h.[176] In the series of dipeptides D-Leu-L-Phe elaborated at the C-terminus with a heterocyclic moiety the 4-phenylpiperidino and 4-phenylpiperazino derivatives inhibited chymotrypsin fairly strongly ($K_i = 7.5 \times 10^{-4}$M and 1.4×10^{-3}M respectively).[177] N.m.r. experiments indicated that the D-Leu and Phe side chains were close in proximity and in the inhibitory conformation this hydrophobic core probably fitted into the S_2 site while the C-terminal aromatic group of the heterocycle fitted the S_1 site. In a pro-drug approach, N-terminal glyoxylation of dipeptides improved the stability of the amide bonds towards cleavage by α-chymotrypsin.[178]

The Reformatsky reaction was involved in the synthesis of the difluoromethylene ketone (72),[179] a potential inhibitor of thrombin (a serine protease that recognises a basic P_1 residue). Nazumamide A (73), a tetrapeptide isolated from Theonella sp. of marine sponge also inhibited thrombin.[180] Fibrinogen-thrombin clotting was inhibited by N-terminal tripeptide analogues of fibrin α-chain, and H-Gly-Pro-Arg-hexamethyleneimine was the most potent of those synthesised and tested.[181]

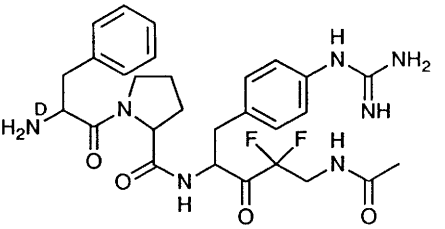
A variety of sulphur functionality was incorporated into tri- and tetrapeptide substrate analogues of human collagenases but no correlation was found between any single structural feature and inhibitory potency.[182] N.m.r. studies on succinyl-Pro-Ala, a competitive inhibitor of bacterial collagenase indicated that the succinyl and alanyl residues were primarily involved in the interaction with the enzyme site.[183]

The tripeptide analogue (74) of pepstatin was found to be as effective as the naturally occuring substrate in inhibiting pepsin.[184] Simulated pepstatin analogues were used in a computational study of the energetics of the pepsin-pepstatin interaction.[185] The fluorescence emission spectrum obtained when Dns-Ala-Phe-Trp-Val-Leu-OCH$_2$Py (Py = 4-pyridyl) was incubated with pepsin enabled low concentrations of the enzyme to be detected.[186]

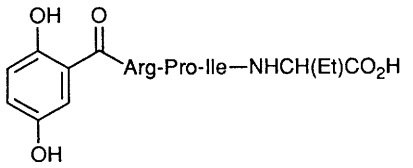
A double labelled ^{14}C inhibitor of human leukocyte elastase has been synthesised.[187] Fragments of eglin c were found to have inhibitory activity against one or more of the enzymes human leukocyte elastase, cathepsin G and α-chymotrypsin.[188-190] A minimum of five amino acids spanning residues 12-16 of aprotinin (BPTI) were necessary for inhibition



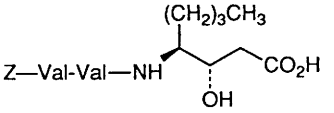
(71)



(72)



(73) Nazumamide A



(74)

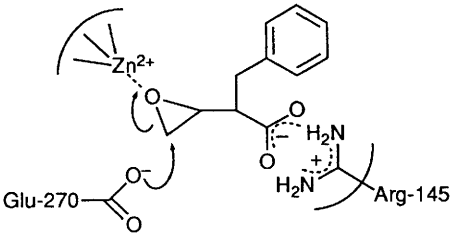
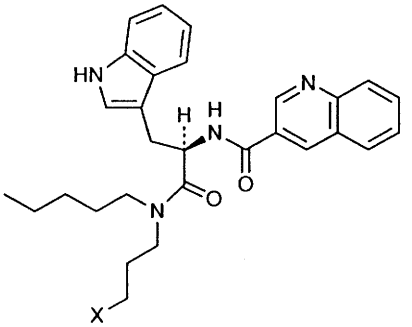


Figure 1



(75) X = CH_3, OCH_3

of porcine pancreatic kallikrein.[191] Enhanced selectivity for inactivation of cathepsin B over cathepsin L was shown by the dipeptide derivatives Z-Ala-His-CHN_2 and Z-Ala-His-CH_2F.[192]

On the basis of structure-activity studies a model was proposed for the interaction of the potent inhibitor Z-thiopro-thiazolidine ($IC_{50}=2.1\mu M$) with the active site of prolyl endopeptidases.[193] An octapeptide fragment of human β-caesin Ile-Tyr-Pro-Phe-Val-Glu-Pro-Ile was also found to be an inhibitor of this endopeptidase ($IC_{50}=8\mu M$).[194]

The nitrile group of the papain inhibitor Ac-Phe-Ala-CN could be converted into other functionality by nucleophilic addition to the papain-nitrile thioimidate ester complex.[195] The reactions were followed by n.m.r. and only the complex derived from the LL diastereoisomer was found to undergo this transformation. Nucleophilic attack on the LD complex was blocked by the methyl group of the alanine residue and the backbones of Trp^{26} and Cys^{24} of the enzyme. 2-Benzyl-3,4-epoxybutanoic acid (BEBA) was an inhibitor of carboxypeptidase A (CPA).[196] Kinetic experiments indicated that, as expected, the interaction was active-site directed (see Figure 1 for hypothetical complex formed upon interaction of BEBA with CPA).

The iterative cycle of crystallographic analysis, design, synthesis, and evaluation in drug discovery was applied to the design of an inhibitor for the *E. coli* enzyme thymidylate synthase.[197] With no prior knowledge of structure/activity relationships the crystallographic analysis of the receptor-ligand interaction gave lead compounds which were improved upon by 100-1000 fold by further development.

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

The biosynthetic mechanisms and the *in vitro* production of bioactive peptide analogues have been reviewed.[198]

6.1 *Peptides with 'Opioid Characteristics'*

The design and synthesis of enkephalin analogues that bind selectively and specifically to opiate receptor subtypes has been the subject of a review.[199] The conformation and receptor studies of cyclic opioid peptides is covered in section 3. Linear residue substitutions in the enkephalins is covered in this section but 1991 was not a very productive year for this type of modification.

None of the compounds in the N-alkylated series R-Tyr-D-Met-Gly-Phe-$[NH(CH_2)_5CO]_n$-OH ($R=CH_2Ph$, $CHMe_2$, Pr, Bu, $n=1,2$)

showed any antagonist activity. The propyl derivative ($n=2$)[200] was however selective for the μ receptor and the benzyl derivative ($n=1$)[201] was a weak analgesic. Analgesic activity was also observed for the D-4-Cl- or 4-F-Phe[4] Met enkephalin analogues although some of the compounds caused hypothermia.[202] Disappointing results were also obtained by extending the C-terminus of the message domain (Tyr-Gly-Gly-Phe) of enkephalin with a long polycationic ligand to mimic dynorphin.[203] The compounds H-Tyr-Gly-Gly-Phe-NH[$(CH_2)_nNR]_mH$ ($R=COCH_2CH_2CH_2NH_2$; $n=2,m=1$-3; $n=3,m=2$) bound only weakly to receptors and the pharmacological activities in the guinea pig ileum were marginal. The length of the hydrophilic spacer in [Tyr-D-Ala-Gly-Phe-NH-CH_2-$(CHOH)_n$-]2 ($n=1,2$) did not affect the affinity of the compound for the δ-receptor whereas shorter spacer lengths increased the affinity for the μ and κ receptors.[204]

The structure-activity relationships of dynorphin A analogues including the metabolically stable analogue [N-MeTyr[1], N-MeArg[7], δ-Leu]dynorphin A (1-8)ethylamide has been reviewed.[205] Elephant β-endorphin is a more potent analgesic than human β-endorphin but like the human counterpart the (6-31) analogue lacking the Met enkephalin moiety was found to be inactive.[206] The opiate binding affinities of the Glu[8] and Leu[8] analogues of human β-endorphin (1-27) were higher than the parent compound.[207]

In recent years several peptides with opioid activity have been isolated from the skins of frogs belonging to the genus *Phyllomedusa*. Unfortunately these peptides have been assigned similar sounding names. To clarify:

dermorphin (μ-selective)	H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2
deltorphins (δ-selective) - family of three heptapeptides	
deltorphin/dermenkephalin	H-Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2
deltorphin I	H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2
deltorphin II	H-Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2

The fluorinated dermorphin analogue Tyr(3-F)-D-Arg-Phe-Lys-NH_2 retained μ-receptor selectivity although affinity for both μ and δ receptors was reduced compared with the non-fluorinated parent peptide.[208] Nevertheless the ^{18}F or ^{19}F-labelled derivative should prove useful in biochemical studies. Ease of introduction of label, high opioid affinity combined with a potential application in PET scanning were the reasons behind the preparation of H-Tyr-D-Met(O)-Phe-Gly-$^{13}NH_2$.[209] A 5 min ammonlysis, with $^{13}NH_3$, of the precursor ester followed by a straightforward h.p.l.c. purification procedure enabled the labelled peptide to be available within a short period of time (half-life $^{13}N=9.96$min). Binding

studies on synthetic dermorphin precursor peptides found that the 15-residue peptide H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-Gly-Glu-Ala-Lys-Lys-Ile-Lys-Arg-OH had the best affinity (half that of dermorphin) for the μ and δ receptors.[210] Futher extension at the C-terminus resulted in lower affinity and the receptor binding of the L-Ala^2 derivatives were between one and three orders of magnitude lower than their D-counterparts.

Several dermenkephalin analogues have been prepared by (4+3) fragment coupling and preliminary receptor binding studies undertaken.[211] In a separate study 15 analogues of the deltorphin family were synthesised and binding studies suggested that the high δ-selectivity of these peptides was due in a large part to the conformation of the C-terminal hydrophobic region.[212] Futher evidence, by the same group of workers, to support this view was obtained from a detailed conformational (n.m.r.) study of dermorphin and three deltorphin analogues.[213]

In a study designed to try and enhance δ-receptor selectivity a number of DPDPE-dermenkephalin hybrid peptides were constructed in which the C-terminal amino acids (putative δ-message) of dermenkephalin were progressively added to the δ-selective DPDPE sequence.[214] Some of the hybrid peptides retained δ-selectivity but there was a steady decrease in potency at both δ and μ receptors.

In the morphiceptin series (H-Tyr-Pro-Phe-X-NH_2) only the Thr(Bzl) derivative showed an increase (10x) in binding affinity for the μ receptor compared with the parent molecule (X = Pro).[215] Substitution of Pro^4 by Hyp^4 or a glycosylated derivative of hydroxyproline resulted in a decrease of biological activity.[216] High selectivity for the μ-receptor was found with Tyr-(1*S*, 2*R*)-Ac^{5}c-Phe-Val-NH_2 (Ac^{5}c = *cis*-2-aminocyclopentane carboxylic acid) and the corresponding δ-Val^4 peptide, whereas the (1*R*, 2*S*)Ac^{5}c analogues were inactive at both δ and μ receptors.[217] From conformational studies it was concluded that only the analogues incorporating the (1*S*, 2*R*)Ac^{5}c residues displayed the relatively large separation of the Tyr and Phe side chains required for μ-opioid receptor activity.

The best reagents to promote the formation of the lactam bond in the tetra- and pentapeptides β-casomorphin analogues Boc-X-D-Orn-Phe-D-Pro-Gly- [X = null, Tyr(CMe_3)] were diphenylphosphoryl azide or norborn-5-ene-2,3-dicarboxyimido-diphenylphosphate.[218]

6.2 *Cholecystokinin Analogues*

Substituting the tryptophan (position 30 of CCK) of the heptapeptide CCK fragment with either a coded amino acid[219] or 1- or 2-naphthylalanine,[220] or introducing a reduced peptide bond[219] in the Trp^{30}

region or replacing the C-terminal amide by an ester function[219] led to a reduction in biological activity compared with the parent compounds. Reduced activity (by two to three orders of magnitude) was also observed by substituting a number of the amino acids in the central part of CCK-8.[221] However, replacing the methionine at position 5 of Boc-CCK-7 with neopentylglycine gave a compound which, although less analgetically active, retained the full gall bladder and sedative activity of CCK-8 and was substantially higher in anorectic activity (400x) than the octapeptide parent.[222] Modifying the C-terminal Phe of CCK-7 by incorporating methylated aromatic side chains had little effect on the biological activity.[223]

The effect of modifying the sulphated tyrosine has also been investigated. The modified amino acid Phe(*p*-CH_2SO_3Na) is able to mimic the Tyr(SO_3H) residue. CCK-8 analogues containing either L-or D-Phe(*p*-CH_2SO_3Na) showed high affinity for the peripheral (CCK-A) and central (CCK-B) receptors and were full agonists to the stimulation of pancreatic amylase secretion.[224] Ac-CCK-7 analogues in which the sulphate was replaced by a carboxymethyl or tetrazolyl group were less potent than the Tyr(SO_3H) parent but were still able to suppress appetite.[225] Enhanced satiating potency, increased selectivity for CCK-A receptors, increased resistance to peptidergic degradation, and a long duration of action were reported for the pentapeptide analogue Ac-Tyr(SO_3H)-Met-Gly-Trp-Met-Thr(SO_3H)-NMePhe-NH_2.[226]

Boc-CCK-4 derivatives Boc-Trp-Lys(CONHR)-Asp-Phe-NH_2 containing side chain ureas were, in general, potent and selective CCK-A receptor agonists.[227] Binding potency at the CCK-A receptor was reduced by converting the urea to a thiourea or by replacing the lysine with ornithine or homolysine. Incorporation of *trans*-3-*n*-propyl-L-proline (conformationally restricted analogue of norleucine) in place of Met in the CCK tetrapeptide Boc-Trp-Met-Asp-Phe-NH_2 resulted in a significant increase in binding to CCK-B receptors and improved the selectivity for the CCK-B receptor over the CCK-A receptor.[228] The anticonvulsant activity was retained and gastrin-like activity suppressed by substituting the N-terminal glycine of pentagastrin with iminodiacetic acid.[229]

The structure, and distance relationships between subunits, of the pancreatic (CCK-A) receptor were studied by cross-linking a series of photoaffinity probes. These were designed to mimic 'short' CCK-8 or 'long' CCK-33 molecules by using PEG spacers.[230] As predicted, the short spacers labelled only the hormone-binding subunit with molecular weight 85,000-95,000, but when a longer spacer was used proteins of molecular weight 80,000 and 40,000 were specifically labelled.

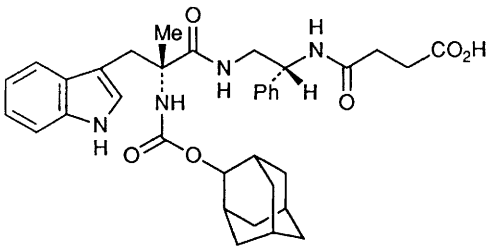
Two groups have combined elements of glutamic acid based CCK antagonists and benzodiazepine CCK antagonists to produce hybrid antagonists.[231,232] In general, the compounds, for example (75), possess a high affinity for the CCK-A (pancreatic) receptor coupled with a low affinity for the CCK-B receptor. Optimisation of the N- and C-terminal structure-activity relationships required for high CCK-B binding affinity led to 'dipeptoid' CCK-B antagonists.[233] Using molecular modelling techniques the 'dipeptoid' (76) was compared with energy minimised conformations of CCK-4. As a result the α,α-disubstituted tryptophan derivative (77), predicted to have close structural and conformational analogy to endogenous CCK, was synthesised *via* alkylation of an isonitrile derivative of tryptophan.[234]

6.3 *Angiotensin and Analogues*

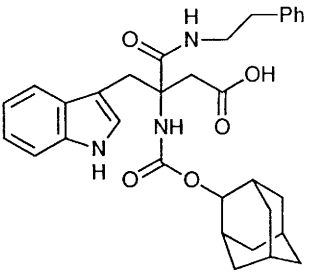
Angiotensin II analogues [X^1, $Tyr(Me)^4$, $Phe(4\text{-}NH_2)^8$]ANG II with either a sarcosine or β-alanine residue at the N-terminus and a carboxylic acid or ethyl ester function at the C-terminus were prepared by stepwise solution couplings.[235] All were inactive except for the N-terminal sarcosine/C-terminal ethyl ester derivative which showed weak agonistic activity. A reduction, by approximately one unit, of the pA_2 value (pA_2 is a measure of the inhibitory activity) was observed when the positively charged Arg^2 residue in Sar-Arg-Val-Tyr-Ile-His-Pro-Ile was replaced by an uncharged amino acid.[236] From structure-activity data on 19 angiotensin II analogues with cyclohexylalanine in positions 4 or 8 it was concluded that de-aromatisation at position 4 resulted in loss of binding activity whereas de-aromatisation at position 8 resulted in antagonists with variable agonist activities.[237] A substantial loss of activity was observed when the dipeptide mimic Valψ[$CH(CONH_2)NH$]His replaced the Val^5 and His^6 residues in the angiotensin II antagonist Sar-Arg-Val-Tyr-Val-His-Pro-Ile.[238] The K_m for renin catalysed hydrolysis of the N-terminal heptadecapeptide of human angiotensinogen (H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Asn-Glu-Ser-Thr-NH_2) was similar to that of the (1-13) analogue without the carbohydrate binding site.[239] Also, V_{max} was one order of magnitude higher for the heptadecapeptide.

6.4 *Oxytocin and Vasopressin Analogues*

Analogues of 1-desaminovasopressin with D-homoarginine in position 8 and modified phenylalanine derivatives in position 2 were synthesised using solid phase methodology.[240] All analogues had low vasopressor (V_1) and antidiuretic (V_2) activities but were inhibitors of the oxytocic uterotonic response, the most potent being the *p*-ethyl-D-



(76) ["dipeptoid"]



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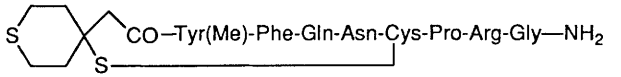

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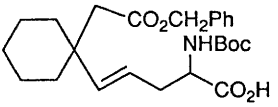

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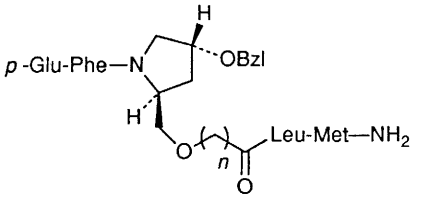
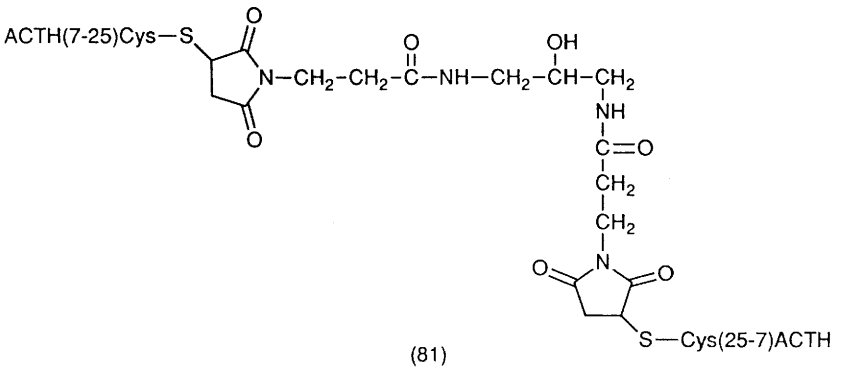


(80) $n = 1, 2$



(81)

YPSK—Aca-RHYINLITRQRY—NH_2

(NPY 1-4 Aca-25-36, Aca = ε-amino caproic acid)

(82)

phenylalanine derivative with a pA_2 of 8.3. Uterotonic inhibitors, but with low inhibitor potencies, were obtained by incorporating a *p*-fluorophenylalanine into position 2 of arginine vasopressin (AVP) and a number of analogues.[241] A solution synthesis of the desamino derivative [Mpr[1], D-Arg[8]]vasopressin (Mpr = β-mercaptopropionic acid) has been reported.[242]

The presence of arginine at position 4 in a number of cyclic and linear vasopressin antagonists had relatively minor effects in the vasopressor (V_1) and antidiuretic (V_2) potencies.[243] Replacing Gln[4] with homoglutamine in AVP gave a compound with high pressor activity and strikingly low uterotonic and milk-ejecting activities. The uterotonic activity and the pressor response both decreased while a considerable level of galactogogic activity was retained by making a similar substitution in oxytocin.[244] Incorporation of a sulphur atom into the cyclohexyl ring of $d(CH_2)_5$Tyr(Me)AVP, a non-competitive V_1 antagonist, gave (78) which was a potent competitive V_1 antagonist.[245]

Evidence obtained from a 2D n.m.r. study of [Lys[8]]vasopressin homologues (i.e. additional glycine residues at the N-terminus) suggested that the 20-membered ring adopted a more or less rigid conformation with the Phe-Gln-Asn residues forming an inverse γ-turn.[246] With a view to the synthesis of cyclic dicarba vasopressin antagonists the suberic acid derivative (79) has been prepared and the authors anticipate that the *trans* double bond will limit the number of conformations available to the cyclic peptide.[247]

An extensive study of cyclic and linear oxytocin analogues has been carried out in which each residue in [Pmp[1], D-Trp[2], Arg[8]]oxytocin[248] was replaced with L-[249] and D-tryptophan.[250] (Pmp = β,β-pentamethylene-β-mercaptopropionic acid). All substitutions in the ring sequence resulted in uterotonic antagonists of lower potency than the parent compound but substitutions in the tail sequence gave oxytocin antagonists of equal or better potency than the parent. Furthermore, the compounds with D- or L-Trp at positions 7, 8, or 9 were weak V_2 (antidiuretic) antagonists of AVP. In the penicillamine series [L-Pen[1]]oxytocin substituting Tyr_2 by *p*-L-hydroxyphenylglycine gave a derivative with antioxytocic activity but the potency was less than that of the parent compound.[251] A reduction in the antagonist potency was observed by substituting Asn[5] in [Pen[1], D-Phe[2], Thr[4], Orn[8]]oxytocin with Thr, Leu, Asp, or Tyr.[252] Restricting the conformational freedom by incorporating, *inter alia*, α,α-disubstituted amino acids at position 2 of oxytocin resulted in a decrease in affinity for the receptors.[253]

6.5 *Luteinising Hormone-Releasing Hormone (LHRH) Analogues*

To reduce/eliminate the histamine side effects of some LHRH antagonists various arginine and lysine residues were replaced with less basic derivatives including N^{ω}-cyano-$N^{\omega'}$-butyl (bCN), homoarginine (Har), N^{ε}-isopropyllysine (ILys), or N^{ε}-triazolyllysine [Lys(atz)]. Azaline, [Ac-D-Nal[1], D-Cpa[2], D-Pal[3], Lys(atz)[5], D-Lys(atz)[6], ILys[8], D-Ala[10]]LHRH, completely inhibited ovulation at 2-3μg/rat, was equipotent to LHRH in releasing histamine (in rats), and was readily soluble in dilute buffers at pH 7.[254,255] The unnatural amino acids were obtained by modifying lysine or ornithine residues at the resin-bound stage of the synthetic procedure. Unnatural hydrophilic amino acids were incorporated into a number of positions in antide [Ac-D-2-Nal[1], D-*p*ClPhe[2], D-3-Pal[3], Lys(Nic)[5], D-Lys(Nic)[6], ILys[8], D-Ala[10]]LHRH to increase water solubility. Several potent analogues were identified including the N-Ac-D-3-Qal[1], *c*-PzACAla[5], D-PicLys[6] derivative [3-Qal = 3-(3-quinolyl)alanine, *c*-PzACAla = *cis*-3-(4-pyrazinylcarbonylaminocyclohexyl)alanine, PicLys = N^{ε}-picolinoyllysine].[256] For the hydrophilic quinolylalanine to be effective, its hydrophilicity had to be balanced by an increase in lipophilic character elsewhere in the molecule at the *c*PzACAla[5], D-PicLys[6], Leu[7] sequence. A series of novel phenylalanine based amino acids have been prepared and incorporated into a related antagonist.[257] The pentapeptide analogue pGlu-His-Arg-Pro-Gly-NH_2 (20% of gonadotropin releasing activity of LHRH) has been prepared using three different coupling routes.[258] To study the long-term degradation pathways for a formulation of histrelin [D-N^{im}-bzl-His[6], des-Gly[10]]LHRH ethylamide an aqueous solution of the peptide was heated at 87°, pH 5.4 for 18 days. The degradation products (amounting to 47% of the original histrelin) resulted from the hydrolysis of the pGlu-His and Trp-Ser amide bonds and racemisation of the serine and histidine residues.[259]

6.6 *Substance P and Analogues*

The C-terminal hexa- and hepta- substance P analogues [Glu-$(OCH_2Ph)^{11}$]SP_{6-11}, and [$Glu(OCH_2Ph)^{11}$]SP_{5-11} were both equipotent to substance P and were mainly active through the NK-1 receptor.[260] Enhanced agonist activity at each of the NK-1, NK-2, and NK-3 receptor subtypes was observed by substituting $Glu(OBu^t)$ for Met in [Orn[6]]SP_{6-11}.[261] One of the diastereoisomers obtained by replacing Phe[8] in pGlu[6]-Phe[7]-Phe[8]-Gly[9]-Leu[10]-Met^{11}-NH_2 by the α-hydroxy β-amino acid (2*RS*, 3*S*)-$PhCH_2CH(NH_2)CH(OH)$-CO_2H was a potent inhibitor of substance P degradation and had an IC_{50} of 20μM.[262] [X^6]-SP_{6-11}

(X = Ala, Val, Leu, Pro, Trp, phenylglycyl) analogues have also been prepared.[263]

Molecular modelling to suggest appropriate substitution patterns for the bioactive topography of substance P led to the design and incorporation of 4-hydroxyproline and 1,4-piperazine 'molecular scaffolds'.[264] Although the most active Hyp-based SP mimetics (80) were 1 to 2 orders of magnitude less potent than the parent [pGlu^6]SP$_{6\text{-}11}$ they showed a marked preference for the NK-3 receptor.

Replacing the Gly at position 8 of scyliorhinin (H-Ala-Lys-Phe-Asp-Lys-Phe-Tyr-Gly-Leu-Met-NH_2), a decapeptide belonging to the tachykinin family, with either Sar or Pro led to an increase in the agonist activity although the level was still less than substance P.[265] Preliminary pharmacological studies on ranamargarin (H-Asp-Asp-Ala-Ser-Asp-Arg-Ala-Lys-Lys-Phe-Tyr-Gly-Leu-Met-NH_2) showed that this frog-skin peptide was highly selective for the substance P P-subtype receptor.[266] Studies with bombesin peptides indicated that the 10 C-terminal amino acids were important in antibody recognition but the C-terminal dodecapeptide probably constituted the complete epitope.[267]

6.7 *Thyrotropin-Releasing Hormone (TRH) Analogues*

A more lipophilic derivative of TRH was obtained by attaching lauric acid to the N-terminal pGlu group of TRH (pGlu-His-Pro-NH_2). CNS and endocrine activity of the lauroyl derivative was slightly reduced to 81 and 64% respectively of the parent TRH compound.[268] *Cis* and *trans* hydroxy proline derivatives of TRH have been prepared and n.m.r. studies indicated free rotation of the histidyl side chain in pGlu-His-*c*Hyp-OH but restricted rotation in the *trans* diastereoisomer.[269]

In a pro-drug study *N*-phthalidylation of the imidazole group in TRH protected the tripeptide against cleavage by TRH-specific pyroglutamyl aminopeptidase (PAPase) but not against unspecific PAPase or intestinal prolyl endopeptidase.[270] Tritium labelled TRH peptides were obtained by coupling [^{3}H]-pyroglutamic acid as a mixed anhydride to appropriate dipeptides using solid phase procedures.[271] Solution techniques were used to prepare L-pGlu-L-Ser-D-Leu-NHPr^i.[272]

6.8 *Somatostatin Analogues*

Potent and cyclic analogues of somatostatin, cyclo[-Phe-Pro-Tyr-D-Trp-Lys-Orn-] and cyclo[-Lys-MePhe-Tyr-D-Trp-Lys-Val-] have been synthesised. A convenient amino group (ornithine or lysine) was incorporated into the design so that the peptides could easily be attached to affinity gels.[273]

6.9 *Bradykinin Analogues*

Acylation of the N-terminus of the potent bradykinin antagonist [D-Arg^0, Hyp^3, Thi^{5,8}, D-Phe^7]bradykinin with 1-adamantaneacetic acid resulted in an analogue with >10x the potency and efficacy of the parent compound.[274] The importance of the Phe at positions 5 and 8 and Arg at positions 1 and 9 in bradykinin was emphasised following a study in which each adjacent pair of residues was replaced with 5-aminovaleric acid.[275] Futhermore an n.m.r. study indicated that the peptide lost conformational rigidity on replacement of the Phe^8 by tyrosine.[276] Loss of biological activity was also observed when either the C-terminal carboxylic acid was replaced by the amide or the Gly replaced at position 4 by a D-Ala.[277]

6.10 *Glucagon/Gastrin-Releasing Peptide*

Substituting His^1 by desaminoHis or Tip in the superagonist [Lys^{17,18}, Glu^{21}]glucagon or the antagonist [D-Phe^4, Tyr^5, Arg^{12}]glucagon caused a reduction in the binding potencies.[278] The conformation restriction imposed at the N-terminus by Tip in [Tip^1, Lys^{17,18}, Glu^{21}]glucagon also affected the ability of the peptide to activate adenylate cyclase (AC) (15% relative to glucagon = 100%). The antagonists were unable to activate the AC system. The Trp-Ala-Val sequence in the bombesin/gastrin-releasing peptide [D-Ala^{24}]GRP(20-26) was found to be important for antagonist activity. Amino acid replacements in other parts of the molecule led to a marked increase in activity, for example, 4-pyridylcarbonyl-His-Trp-Ala-Val-D-Ala-Lys(X)-Y (X = $COCH_2CH_2Ph$, Y = LeuNHMe; X = Z, Y = MeLeuOMe) had IC_{50} values $<20\mu g/kg$ *s.c. in vivo* and effects lasting >3H.[279] Improved *in vivo* activity was also observed by substituting D-Trp or D-Tpi (Trp analogue) into position 6 of Leu$^{13}\psi$[CH_2N-H][Leu or Phe]^{14}-bombesin(6-14).[280] Modification or deletion of amino acids residues at positions 12 and 14 of the bombesin nonapeptide shifted the biological activity from agonism to weak antagonism.[281]

6.11 *Miscellaneous Examples*

Bivalent ACTH antagonists, the most potent of which was (81), were prepared by reaction of the sulphydryl group of Cys^{25}ACTH(7-25) or Cys^{39}ACTH(7-39) with bis(maleimide) compounds. The bivalent ACTH peptide (81) was 28x more potent than the monovalent analogue Cys^{25}ACTH(7-25)-*S*-*N*-ethylsuccinimide.[282] Flexible spacers were also used to link the N-terminal tetrapeptide of neuropeptide Y to the C-terminal dodecapeptide. The increased receptor binding and increased α-helical content, particularily for (82), of these discontinuous analogues was thought to be due to hydrophobic interactions stabilising the α-helix

between residues 25 and 32.[283] The relative efficiency of cross-linking α-conotoxin to the acetylcholine receptor was partly dependent on the position of the photolabile group on the toxin. More intense labelling of the receptor subunits was obtained if the radiolabelled azido-salicylic acid group was attached to the ε-amino group of the lysine residue at the C-terminus of the toxin rather than the amine at the N-terminus.[284]

The parathyroid hormone analogue desamino[Nle^{8,18}, D-Trp^{12}, Lys^{13}(ε-3-phenylpropanoyl), Tyr^{34}]bPTH(8-34)NH_2 was an antagonist with high *in vitro* potency.[285] The activity of α-hANP (human atrial natriuretic peptide) analogues in which homo-amino acids replaced Asp^{13} and Arg^{11,14,27} residues suggested that the length of the methylene chain in the Asp residue was critical for binding whereas the homoarginine residues did not strongly affect the binding potencies.[286] Futhermore the enhanced vasorelaxant activity observed with the di-homoarginine analogue [Har^{11,27}]α-hANP(7-28) was partially a consequence of strong ionic stabilisation with the carboxyl group of Asp^{13} and the C-terminus.

Substituting each residue of neuromedin U-8 (H-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-Asn-NH_2) with glycine or the appropriate D-amino acid was detrimental to the biological activity. However, [D-Tyr^1]NMU-8 was twice as potent as NMU-8 and [D-Pro^6]NMU-8 and [D-Leu^3, D-Pro^6]NMU-8 were non-competitive antagonists.[287] Structure-activity relationships with the larger pentacosapeptide, neuromedin U-25, indicated that the sequence Phe-Leu-Phe-Arg-Pro-Arg may be essential to contractile activity and that positions 6 to 9 and 13 to 15 appeared to be of special importance for potent activity.[288] Studies with the N-terminal (1-16) fragment of galanin in which each amino acid was replaced with alanine identified that residues Gly^1, Trp^2, Asn^5, Tyr^9, and Gly^{12} were important for high affinity binding to the receptor.[289]

The increased diuretic activity of [Hyp^3]tuftsin (H-Thr-Lys-Hyp-Arg-OH) was attributed to the presence of the hydroxyl substituent on the pyrrolidine ring.[290] The C-terminal elongated derivative H-Thr-Lys-Pro-Arg-Thr-Thr-OH maintained 60% tuftsin activity on the phagocytosis process but other analogues in the series were inactive.[291] Tuftsin with various sugar moieties attached to the hydroxyl group of threonine were synthesised[292] and several derivatives were equivalent or better than tuftsin at stimulating the release of cytokines.[293] The glycosylated undecapeptide (H-Thr-Lys-Pro-Arg-Glu-Gln-Gln-Tyr-Asn(β-D-GlcNAc)-Ser-Thr-OH) which corresponds to the 'tuftsin region' at the Fc domain of IgG was also prepared but this peptide was less active than tuftsin.

The acid-base properties of thymopoietin have been explored using 21 tri- and tetrapeptide analogues.[294] The structure of the immunoactive lipopeptide *S*-[2,3-bis(palmitoyloxy)propyl]-N^{α}-palmitoyl-Cys-Asn-Ser-

Gly-Gly-Ser-OH which stimulates proliferation of bone marrow cells has been confirmed by total synthesis.[295] Addition of lipid moieties to the N-terminus of peptides belonging to the envelope protein of hepatatis B virus were aimed at increasing the immunogenicity of the peptide by increasing entrapment yields into liposomes.[296] Lipophilic diesters of muramoyl dipeptide have been prepared.[297]

The antineoplastic agents (-)-dolastatin 10[298,299] and (-)-dolastatin 15[300] from the Indian Ocean sea hare *Dolabella auricularia* have been chemically synthesised. (-)-Dolastatin 15 is currently undergoing pre-clinical development as a potential anticancer drug.

7 Conformational Information Derived from Physical Methods

The use of high field 1D and 2D n.m.r. techniques and/or X-ray crystallography to establish structure is now widespread. Where appropriate, therefore, the conformational properties of peptides or their analogues are dealt with in earlier sections. The papers cited under this sub-heading are those which concentrate on spectral analysis or those which use a theoretical approach to investigate structure.

The n.m.r. of peptides and proteins is included in the comprehensive review on natural macromolecules in another RSC publication.[301] The use of n.m.r. as a tool in drug design has been discussed in two reviews.[302,303]

A combination of 1H n.m.r., c.d. and molecular modelling revealed that endothelin CSCSSLMDKECVYFCHLDIIW formed a compact structure in DMSO with an extended helix-like region between Lys^9 and Cys^{15}.[304] A similar 3D structure was found for [Nle^7]endothelin in both DMSO and in an acetonitrile-water (1:1) mixture.[305] Protein structure prediction and molecular dynamic simulations have been used to study the 3D structure of big endothelin (38 amino acids).[306] Similar studies with salmon calcitonin CSNLSTCVLGKLSQELHKLQTYPRTNTG-SGTP found that the core of the molecule, between residues 8 and 22, adopted an amphiphilic α-helix like structure in trifluoroethanol (TFE)/H_2O (9:1).[307] Amino acids 4 to 7 were also involved in the helix whilst the C-terminus was more mobile but folded back towards the core forming a large loop. In the TFE-based structure of the linear VIP molecule two helical segments involving residues 7-15 and 19-27 were linked by a random coil portion.[308]

ROESY cross peaks and 1D nOe enhancements of angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) and analogues in DMSO-d^6 indicated a clustering of the Phe, Tyr, and His aromatic rings.[309] The absence of Tyr-OH and imidazole NH protons in spectra at neutral pH suggested

fast exchange between different enviroments. These findings supported the proposed charge relay system involving the triad Tyr, His, Phe (Figure 2).

β-Casomorphin-5 (H-Tyr-Pro-Phe-Pro-Gly-OH) has an affinity for the μ opioid receptor and an equilibrium between four conformers (originating from two Xxx-Pro bonds) was observed in the n.m.r. spectrum with the biggest population arising from the *t-t* isomer.[310] nOe data indicated a stacking arrangement of the aromatic side chains in the all *trans* isomer. In contrast, the theoretical low energy conformations of the μ-selective peptide (83) suggested that the aromatic rings were not in a tilted stacking orientation.[311] However the more or less unhindered side chain rotations made it difficult to select the most probable receptor bound conformation. In [Trp^3]β-casomorphin analogues, fluorescence energy transfer experiments enabled distances between the Tyr and Trp side chains to be estimated. Three peptides were studied and a correlation was found between the Tyr-Trp intramolecular distance and biological activity; the shorter distances corresponded to higher biological potency.[312]

Topographical requirements for δ-receptor selectivity were also explored using low energy conformers of DPDPE (Tyr-D-Pen-Gly-Phe-D-Pen), DCFPE (Tyr-D-Cys-Phe-D-Pen) and dermenkephalin. The results suggested a more or less defined position in space for the Phe side chains. The positions of the Tyr side chains could not be specified so precisely but a distant spacing of the aromatic side chains was indicated.[313]

By using a transverse relaxation (WATR) method to completely eliminate the water peak from the n.m.r. spectrum of reduced AVP (Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2) a complete assignment of the resonances was possible.[314] In the n.m.r. of [Cpp^1, Sar^7]AVP a long range nOe was observed between the αCH protons of Cpp^1 and Cys^6 thus indicating a β-turn around the Phe^3/Gln^4 residues. The acyclic portion of the molecule was more flexible and the double set of resonances in the ROESY spectrum for the C-terminus tripeptide sequence was due to *cis-trans* isomerism of the Cys^6-Sar^7 amide bond.[315] The theoretical conformational analysis using ECEPP/2 and AMBER force field calculations was generally consistent with the experimental data for the cyclic portion. 2D n.m.r. studies on pentapeptide analogues of vasopressin have also been reported.[316]

Analysis of the fluorescence parameters of the vasotocin analogue (84) bound to isoforms of bovine neurophysins (carrier proteins) suggested that the dansyl group in the NP II complex was in a more polar environment than in the NP I complex.[317] Results of 2D homo- and

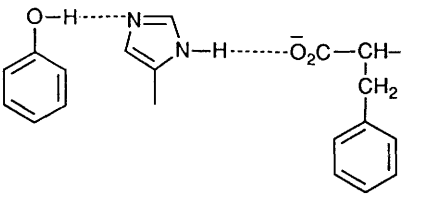
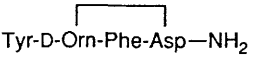


Figure 2



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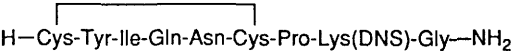

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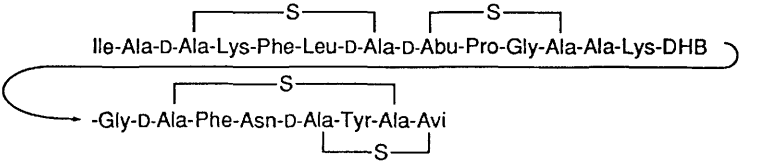

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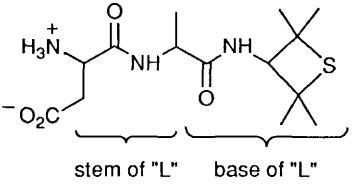
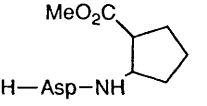


stem of "L" base of "L"

(86)



(87)

hetero-nuclear n.m.r. experiments of eledoisin (pGlu-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH_2) concluded that the molecule had a highly flexible conformation in DMSO solution.[318] Experimental nOe and hydrogen bond connectivities were included in molecular dynamic calculations of N-acetylmuramyl dipeptide. Many conformations satisfied the distance constraints including one with the S-shaped conformation proposed by previous n.m.r. studies.[319]

The conformational flexibility of bombesin antagonist spantide (D-Arg-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH_2) and the bombesin nonapeptide analogue (Thr-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH_2NH]Met-NH_2) have been studied using c.d. and n.m.r. techniques. In aqueous solution the reduced nonapeptide existed as an ensemble of flexible conformers but in apolar media (TFE, SDS - a membrane mimic environment) c.d. measurements indicated a shift towards folded structures.[320] In contrast, spantide showed evidence of folded structures in both polar and apolar solutions.[321]

The conformational flexibility of neurotensin (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) was reduced in SDS compared with aqueous or methanolic solutions due to electrostatic interactions between the cationic segment and the anionic sulphate groups of the micelle.[322] The tumbling rate of the peptide decreased due to the binding interaction and this was manifested in the n.m.r. spectra by differing intensities of the cross peaks; in the HOHAHA spectra in SDS the αCH-NH cross peaks for Lys^6, Arg^8, Arg^9, Tyr^{11} were very weak compared with those for the residues on either side of this segment or when compared with the strong interactions in aqueous and methanol solutions.

Addition of a phospholipid or TFE to an aqueous solution of hGRF(1-29) caused an essentially unordered peptide to fold into a helical conformation. C.d. spectra indicated that the peptide formed an almost complete helix in 50% aqueous TFE whereas in the lipid environment a maximum of 65-70% helicity was obtained.[323] Similar studies with GRF showed that in buffer the hormone was mainly in the random coil configuration but the presence of negatively charged lipids induced a degree of secondary structure.[324]

Spin-lattice relaxation experiments of gramicidin A in SDS micelles in the presence of Mn^{2+} indicated that the Mn^{2+} ions retained their hydration shells and were near (6-8 Å) the C=O groups of D-leucine at positions 10, 12, and 14.[325] Analysis of the 2D n.m.r. of a synthetic 32-residue peptide analogue of the membrane spanning segment B of *Halobacterium halobium* bacterioopsin in SDS micelles showed[326] a right-handed α-helical stretch from Lys^8 to Leu^{29} with a kink at $Pro17$.

Support for the view that the ion channel properties of the lantibiotic gallidermin (85) were due to an elongated amphiphilic shape was obtained from a study combining n.m.r. and molecular dynamics techniques. A set of five converging structures were obtained in which the peptide adopted a screw-like conformation (30 Å in length and 8-10 Å in diameter).[327]

The proposal that an L-shaped structure is the key to the sweet taste of aspartyl sweetners was further explored using n.m.r. and computer simulations of alitame (86, L,D isomer) and the L,L (bitter) and D,D (tasteless) stereoisomers. In the mimimum energy conformations of the L,L and D,D isomers the thietane ring system projected behind, and in front, respectively of the stem of the 'L'-shaped peptide backbone thus deviating from the optimum 90° angle with the zwitterionic ring of the aspartyl moiety.[328] Molecular mechanics calculations correctly predicted, and X-ray crystal structures confirmed, that the *trans*-(1*R*, 2*R*) and *cis*-(1*S*, 2*R*) stereoisomers of (87) preferred an 'L' shaped conformation.[329] Both isomers were sweet. Aspartame (L-Asp-L-Phe-OMe) in the hydrated state has been subjected to conformational calculations.[330] QSAR models developed from the mimimum analogue peptides sets (MAPS) of bitter-tasting dipeptides and dipeptide ACE inhibitors appeared to be similar to those based on the original whole sets.[331]

The possibility of a link between substance P-induced histamine release and an increase in copper concentration in blood plasma prompted a spectroscopic study of the coordinate properties of substance P.[332] SP and $SP_{1\text{-}5}$ analogues formed highly stabilised complexes with Cu(II) ions by chelation through the N-terminal nitrogen and the ε-amino nitrogen of the lysine residues. Furthermore the peptide was forced into a bent conformation by the prolyl residues.

Mimimum energy conformations of Met-enkephalin,[333] statine,[334] and CCK fragments[335] have been obtained using the Monte-Carlo technique. Leu-enkephalin has been modelled[336] using PLEC, a program which uses distance constraints from an X-ray structure. The possible role of the membrane in mediating receptor subtype selectivity was highlighted in a review on the 'active conformations' of peptides.[337]

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4
Cyclic, Modified, and Conjugated Peptides

BY J. S. DAVIES

1 Introduction

The format, adopted for this Chapter last year (Volume 23), has been retained. The coverage is representative of studies on naturally-occurring cyclic and modified peptides, and cyclic structures investigated for structural/conformational purposes. No attempt has been made to review the very active area of cyclic analogues of biologically active domains, and the use of surrogate peptide bonds, which are topics covered in Chapter 3 of this Volume. However, post-translational modification, as in phosphorylated and glycosylated peptides remain topics covered here.

The number of papers published in this area showed a modest increase from last year. Again most of the mainstream journals were scanned, but the reviewing was greatly helped by access to a complimentary bi-weekly copy of CA Selects[1] on Amino Acids, Peptides and Proteins, produced by Computer Search. Abstracts which appeared up to Issue 13 (1992) have been included in this Report. Although CA Selects do access the patent literature, these have not been reviewed, so the reporting has again concentrated on refereed papers. Relevant papers contributed to the 12th American Peptide Symposium, Boston[2] and to the Proceedings of the 1st International Workshop on Lantibiotics[3] at Bonn, have therefore not been reviewed.

Again, the literature for 1991 confirms the very significant inroads made by high field nmr techniques to structural elucidation. The very brief comments allowed in a review of this kind will obviously not do justice to the effort required in producing detailed structural assignments using physical methods, but many papers nowadays often seem to reflect the methodology as being routine. While nature continually reveals its extensive repertoire of complicated cyclic peptides and depsipeptides, human endeavour is increasingly being concentrated on the carbohydrate-peptide interface.

2 Cyclic Peptides

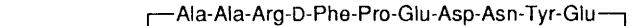
2.1 *General Considerations*

As mentioned in the Introduction, advanced nmr techniques are providing invaluable structural information in this field. Typical of the power of the 2D- and 3D-techniques are the reports on a variety of cyclic peptides[4], e.g., *cyclo*(Ala-Ala-Ala-Pro-Ala-Pro), *cyclo*(D-Pro-Phe-Phe-Lys(Z)-Trp-Phe) and the peptide macrolide FK 506[4]. Several new proton-detected heteronuclear 2D-techniques have been applied[5] to the thio-analogue of cyclosporin A. Very indicative of the future potential of the technology are the results[6] of a nmr study of cyclosporin A when it is bound to its putative target protein, cyclophilin. The application of nmr techniques to the conformation of peptides and proteins in general has been reviewed[7].

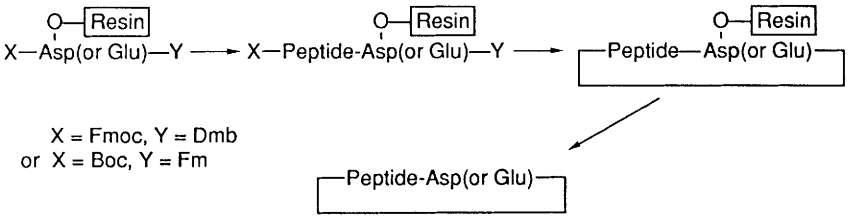
Traditionally head to tail cyclisation of linear peptides has remained the territory of solution phase methodologists. Two separate reports utilising the side-chain carboxyls of Asp and Glu residues for resin attachment have now revealed that the solid phase can also be used. In the synthesis of (1)[8] the linear precursor was attached *via* the side-chain carboxyl groups of either Fmoc-Asp-Dmb or Fmoc-Glu-Dmb where Dmb is the 2,4-dimethoxybenzyl group. Assembly of peptide was then carried out *via* the Fmoc-synthetic strategy, and when complete, the Dmb ester group was removed by 1% tri-fluoroacetic acid in dichloromethane, the Fmoc group by piperidine, prior to cyclisation with BOP/HOBt/N-methylmorpholine or diisopropylcarbodiimide. For the synthesis[9] of the tachykinin peptide antagonist MEN10207, *cyclo*(Asp-Tyr-D-Trp-Val-D-Trp-D-Trp-Arg), the aspartyl β-COOH group was linked to the PAM resin using Boc-Asp-OFm (Fm = fluorenylmethyl group). After assembly *via* the Boc/Bzl strategy, the Fm ester is converted to the acid using piperidine, Boc is removed by TFA, prior to cyclisation using the BOP reagent. Deprotection of all side-chains and release of the resin was achieved using HF. Both these methods assume the need to have Asp or Glu in the final sequence. The overall approach is summarised in Scheme 1.

2.2 *Naturally occurring Dioxopiperazines (Cyclic Dipeptides)*

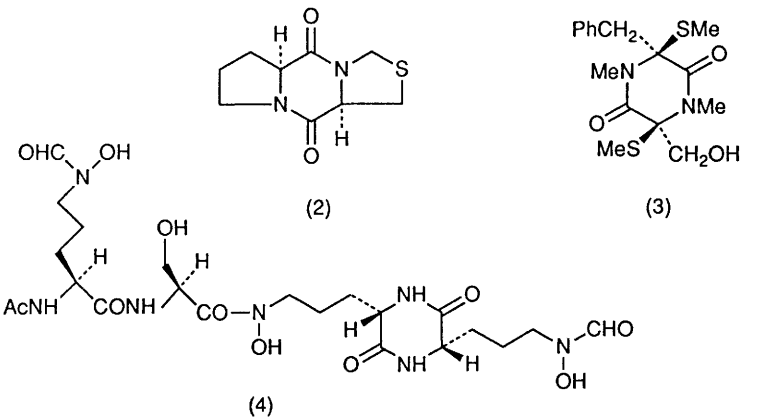
A new sulfur-containing dioxopiperazine, *cyclo*(Pro-ThioPro) (2) from the Bermudian sponge *Tedania ignis*, has been characterised[10] and its structure confirmed by synthesis. Biosynthetic steps from the dioxopiperazine stages to (3*S*,6*S*) epidithiodioxopiperazines have been clarified further[11] by the efficient incorporation (41%) of *cyclo*(L-[4′-^{3}H]Phe-L-[3-^{14}C]-Ser) by *Hyalodendron* sp (FSC-601) cultures to the 3*S*,6*S* meta-



(1)

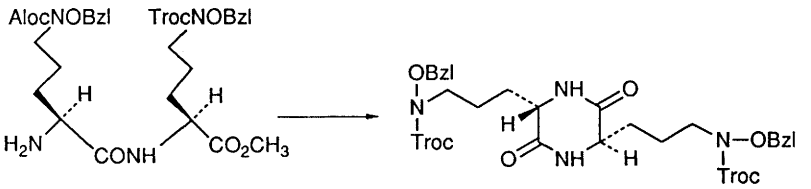


Scheme 1



(2) (3)

(4)



Aloc = allyloxycarbonyl
Troc = trichloroethoxycarbonyl

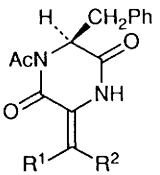
Scheme 2

bolite didenthiobis(methylthio)hyalodendrin (3). The first total synthesis[12] of foroxymithine (4) from *Streptomyces nitrosporeus* - a Fe^{3+} ion chelator and ACE inhibitor contradicts the widely held view that "DKP's" are easily formed from dipeptide precursors. The key step (Scheme 2) could only be achieved (70% yield) using imidazole (10 eq) in methanol for about 100 hr.

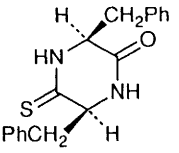
2.3 *Other Dioxopiperazines*

Crystal structures[13] of the geometric isomers (5) and (6), show that both molecules are in the folded conformation with the aromatic rings of the benzyl groups facing the dioxopiperazine ring. In the (*Z*)-form (6) the dioxopiperazine ring is in the boat conformation, while in the (*E*)-form (5) it is twist-boat with the benzyl side-chains occupying the flagpole position. Comparison of the physical data[14] of the monothioxo cyclodipeptide (7) with its all oxygen analogue, has revealed a low frequency shift of the amide I mode in the ir spectra and low field shifts in resonances of groups close to the CS group. A series of cyclic retro-inverso dipeptides *cis*- and *trans*- (8) ($R = H$, NH_2, $R^1 = OH$, $R^2 = H$; $R = R^1 = H$, $R^2 = OH$) have been synthesised[15] to serve as models to define structural requirements for binding affinity with opiate receptors. The compounds were inactive in the guinea pig ileum and mouse vas deferens assays. When the conformations of the compounds represented by (8) were studied[16] by ^1H nmr and semi empirical energy calculations and compared with the parent cyclodipeptides *cyclo*(L-Tyr-L-Phe) and *cyclo*(L-Tyr-D-Phe), in the *cis*-forms both aromatic side chains share the space over the dioxopiperazine rings in a 'face to face' fashion. A 'sandwich' conformation predominated in the *trans*-forms. The cyclic compounds (8) in the *trans*-form show only one type of boat structure in which the malonyl side chain is pseudoequatorial, and the gem-diamine side chain is pseudoaxial. Further refinements have been possible[17] in the conformations of cyclodipeptides with two identical L-aromatic amino acid residues, as in *cyclo*[-L-5(OH)Trp-L-5(OH)Trp] and *cyclo*[-L-Phe-L-Phe]. Using nmr it is shown that one aromatic residue occupies the folded antiperpendicular and the other the 'extended to nitrogen' perpendicular conformers, with each of the individual residues in a fast conformation equilibrium between the two conformers. Hence the one set of resonances observed in the nmr spectra.

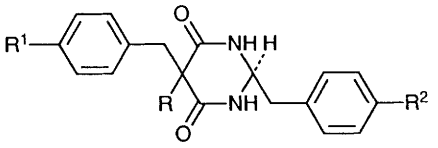
The dioxopiperazine ring has also found useful roles in other situations. In a method devised[18] to give peptide solutions suitable for immediate biological testing, the ready formation of a dioxopiperazine ring (10) from a linker such as (9), allows facile cleavage of the peptide off the pins in the multipin methodology. Coupling of the hydrophobic cavity of β-cyclodextrin to *cyclo*(-L-His-L-His) as in (11)[19] has been achieved



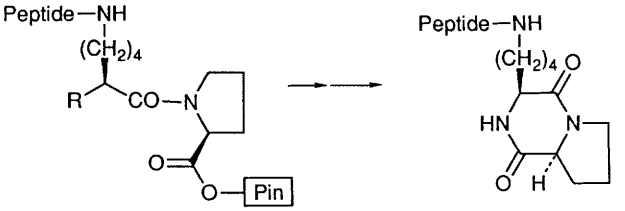
(5) R^1 = *p*-ClPh, R^2 = H
(6) R^1 = H, R^2 = *p*-ClPh



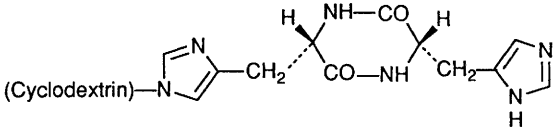
(7)



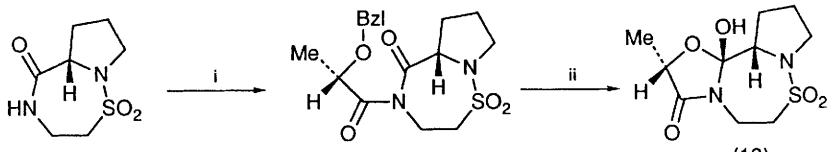
(8)



(9) R = BocNH (10)



(11)



(12) (13)

Reagents: i, (*S*)-$PhCH_2OCHMeCOCl$; ii, Pd/C

Scheme 3

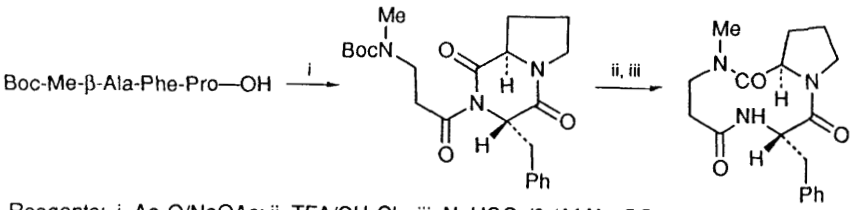
using 6-deoxy-6-iodo-β-cyclodextrin and the cyclodipeptide. Conformational evidence from nmr allows a model to be produced to explain the coordination properties of (11) with metal ions. A similar cyclodipeptide *cyclo*(-(*R*)-Phe-(*R*)-His) has given rise[20] to an interesting enantioselective autoinduction catalysis in the hydrocyanation of phenoxybenzaldehyde to (*S*)-2-hydroxy-2-(3-phenoxyphenyl)acetonitrile.

Cu(II) ternary complexes with L- or D-amino acids and *cyclo*(His-His) have been studied[21] spectroscopically in aqueous solution. Complexes from the L form are more entropy favoured than the D analogues, and the differences seem to increase in comparing a series ranging from phenylalanine to tryptophan, suggesting that there is aromatic ring stacking interactions between one of the dioxopiperazine imidazoles and the aromatic rings of the amino acids. A crystal structure[22] of the sodium salt of the dioxopiperazine derived from aspartame, *cyclo*(-L-Asp-L-Phe).$4H_2O$, revealed a 3D network due to the ligand binding of the metal sodium atoms, and H-bonding involving the amide, carboxylate groups and the water molecules. Insertion of the (*S*)-lactyl residue into *cyclo*(Tau-Pro) (12) *via* Scheme 3 leads to good yields[23] of the stable oxa-cyclol (13) and not the cyclodepsipeptide analogue.

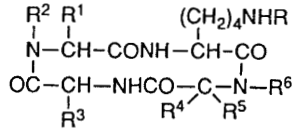
2.4 *Cyclotripeptides and Cyclotetrapeptides*

Only one reference[24] to the cyclotripeptides was retrieved this year, and involved the synthesis and conformational study of the 10-membered ring diastereoisomers *cyclo*-(NMeCH_2CH_2CO-Phe-D-Pro) (14) and *cyclo*(NMeCH_2CH_2CO-Phe-Pro) (15). Both were prepared by cyclisation from the same linear precursor with the L-proline at C-terminal position. Cyclopeptide (15) was obtained from the p-nitrophenyl ester of its precursor, while (14) was obtained using the β-amino-acyl incorporation method summarised in Scheme 4. Both forms seem to exist in the same conformations in the solid state and in solution, and both exhibit *cis-cis-trans* backbone conformations, the Pro and NMeβAla bonds being *cis* and the CONH remaining *trans*.

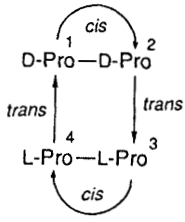
The structure of a new cyclotetrapeptide, trapoxin A, has been worked out[25] to be *cyclo*[(*S*)-Phe-(*S*)-Phe-(*R*)-pipecolinyl-2(*S*), 9(*S*)-2-amino-8-oxo-9,10-epoxydecanoyl] while the marine ascidian *Cystodytes delle chiajei* produces[26] *cyclo*(L-Pro-L-Leu)$_2$, *cyclo*(L-Pro-L-Val)$_2$ and *cyclo*(L-Pro-L-Phe)$_2$. The structures were confirmed by synthesis from linear precursors with Pro at the C-terminus and using the BOP reagent for cyclisation, although yields quoted were low (20%). FAB Mass Spectra in positive and negative modes failed to supply[27] sequence information to distinguish between the normal and retrosequences in a series of chlamydocin analogues (16). The most significant structural infor-



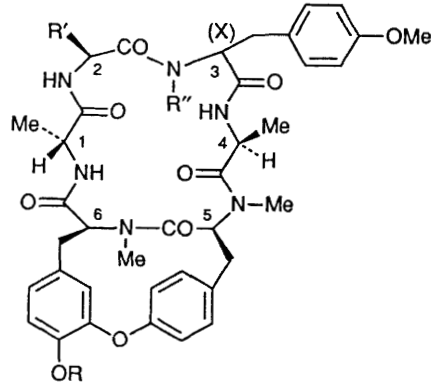
Scheme 4



(16) R = H, Z, $R^1 = R^3$ = Me, R^2, R^4 = H, R^5, $R^6 = (CH_2)_3$;
R = H, Z, epoxypropionyl, R^1, $R^2 = (CH_2)_3$, R^3 = Bzl, R^4, R^5 = Me, R^6 = H



(17)



(18) X = L, R = Me, R′ = Me, R″ = Me
(19) X = L, R = H, R′ = Me, R″ = Me
(20) X = L, R = Me, R′ = Me, R″ = H
(21) X = D, R = Me, R′ = CH_2OH, R″ = Me
(22) X = L, R = Me, R′ = MeCHOH, R″ = Me
(23) X = L, R = Me, R′ = CH_2OH, R″ = Me

mation came from Collision Activated Decomposition (CAD) spectra in tandem mass spectrometry.

The results of cyclisation reactions[28] on two diastereoisomeric tetraprolines as shown in the Table reveal that the cyclic tetrapeptide *cyclo*-(D-Pro-D-Pro-L-Pro-L-Pro) can only be obtained from DLLD-Pro_4 as precursor. In other work *cyclo*(DLDL-Pro_4) was obtained in 80% yield under the same conditions. The X-ray analysis shows that the cyclic(D-DLLPro_4) is in a strained C_i symmetrical conformation with alternating *cis-trans* peptide bonds as in (17). The strain probably explains the quantitative ring opening of the compound with trifluoroacetic acid at room temperature.

Table

Linear Precursor	Total Yield Method	%	Yield of *cyclo*(DDLLPro$_4$)$_n$ (%) n=1	n=2	n=3	n=4	n=5
DLLD-Pro_4	†DPPA/DMF	83	41	39	2	1	–
DLLD-Pro_4	†DPPA/CH_2Cl_2	70	42	15	10	3	–
DDLL-Pro_4	†DPPA/DMF	86	–	69	8	5	3
LLDD-Pro_4OPfp*	active ester/DMF	76	–	57	9	7	3

* pentafluorophenyl ester. †DPPA = diphenylphosphoryl azide

2.5 *Cyclopentapeptides*

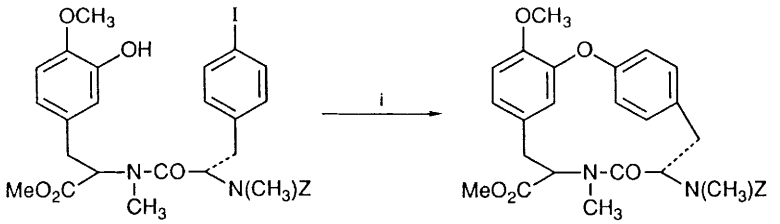
The structures[29] of two novel endothelin-binding inhibitors, BE18257A and BE18257B have been proven to be *cyclo*(D-Glu-L-Ala-D-Val-L-Leu-D-Trp) and *cyclo*(D-Glu-L-Ala-D-alloIle-L-Leu-D-Trp) respectively, by spectral analyses and confirmatory solution phase synthesis. Classical solution phase methodology[30] also provided *cyclo*(-L-Pro-L-Pro-L-Phe-βAla-βAla) for further X-ray studies. The final link in the ring was constructed at the βAla-βAla amide using DCCI in dichloromethane under dilution which gave a 20% yield of the cyclic peptide. The Pro^1-Pro^2 bond was found to be *cis* and the molecular conformation is stabilised by an intramolecular H-bond between $βAla^5$-CO and the Phe^3-NH. The Pro^1-Pro^2 segment occupies the relative positions 2 and 3 of a type VIa β-turn. Intramolecular H-bonds characteristic of β- and δ-turns have been picked out[31] in thirteen energy minimised conformations of *cyclo*-$(Gly)_5$. Peroxide oxidation[32] of the pseudocyclopentapeptide *cyclo*(Gly-Proψ[CH_2S]Gly-D-Phe-Pro), prepared from a linear precursor using diphenylphosphoryl azide/$NaHCO_3$ cyclisation conditions, yielded two diastereomeric sulfoxides *cyclo*-[Gly-Proψ[CH_2-*R* or *S*-SO]Gly-D-Phe-Pro). A single γ-turn centred around Pro^5 was revealed by nmr studies in $CDCl_3$, but a second minor conformation type II′ β-turn is stabilised in

d_6-DMSO, and is also the main form in the X-ray structure. There is no evidence that the sulfoxide surrogate participates in any intra- or intermolecular H-bonding.

2.6 *Cyclohexapeptides*

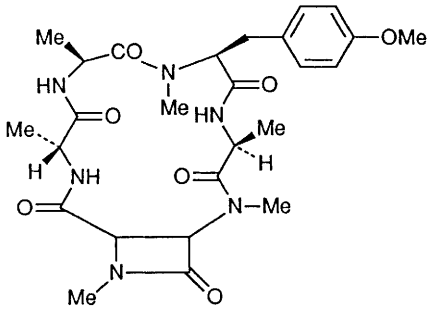
Pole position in this category goes to the growing class of potent antitumour antibiotics, the RA series isolated from *Rubia akane* and *R.cordifolia*, and bouvardin and analogues from *Bouvardia ternifolia*. In the synthesis of these 'bicyclics' the macrocyclisation of the 14-membered diaryl ether ring has been a major stumbling block. This has now been overcome[33] for RAVII (18) and deoxybouvardin (19) using a modified Ullman reaction as summarised in Scheme 5 for a model reaction. The structures of the two new antitumour agents from *Rubia cordifolia* have been elucidated[34]. Using a combination of 2D techniques and nOe relationships RAVI (21) was characterised and found to have a β-turn involving a Ser at position 2 and D-MeTyr at position 3, while RAVIII (22) had a Thr and L-MeTyr in these positions. In the solid state RAVI (21) was shown to have a type V β-turn at the Ser^2-$MeTyr^3$ positions in contrast to the type II turns found in other members of the series. Differences between the solid state and solution conformations were also highlighted in restrained molecular dynamics calculations using an AMBER programme. RAVIII was of interest because it has a lower biological activity and less β-turn character, so knowledge of the ^{13}C spin relaxation times of the $MeTyr^3$ aromatic ring over the β-turn could assist to clarify the role of the type II β-turn in the biological activity of this series. A higher field (500 MHz) nmr study[35] has enabled a complete assignment of the ^1H and ^{13}C signals in RAVII (18). The two conformers found in $CDCl_3$ solution have been rationalised as being due to *cis-trans* rotational isomerism of the Ala^2-$MeTyr^3$ bond. With the higher precision in the nmr technology, it is speculated that the highly strained 14-membered ring is necessary to maintain the typical type II β-turn. This is confirmed[36] by the much reduced biological potency of the β-lactam deoxybouvardin analogue (24), although it possesses an accessible β-turn conformation. The N-demethylated RAVII (20) formed by hepatic microsomal biotransformation[37], removes the chance of *cis-trans* isomerism at the β-turn. The solution conformation confirms this restricted conformational state. A more detailed hplc analysis of the *Rubia cordifolia* extracts have revealed[38] minor components RAI-III and RAI-VI which have been identified as δ-turn conformational isomers of RA-III (23) and RA-VI (21), respectively.

The nmr derived solution conformation of *cyclo*[-D-Ala-Phe-Val-Lys(Z)-Trp-Phe], has been simulated[39] by molecular dynamics calcula-



Reagent: i, 2 eq. NaOH, 10 eq. $CuBr{\cdot}SMe_2$, collidine, 130 °C

Scheme 5



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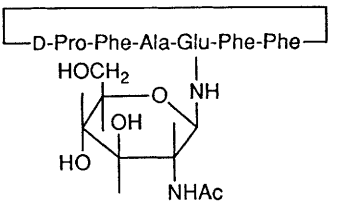

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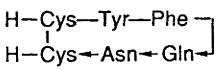

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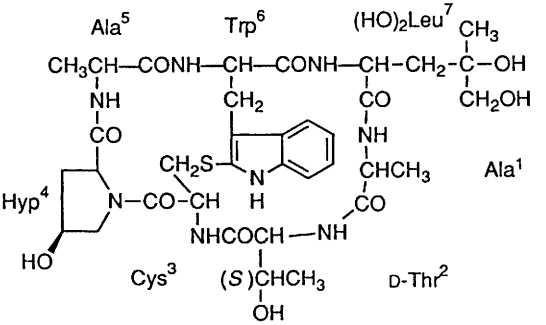

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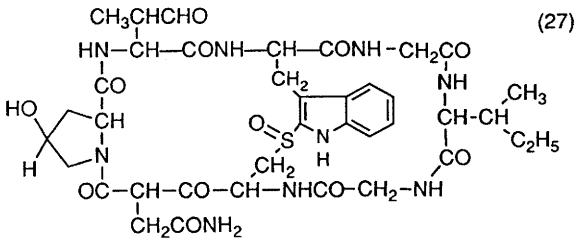


(28)

tions including DMSO as solvent. Intermolecular H-bonds are formed between the solvent, the backbone amide protons and the Lys side chain. Similar calculations, and an nmr analysis[40] of the N-glycosylated cyclohexapeptide (25) have been compared with the non-glycosylated analogue. For both compounds distance constraints derived from 2D nOe measurements could not be satisfied by one conformation, so interconverting conformers are assumed. The N-glycosylation does not affect the conformation or the overall shape of the peptide backbone or side chains, and has no influence on the H-binding pattern or on the fast dynamical equilibrium of the molecules. The syntheses of the compounds were achieved by cyclisation with water soluble carbodiimide/HOBt or by azide coupling. The cyclic hexapeptide *cyclo*(-L-Val-L-Pro-D-Ala-L-Val-L-Pro-D-Ala) exhibits[41] exact C_2-symmetry in the crystalline state with *cis* amide links between the Val and Pro residues. As part of an on-going project,[42] on β-turn conformations in proteins and their correlation with physical data, the pseudo-cyclohexapeptides *cyclo*[Gly-Pro-Ser(R)-Gly-$NH(CH_2)_4CO$-] with R = H, or Bu^t, have been synthesised, and analysed by X-ray crystallography, quantitative nOe and cd measurements. The Bu^t derivative adopted a type I β-turn involving the Pro-Ser sequence, and the nOe data confirmed the ratios of the cd component curves of the two major β-turn types (type I and II).

A previously published mass-weighted molecular dynamics simulation has been applied[43] to the disulfide linked hexapeptide (26) and its linear analogues, and shows potential for generating plausible structures for loop modelling of protein surfaces.

2.7 *Cycloheptapeptides and Cyclo-octapeptides*

A nmr solution determination of the conformation of evolidine, *cyclo*(Ser-Phe-Leu-Pro-Val-Asn-Leu) carried out in 1971, has been shown to be very close to the crystal structure[44] of evolidine. A type I β-turn involving Leu-Ser and a type VIa incorporating the *cis* Leu-Pro bond, suggest that a two β-turn backbone bulge can be a favourable motif in cycloheptapeptides. This has been confirmed[45] using a constrained distance geometry search in conjunction with the ^1H nmr data on evolidine.

Homo- and hetero-nuclear nmr techniques have been used[46] to assign all the ^1H and most of the ^{13}C resonances in phalloidin (27) the main representative of the toxins from *Amanita phalloides*. NOESY/ROESY and TOCSY determinations at 500 MHz have been utilised, as well as the temperature dependence of the NH protons. The latter coefficient for D-Thr^2-NH and $(OH)_2Leu^7$-NH indicate external orientation of the NH towards solvent, while values for Trp^6-NH and Cys^3-NH suggest intramolecular H-bonding. Intramolecular H↔H distances derived from

rotating frame nOe experiments and calibrated with respect to the indole NH-H_7') proton distance have been used as constraints in energy minimisation and molecular dynamics calculations using the GROMOS programme. There are no conformational changes detectable by nmr but fast conformational interchange cannot be excluded.

Another family of toxins from various mushroom species are the amatoxins, which act as inhibitors of the nuclear RNA polymerases of most eukaryotic organisms. Several new amotoxin derivatives have been prepared[47] utilising the 6′-O-methylaldo α-amanitin (28), which was generated by periodate oxidation of 6′-O-methyl α-amanitin. Addition of ammonia, glycine and proline in the presence of sodium cyanoborohydride to (28) gave derivatives which in turn gave K_i values for calf thymus RNA polymerase II of 1.7×10^{-7}, 2.5×10^{-7} and 7.0×10^{-6} M, respectively. The corresponding carboxyl or cyano group instead of the aldehyde gave K_i values of 1.0×10^{-7} and 3.0×10^{-9} M, respectively. Only the cyano group therefore (uncharged) is a very potent inhibitor, with the other derivatives which all carry charged functions being much poorer.

The cyclic octapeptide, *cyclo*(-Ala-Gly-Pro-Phe-Ala-Gly-Pro-Phe) as its tetrahydrate from methanol/water has undergone X-ray studies[48]. Two β-turns encompassing the residues Pro-Phe, one of type I, the other of type II were found, which is a similar conformation to that found for *cyclo*-bis(-L-Cys-Gly-Pro-Phe). This suggests that the disulfide bridge of the Cys analogue does not impose conformational constraint on the ring backbone. Host-guest binding studies[49] have been made possible from ^{13}C nmr conformational analysis of *cyclo*(-L-Phe-L-Pro-Gly-L-Pro)$_2$ synthesised from a linear precursor by cyclisation of a N-hydroxysuccinimide ester in pyridine. The parent molecule before complexing existed in two kinds of C_2-symmetric conformations with *trans-trans-trans-trans* and *cis-trans-trans-trans* forms in $CDCl_3$. When CsSCN is added the 1:2 complex Cs^+:A in $CDCl_3$ reverts to an all *trans* conformation. Addition of DL-PheOMe.HCl formed a 1:1 complex with C_2-symmetric all *trans* characteristics but separate resonance were indicative that the cyclopeptide could distinguish between the D- and L-forms of the PheOMe.HCl. The formation of complexes was the main thrust of a cd and nmr study[50] on cyclic octapeptide (29). Only weak interactions with alkali metal ions were indicated by the cd spectra, but with Mg^{2+} and Ba^{2+}, 1:1 complexes were formed. Complexes of both 2:1 and 1:1 ratios were indicated with Ca^{2+}.

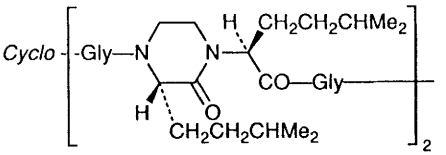
2.8 *Cyclononapeptides*

Cyclolinopeptide A isolated from linseed oil and recently shown to

have cytoprotective properties *in vitro* has been the subject of extensive structural analysis over many years, since its structure was identified as *cyclo*-(Pro-Pro-Phe-Phe-Leu-Ile-Ile-Leu-Val). However, the high degree of mobility, both of its side chains and peptide backbone requires the application[51] of high field nmr techniques (500 MHz) to rationalise conformational aspects. In acetonitrile solution the backbone contains a *cis* Pro-Pro bond with all other amides *trans*. Ba^{2+} binds more tightly than other ions to cyclolinopeptide A, giving complexes in the ratio 1:2 or 1:1 depending on the Ba^{2+} ion concentration. Nmr spectra of the 1:1 complex suggest that the backbone changes to all *trans* peptide bonds, a type I 6→3 β-turn and a 3→1 γ-turn giving an overall bowl shape with the concave side hosting Ba^{2+} ions. In two separate reports[52,53] using molecular dynamics the starting point for all simulations was a recently pubished X-ray structure. One report[52] used the GROMOS programme for energy minimisation while the other work[53] utilised a KGNMD programme from a MOTTEC package. Restrained and unrestrained dynamics simulations *in vacuo* and in CCl_4, reveal that the orientation of the side-chains in the crystal structure is better reproduced if the CCl_4 is involved and confirms the observation of a unique conformation in $CDCl_3$ at 214K as deduced by nmr. When water molecules are simulated into the interactions, several conformers are observed with high flexibility in the two Xxx-Pro bonds.

2.9 *Cyclodecapeptides*

[L-$Lys^{2,2'}$]-Gramicidin S has been known to possess the same activity and conformation as gramicidin S which has ornithine in the 2,2′ positions. To investigate the effect of configuration on this position two analogues [D-$Lys^{2,2'}$]- and [D-Lys^{2}, L-$Lys^{2'}$]-gramicidin S (30 and 31) have been synthesised[54] in the solution phase. Cd spectra showed patterns suggesting β-turns around D-Phe-Pro resembling that of the parent, but these analogues had weak or no activity against Gram-positive microorganisms. Three cyclic octapeptides [des$Val^{1,1'}$]-, [des$Leu^{3,3'}$]- and [desVal^{1}, $Leu^{3'}$]-gramicidin S showed[55] no antibiotic activity. The cd spectra although resembling each other, were different from the parent, suggesting a conformational role for Val and Leu residues. A β-turn mimetic, replacing the i+1 and i+2 residues of the turn has been inserted[56] into the [$Lys^{2,2'}$]-gramicidin S sequence as in (32). Boc-Protection on a PAM resin was used to synthesise the linear precursor with the BOP reagent used to link the two ends at the $Pro^{5'}$-Val^{1} bond. The intramolecular H-bonds, interresidue nOe's and $J^{N\alpha}$ coupling constants were equivalent to the values of native gramicidin S, confirming a type II′ β-turn. The pattern of H-bonding is represented diagrammatically in (32) using circled H's. A heterodetic bicyclic decapeptide *cyclo*-(Glu-Leu-Pro-



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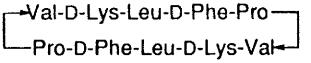

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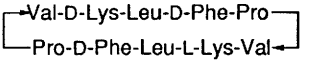

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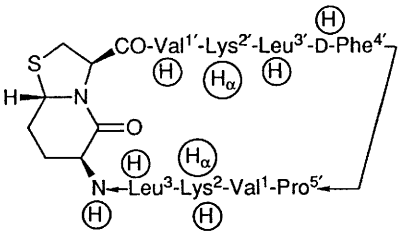

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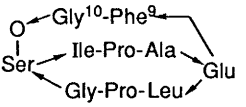

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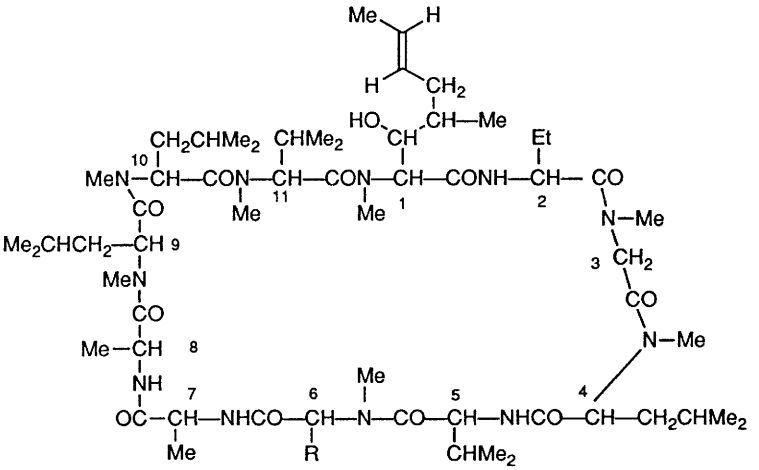


(34) R = CH_2CHMe_2
(35) R = Me

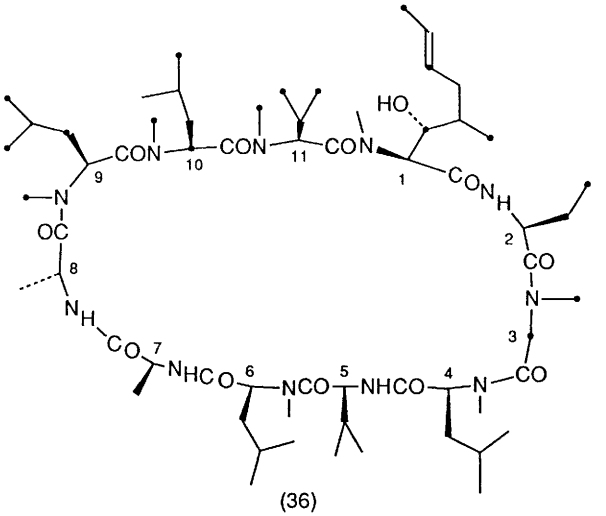
Gly-Ser-Ile-Pro-Ala)*cyclo*-(1δ-5β)Phe^9-Gly^{10} (33) has been synthesised[57] in the solution phase, using isobutylchloroformate or DCCI/HONSu for final cyclisation in 30% and 20% yield, respectively. Nmr data, involving NH temp coefficients and measured nOe contacts, confirm H-bonding networks at Glu^1, Gly^4, Ile^6 NH's and a model structure which includes a cavity which can be a binding site for Ca^{2+}, Mg^{2+} and Ba^{2+} ions through co-ordination to Leu^2, Gly^4, Glu^{1}γ-CO, Phe^9 and Ile^6 CO groups.

2.10 *Higher Cyclic Peptides*

Leading exponents in the field of peptide conformation feel[58] that with the improved understanding of the 3D molecular structure of key immunosuppressive agents such as cyclosporin A (34) and FK506 that the "fog is slowly lifting on the understanding of immunosuppression". The conformation[59] of the MeAla-6-cyclosporin A analogue (35) is therefore pertinent since it shows weak immunosuppressive activity and yet binds strongly to the proposed protein receptor, cyclophilin. A detailed nmr study[59] showed the backbone conformation of the analogue to be identical to cyclosporin A but differences did occur in the side-chain motions of the MeBmt^1, MeLeu^9 and MeLeu^{10} residues. Temperature studies suggested that the preferred rotamers in these side chains could increase the stability of the H-bond between NH^7 and CO^{11}, but prevent the essential MeBmt^1 side chain from adopting the active orientation for immunosuppressive activity.

Of great significance to the overall understanding of immunosuppression are two independent reports[60,61] on nmr studies on cyclosporin A when it is complexed to cyclophilin, a 17.7 kDa protein with peptidyl-prolyl isomerase activity. When uniformly ^{13}C-labelled cyclosporin A is used ^{13}C-signals in the bound state can be assigned and a molecular structure calculated from nOe values, and distance geometry considerations. The conformation of the bound cyclopeptide is very different from that suggested by X-ray and nmr studies on the uncomplexed molecule. The filled circles in structure (36) indicate the cyclosporin A protons with nOe's to cyclophilin as observed in a 3D HMQC-NOESY experiment. The parts of the molecule involved in binding reflect previous structure-activity studies. Using ^{15}N- and ^{13}C-labelled cyclosporin A nmr data[61], accumulated in aq. solution followed by analysis using the distance geometry programme DISMAN, and energy-refined by the FANTOM programme, again revealed a backbone conformation significantly different from unbound cyclosporin A. All peptide bonds were *trans*, no elements of regular secondary structure and no intramolecular H-bonds could be detected.

Reaction[62] of cyclosporin A with Lawesson's Reagent gives sulfur



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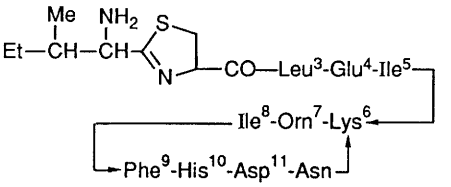

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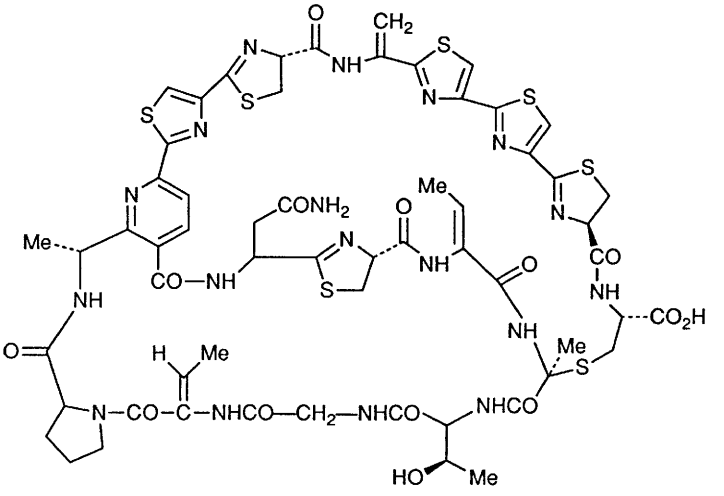


(38)

for oxygen replacement in the amide CO's and in the HO group of the MeBmt[1] side chain. Whereas one conformation dominates cyclosporin A in $CDCl_3$, two conformers, (A) and (B) forms, in the ratio 58:42 are present in [$^1\psi^2$,CSNH] cyclosporin A. The (A) form is identical to the parent molecule's solution state while the minor (B) conformer exhibits a *cis*-form between Sar[3] and MeLeu[4]. None of the nine thio-cyclosporins are as immunosuppressive as cyclosporin A, and the higher the S-content the lower the activity. [$^1\psi^2$,CSNH] Cyclosporin A followed by the [$^7\psi^8$CSNH]-analogue showed the highest activities, and considering that the former is made up of two slowly exchanging forms the result is noteworthy. The paramagnetic relaxation reagent, 4-hydroxy-2,2,6,6-tetramethylpiperidin-yl-1-oxy has assisted[63] in identifying the solvent exposed regions of the bound ligands when cyclosporin A is bound to its putative target protein cyclophilin. A fluorescent derivative[64] of a cyclosporin A isomer, prepared by dansylation points to the possible development of more sensitive means in future to study the binding properties of cyclosporins using hplc.

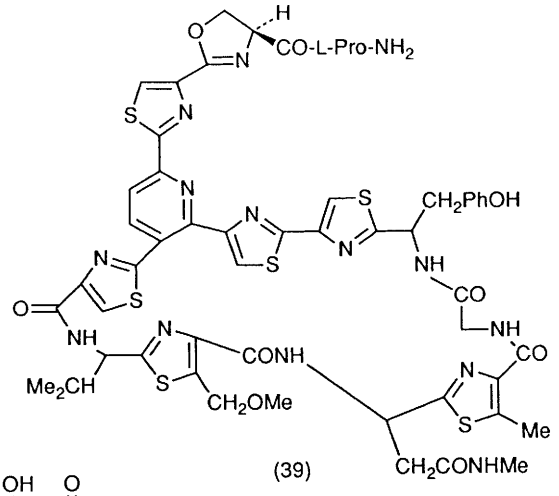
^{1}H Nmr spectra (at 500 MHz) of bacitracin A (37) in water and d_6DMSO have been assigned[65] and compared for the two solvents. The cyclic part of bacitracin A seems to be fairly mobile but a high field shift of the γ-methyl of the Ile[8] suggests this part has a well-defined conformation. It is believed that this is due to close proximity of the Ile[8] and Phe[9] side-chains, which is part of a β-turn (type II) involving Orn[7]-Ile[8]-D-Phe[9]-His[10], which is compatible with the chirality of the central residue. The linear part is folded over the cyclic moiety.

2.11 *Peptides containing Thiazole Type Rings*

As part of a screening programme for natural renin inhibitors the broth of *Streptomyces* NRO516 yielded[66] a unique polythiazole containing peptide, named cyclothiazomycin (38). Fragments obtained by chemical breakdown were subsequently 're-connected' from evidence based on NOESY experiments. Similarities with thiostrepton and nosiheptide can be noted in the structure. Bacterial protein synthesis can be inhibited by antibiotic GE2270A isolated[67] from *Planobispora rosea* ATCC53773. Application of a range of physical methods have led to the structure (39), which seems to have a similar biosynthetic origin to that of nosiheptide and micrococcin.

2.12 *Cyclodepsipeptides*

Decigram quantities of the cytotoxic cyclopeptides, didemnins A-C (40) can be synthesised[68] *via* preformation of the protected linear depsipeptide precursor which has the leucine C-terminal and the proline



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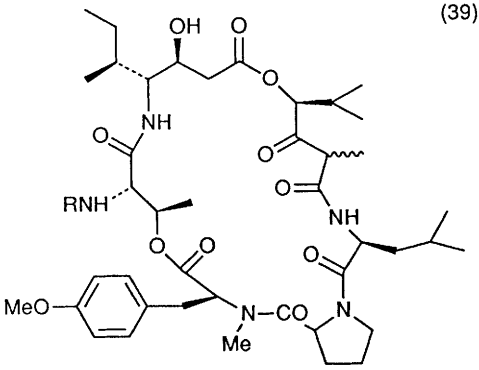
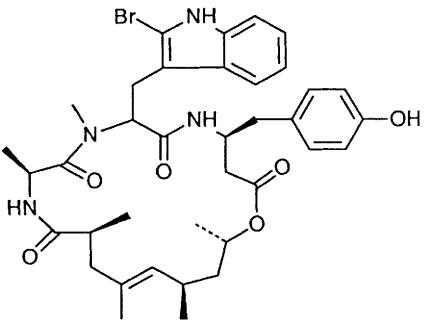


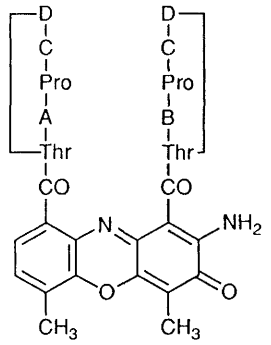
(40) Didemnin A
R = H-(*R*)MeLeu
Didemnin B
R = H-(*S*)-Lac-Pro-(*R*)MeLeu
Didemnin C
R = H-(*S*)-Lac-(*R*)MeLeu



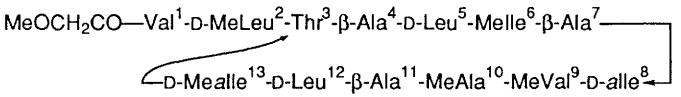
(41)

residue N-terminal. The pentafluorophenyl ester of the C-terminal residue in a two-phase system gave rise to the ring skeleton in 75% yield within a few minutes. The respective side-chains were then attached. A second total synthesis[69] of (+)-jasplakinolide (or jaspanamide) (41) has been published. The final cyclisation step was made at the depside link by activation of the β-Tyr carboxyl using DCC, for nucleophilic attack by the alcohol group. The latter had been temporarily protected by a t-butyldimethylsilyl group during the build up of the linear precursor. Analogues (43-46) of actinomycin D (42) have been synthesised[70] to ascertain whether new side-chains could be tolerated, so that in future carrier molecules for antibody studies could be attached. Nmr data showed that none of the changes made a significant difference to the solution conformation but all had lower antimicrobial activity. Analogue (43) appeared to bind more strongly to DNA, and had 100 times the antitumour activity but only half the antimicrobial activity. The incorporation[71] of α,α-disubstituted amino acid residues using 3-amino-2H-azirines for direct amide cyclisation, has been extended to cyclic depsipeptides.

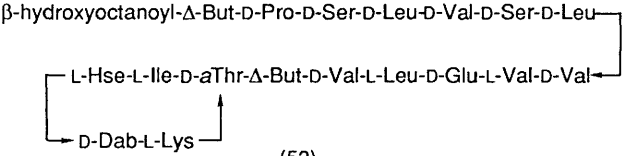
Valinomycin, *cyclo*[-(D-Val-L-Lac-L-Val-D-Hyi)]$_3$ where Lac = lactic acid and Hyi = a-hydroxyisovaleric acid, one of the most researched cyclic ionophores, still commands attention. The crystal structure[72] of the [L-Val^1,L-Val^5] *meso*-valinomycin has now been determined. This analogue with Hyi replacing Lac and a change in chirality at positions 1 and 5, is characterised by a relatively weak complex forming capacity (1/100th that of valinomycin). The structure was found to be asymmetric with four 4→1 and one 6→1 intramolecular H-bonds, but does not have a cavity formed by carbonyl oxygens which is characteristic of the parent molecules. When the 2nd and 4th positions of *meso*-valinomycin were modified to give, [D-Hyi^2,L-Hyi^4]*meso*-valinomycin an X-ray analysis[73], the molecule adopts a distorted bracelet structure, stabilised by six 4→1 type H-bonds. In contrast to *meso*-valinomycin only 4 of the 6 Val carbonyl oxygens are directed inwards to form a coordination centre for the molecule. Carbonyl atoms of D-Val^1 and L-Val^3 point away from the centre, so although the analogue has a partially formed ion-binding centre it is inaccessible because the Hyi residues screen the cavity, and hence explains lack of ion-binding. Molecular dynamics studies[74] have been carried out to scan the possible conformations in the neighbourhood of the bracelet conformation of valinomycin, while FT-IR spectroscopy has been used to study[75] its interactions with ions in organic solvents, detergents and lipids. In $CDCl_3$ and lipid bilayers (K^+ and NH_4^+) complexing occurs, and the ir data confirm a bracelet conformation in these circumstances. In solvents of both medium ($CHCl_3$/DMSO 3:1) and high (pure



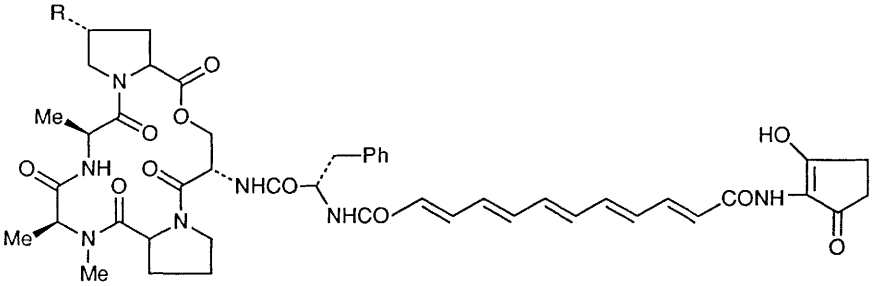
	A	B	C	D
(42)	D-Val	D-Val	Sar	MeVal
(43)	D-Val	D-Val	Sar	MeLeu
(44)	D-Val	D-Val	Meg	MeVal
(45)	D-Thr	D-Thr	Sar	MeVal
(46)	D-Thr	D-Val	Sar	MeVal
	D-Val	D-Thr	Sar	MeVal



(47)
(48) [D-Val^{8}]-(47)
(49) [MeVal^{6}]-(47)
(50) [MeVal^{13}]-(47)
(51) [MeβAla^{4}]-(47)



(52)



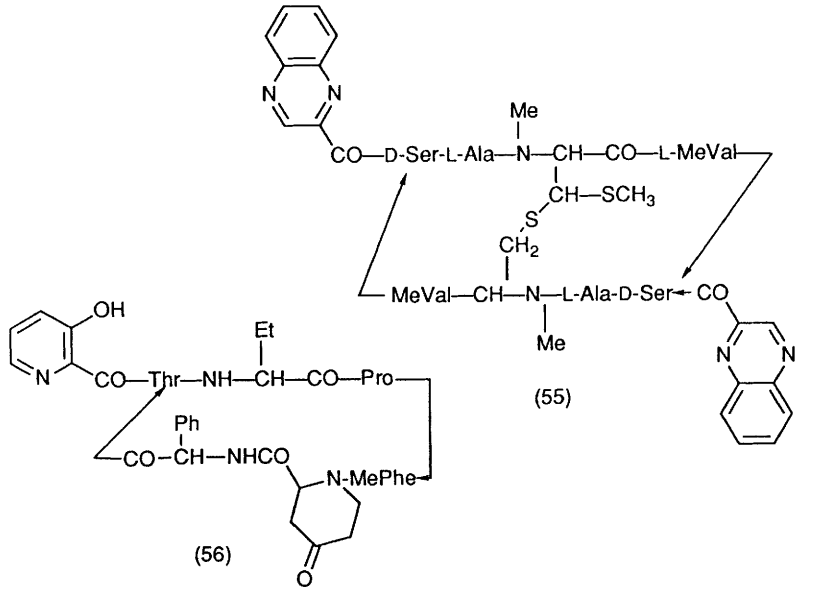
(53) R = Me; (54) R = H

DMSO) polarity there is significant disruption of the internal H-bonding network and no complexation is observed. In detergent micelles, the situation resembles the non-polar $CHCl_3$ conformation but as ^2H_2O penetrated the micelles complexation was reduced due to charge repulsion at the surface of the micelles.

The Okinawan sponge *Theonella swinhoe* has yielded[76] a range of theonellapeptolides of which the main family member exhibits the structure (47). The tridecapeptide lactones (47), (49), (50) and (51) exhibit[77] moderate cytotoxic activity towards L1210 *in vitro* (IC_{50} 1.6, 1.3, 2.4 and 1.4 μg/ml, respectively). Analogue (51) exhibits ion transport activity for both Na^+ and K^+ ions. The principal active component of tolaasin, the *Pseudomonas tolaasii* toxin, which is responsible for brown blotch disease of mushrooms has been shown[78] to be a lipodepsipeptide (52), which can be considered to be related to other antimicrobial toxins such as syringomycin and syringotoxin. However (52) is unique in its high MW and relatively small lactone cycle. Novel depsipeptide antibiotics, enopeptins (A) (53) and (B) (54) have been isolated[79] from the culture broth of *Streptomyces sp* RK-1051. Alkaline methanolysis, together with ^{13}C nmr, nOe, 2D NOESY, and 1D ROESY all contributed to the total structural elucidation. Many different models have already been proposed for echinomycin (55), but the details appear to remain ripe for solving. In a new study[80], nmr data, distance geometry calculations and restraint energy minimisation brought five converged conformations 'into the frame'. The bicyclic depsipeptide adopts a rectangular shape, maintained by the sulfide bridge, the latter pointing away from the quinoxaline rings. The results are consistent with the X-ray 'picture' of triostin and to an earlier modelling study. The picolinic hydroxyl group in virginiamycin S_1 (56) can be protected[81] by the Boc group using di-*tert*-butylcarbonate. Interestingly, once protected mild hydrolysis of the neighbouring lactone bond is prevented suggesting that neighbouring group participation has been blocked. Virginiamycin S has been the subject[82] of a study using multifrequency phase fluorometry, where fluorescence lifetimes could be measured for different protolytic forms. It has been shown[83] that interactions between virginiamycin S (56) and ribosomes is partly provided by a salt bridge between Mg^{2+} ions and the negative form of the picolinic hydroxyl, which is essential for biological activity.

A novel tricyclic depsipeptide structure, microviridin (57) has been identified[84] in a hplc purified fraction from *M.viridis*, while 18 new antifungal antibiotics under the family name aureobasidins (58) have been characterised[85] from the culture medium of *Aureobasidium pullulans No R 106*.

If it wasn't for its importance as an immunosuppressive reagent,

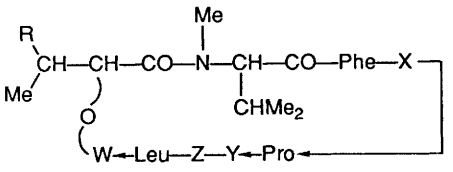


(55)

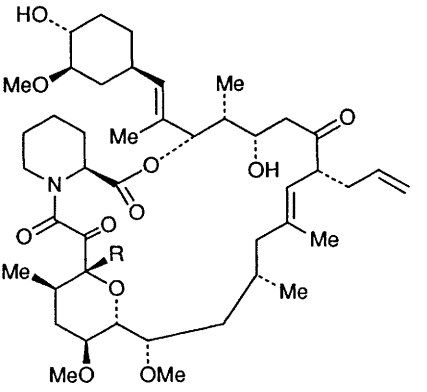
(56)

Ac—Tyr-Gly-Thr-Phe-Lys-Tyr-Pro-Ser-Asp-Trp-Glu-Glu-Tyr—OH

(57)



(58) R = Et, Me; X = MePhe, β-HOMePhe;
Y = aIle, Leu, Val; Z = Val, MeVal;
W = Val, MeVal, β-HOMeVal



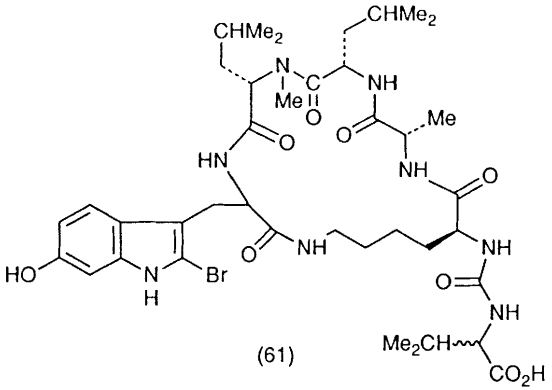
(59) R = OH
(60) R = NHAc

FK-506 (59) would find it difficult to qualify for this section as it only has one amide bond! However, it overlaps with work in cyclosporin A and its possible interaction with *cis-trans* isomerases is of great interest. The immunophilins (e.g., FKBP[86]), receptors for FK-506 and the related rapamycin have been identified as the rotamerases, so the nature of the binding between FK506 and FKBP would be helpful in understanding the mechanisms. The involvement of the pipecolinyl groups has been confirmed[87] by detailed nmr studies of the complex between FK506 and a deuterated FKBP receptor and further refinement of the *cis-trans* conformation around the pipecolinyl residue has been made[88] by utilising the configurational assignments [pro(*R*) or pro(*S*)] of the methylene protons at C-16, 18 and 23. N-Acyl analogues such as (60) of FK506 have been prepared[89] to study the receptor binding effect of the modification. However, the strength of binding was reduced possibly due to insufficient space.

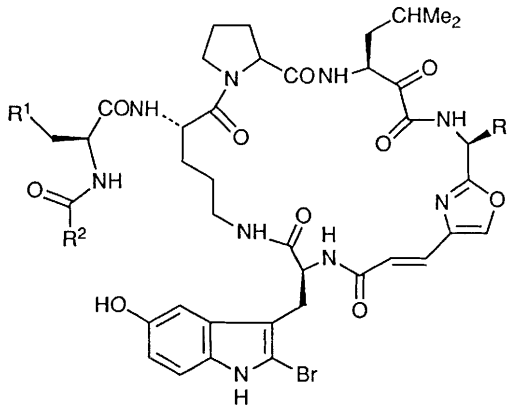
2.13 *Cyclic Peptides containing Other Non-Protein Ring Components*

The Okinawan marine sponge of the *Theonella* species has produced a novel calmodulin antagonist, identified[90] as konbamide (61) which contains 2-bromo-5-hydroxytryptophan as a novel feature. Five other related antagonists have also been isolated[91] from the same source, which includes keramamide A a molecule that contains N-methyl-6-chloro-5-hydroxytryptophan as one of its components. From the same marine sponge have come[92] keramamides B-D (62). Chiral analysis using trifluoroacetyl/methyl ester derivatives confirmed all amino acid units had the L-configuration. A slightly different structure, orbiculamide A (63), has been allocated[93] to a cytotoxic peptide isolated from the *Theonella* source. This is cytotoxic againt P388 murine leukemia cells with an IC_{50} of 4.7 μg/mL.

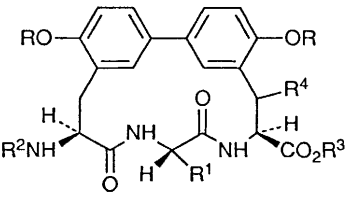
The highly potent antibiotics against β-lactam resistant bacteria, the biphenomycins, have been the subject of two independent syntheses. Biphenomycin B (64) was synthesised[94] using the route in Scheme 6. Cyclisation using the pentafluorophenyl ester gave a yield of 85%. Synthesis[95] of the analogue (65) was achieved *via* a double Heck coupling of 3,3′-diiodo-4,4′-dimethoxy phenyl with two orthogonally protected 2-amidoacrylates followed by the insertion of protected L-Lys to make the last two peptide bonds. Two major and two minor components, named LL-AF283, isolated from *Streptomyces filipinensis*, have been shown[96] to be members of the biphenomycin family. One of the two major components (66) is identical with biphenomycin A. To ascertain what minimal structural requirement would be required for a carboxylate binding product in the vancomycin series, an attempt has been made[97] to synthe-



(61)



(62) R^2 = MeEtCHCHOHCONH—CH(CHMeEt)—
R = R^1 = Et; R = Et, R^1 = Me; R = R^1 = Me
(63) R^2 = EtMeCH—CH_2—, R = Me

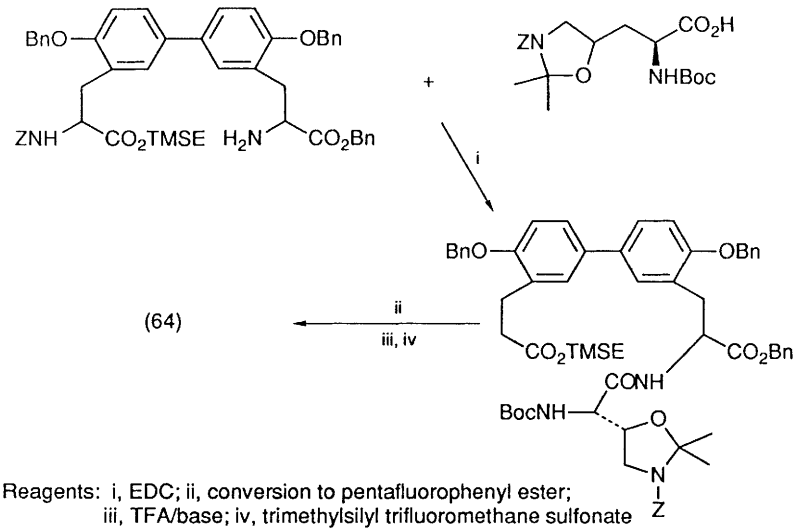


(64) R = R^2 = R^3 = R^4 = H, R^1 = CH_2—CH(OH)CH_2NH_2
(65) R = Me, R^1 = BocNH$(CH_2)_4$—, R^2 = Boc, R^3 = Me, R^4 = H
(66) R = R^2 = R^3 = H, R^4 = OH, R^1 = CH_2—CH(OH)CH_2NH_2 or
= CH_2—CH(OH)—CH_2—NH←Arg

sise (67). The Ullman reaction produced the diaryl ether, but little success was obtained in the lactamisation step. In the hepatotoxin microcystin-LR (68) from *Microcystis aeruginosa* the unique C_{20} amino acid Adda, (2*S*, 3*S*, 8*S*, 9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic) has been implicated in its hepatoxicity properties. A stereo-controlled synthesis[98] of Adda has been completed, and a biosynthetic study[99] has revealed that the C_6 and C_8 of Adda are methionine derived, while the C_2-methyl arises from two sources. The methylaspartyl residue is derived in a manner similar to the biosynthesis of Leu residues. In the echinocandin antibiotic[100] L-671,329 (69) from *Zalerion arboricola*, homotyrosine has been biosynthesised from tyrosine by chain elongation *via* acetate condensation. 2-^{13}C-Acetate labelled all the even numbered carbons in the myristic acid side chain, while methionine was the source of the 10,12-dimethyl residues in the same side chain. Only one proline (the 4 HOPro) was derived from Pro; the other (the 3HO-4MePro) seems to be biosynthesised from L-Leu. Echinocandin (69) has also been obtained[101] from the fermentation broths of *Cryptosporiopsis* sp and *Pezicula* sp.

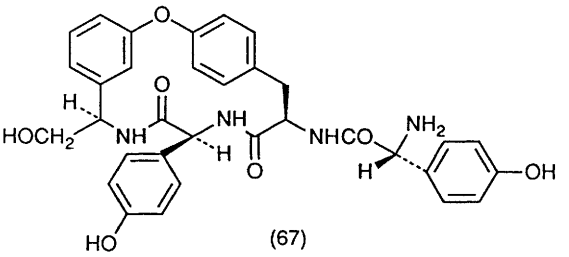
An authoritative review[102] of the lantibiotics has been written. The structural gene, *roc* A, has been identified[103] on isolated chromosomal DNA of the Ro 09-0198 (cinnamycin) - producing strain *Streptoverticillium griseoverticillatum* and has revealed that the first duramycin-type prelantibiotic has an unusually long leader sequence of 59 amino acids. Plasma desorption mass spectrometry (PDMS) and liquid secondary ionisation mass spec (LSIMS) gave the same fragmentation pattern[104] for the lantibiotic nisin, with no reduction of the lanthionine or β-methyl-lanthionine bridges. Gallidermin (70) a potential therapeutic agent against acne has been studied[105] by ^{1}H nmr at 500 MHz in trifluoro-ethanol:water (95:5) using a combination of double quantum filtered correlated spectroscopy, homonuclear Hartman-Hahn, and nOe experiments. A screwlike structure was obtained which is in excellent agreement with the amphiphilic and channel-forming properties of gallidermin on membranes and the susceptibility of an exposed site between residue 13 and 14 to tryptic cleavage.

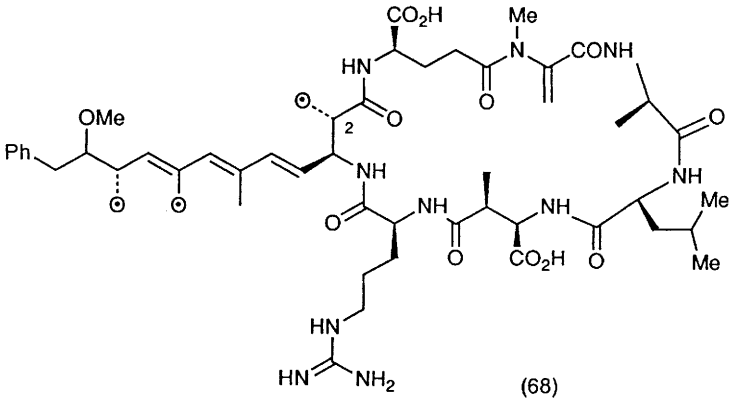
A number of details on the synthesis of the teleocidine, a family of indolactam alkaloids, known for their tumour promoting activity and activation of protein kinase K, have appeared. The initial studies[106] on precursor molecules reaped useful material for subsequent synthesis of teleocidin (A-1) (lyngbyatoxin A) (71)[107], teleocidin A-2 (72)[107,108]. Teleocidin (B-3) (73) and (B-4) (74) have also been synthesised[109], together with a tetramethyl analogue (75)[110]. Microbial conversion, involving attaching the N-methyl group to the indole benzene nucleus has also led[111] to ten indolactam congeners.

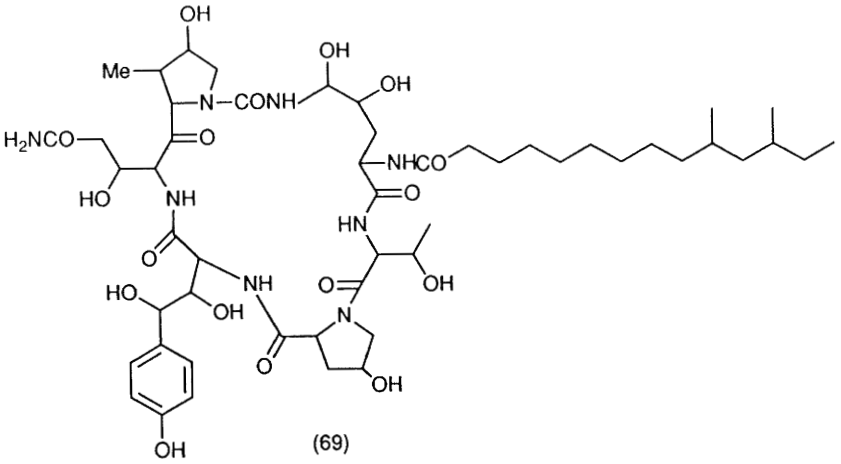


Reagents: i, EDC; ii, conversion to pentafluorophenyl ester;
iii, TFA/base; iv, trimethylsilyl trifluoromethane sulfonate

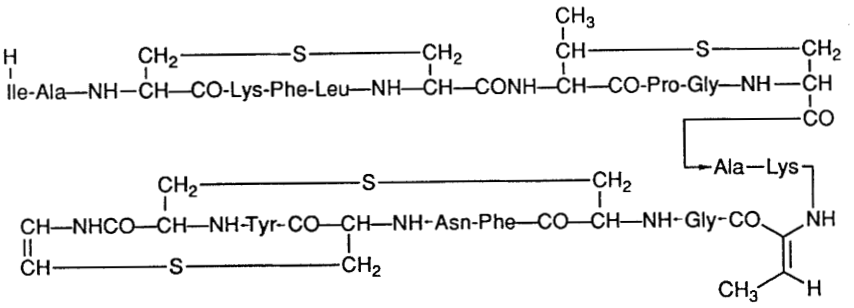
Scheme 6







(69)



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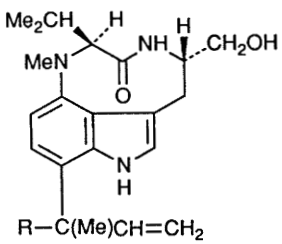
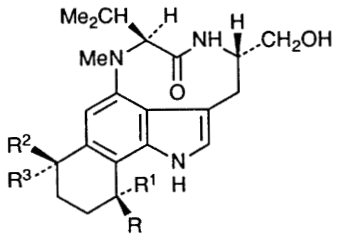


(71) R = (*R*)-$Me_2C{=}CHCH_2CH_2$—
(72) R = (*S*)-$Me_2C{=}CHCH_2CH_2$—



(73) R = R^3 = Me, R^1 = CH=CH_2, R^2 = $CHMe_2$
(74) R = CH=CH_2, R^1 = R^2 = Me, R^3 = $CHMe_2$
(75) R = R^1 = R^2 = R^3 = Me

3. Modified Linear Peptides

In line with a desire to reduce overlap between Chapters 3 and 4, discussion of enzyme inhibitors, dehydropeptides, α,α-dialkyl residues, and amide bond surrogates are now the preserve of Chapter 3.

3.1 *Phosphonopeptides*

A need to prepare phosphorylated peptides for the synthesis of casein-related peptides has provided a very thorough survey of a variety of phosphate protecting groups and reagents. The R groups in Boc-Ser(PO_3R_2)OH and Z-Ser(PO_3R_2)OH have been assessed[112] over the range, Ph, Me, Et, Bzl and Bu^t with the latter the best in conditions of mild hydrolysis. Other derivatives explored included Ppoc-Ser(PO_3Bzl_2)OH[113] its 4-bromobenzyl phosphorylated analogue[114], and the diphenyl phosphate protecting group[115]. The latter protecting group also found favour[116] in the synthesis of multiple phosphoserine containing peptides Ac-[Ser(PO_3H_2)]$_n$OMe where n = 1-3. The potential of the various phosphate protecting groups in solid phase synthesis was also investigated, e.g., the use of global phosphorylation on a resin[117] using either dibenzyl or di-t-butyl-N,N-diethyl phosphoramidite/H-tetrazole followed by *m*-chloroperoxy benzoic acid to oxidise the resultant phosphite triester. The seryl residue has been incorporated on the resin using either Fmoc-Ser(Bu^t)OH followed by Z-Glu(OBzl)OH, with 90% TFA used to cleave the Bu^t group. The potential of Boc-Ser(PO_3Et_2)OH, Boc-Ser(PO_3Ph_2)OH and Ppoc-Ser(PO_3Bzl_2) in solid phase work has also been reported[118]. A potentially useful hydrolytically stable mimetic of phosphotyrosine, Fmoc-DL-Phe-4-[(Bu$^t_2$)$POCH_2$-] has been synthesised[119] for utilisation in synthetic sequences.

H-Ala-Tyr(PO_3H)-Ala-Ser(PO_3H)-Ala-OH was obtained[120] in high yield and purity by phosphorylation of unprotected Tyr and Ser residues using di-t-butyl,N,N-diethylphosphoramidite/1H-tetrazole, followed by oxidation with t-butylhydroperoxide. The same phosphorylating agent was used[121] also in the synthesis of H-Ala-Glu-Tyr(PO_3H)-Ser-Ala-OH when it was attached to the resin. Oxidation of phosphite to phosphate ester in this case was made by *m*-chloroperoxybenzoic acid. The global phosphorylation approach for tyrosyl residues in the last two examples has also been compared[122] to a method which involves insertion of a protected O^4-phospho-L-Tyr as a Fmoc-Tyr(PO_3R_2)-OH derivative where R ranged from Me, Et, Bzl to Bu^t. Overall the conclusion is drawn that the global phosphorylation is the simpler approach.

β-Elimination of alkyl phosphates is a possibility in the Fmoc strategy when piperidine is used to remove the Fmoc group, so a survey of

the use of post-assembly phosphorylating technique has been made[123]. Unprotected amino acid hydroxyl groups were phosphitylated using bis(benzyloxy)(diisopropylamino)phosphine and then oxidised with t-butylhydroperoxide, with final deprotection and cleavage from an acid labile linker being processed by trifluoroacetic acid. The tau protein fragment, H-Lys-Ser-Ser-Pro-Gly-Ser(PO_3H)-Pro-Gly-Thr-OH has been synthesised[124] using the Boc/Bzl protecting strategy in the solid phase, but using 2-methylphenyl and 2,6-dimethylphenyl groups to protect the phosphate moiety on serine. These were more stable to HF and could be removed by hydrogenolysis.

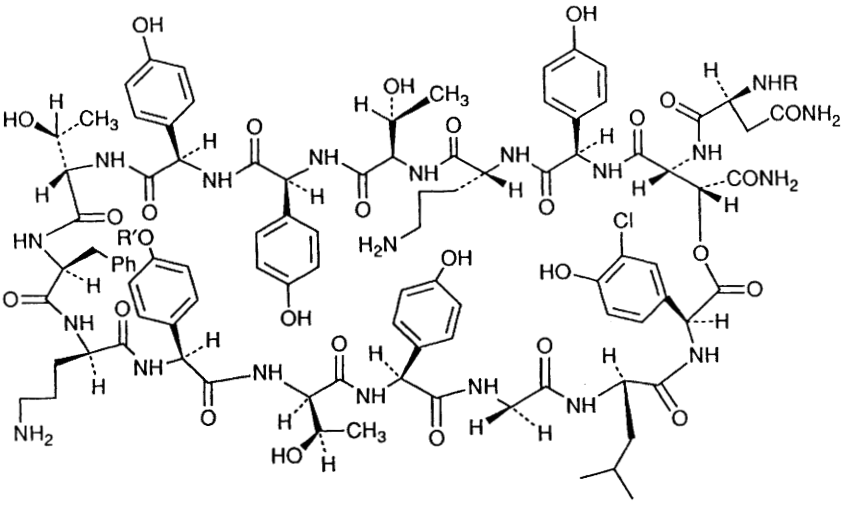
In a comparison[125] of the RGD-adhesion characteristics of the osteopontin active domain, H-Gly-Arg-Gly-Asp-Ser-Leu-OH, to its analogue having a phosphate group on serine, it was shown that the phosphorylated form gave much lower cell binding characteristics. The phosphate group had been incorporated as a diphenylphosphonoserine.

4 Conjugate Peptides

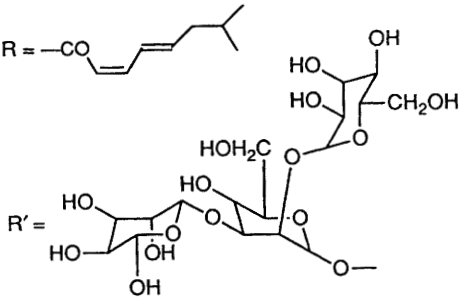
4.1 *Glycopeptide Antibiotics*

Nature appears to be a prolific provider of new members for the vancomycin family of highly modified glycopeptides. Research on vancomycin itself continued by studies[126] on a semiquantitative estimation of binding constants for the antibiotic and the peptido-cell wall analogue Ac-D-Ala-D-Ala. Although uncertainties in treatment are acknowledged, an intrinsic binding energy of ≈ 24 kJ mol^{-1} has been assessed for amide-amide hydrogen bond formation in aq. solution. A report on the synthesis of some of the building blocks of vancomycin has also appeared[127].

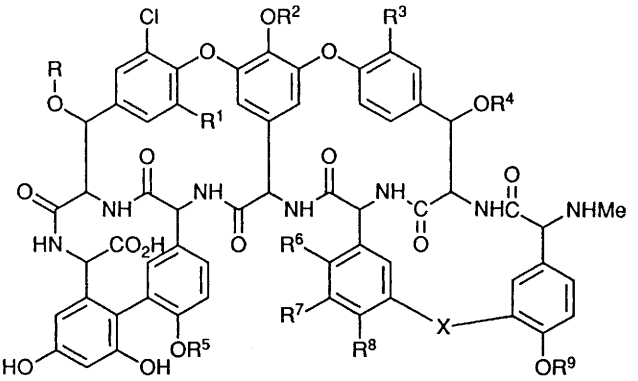
Contemporary technology has been instrumental[128] in providing a full structure for ramoplanose (UK 71,903) (76) which is related to ramoplanin A2. The structure has a branched chain trisaccharide and a *cis-trans* N-terminal dienic fatty acid. Distance geometry and restrained molecular dynamics calculations on the peptide backbone revealed families of conformations with two anti-parallel β-strands connected by seven intramolecular H-bonds and two reverse turns. A 'β-bulge' induced a curvature in the β-sheet structure. New members to the family have been discovered[129] in *Pseudonocardia compacta* subsp *helvetica*. Helvecardins A (77) and B (78) contain the same pseudoaglycone as β-avoparcin, so the nmr and mass spectral details confirmed the helvecardins A and B as β-avoparcin, 1′-*O*-methylated on rhamnose and demannosylhelvecardin A, respectively. The novel glycopeptide UK-69542 from *Saccharothrix aerocolonigenes* has been assigned[130] the structure (79) which is very

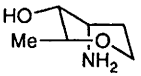
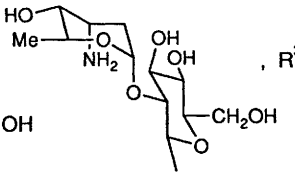


(76) R = —CO



R′ =



(77) R =  , R^1 = H, R^2 =  , R^3 = H

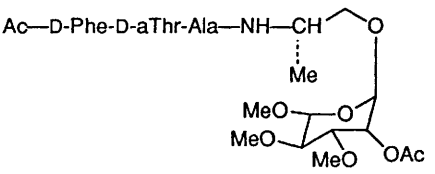
R^4 = mannose, R^5 = H, R^7 = Cl, R^8 = OH

R^9 = 2′-*O*-methylrhamnose

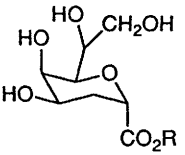
(78) As (77) but R^4 = H and the bridge X is not present

(79) R =  , R^1 = Cl, R^2 = H, R^3 = Cl, R^4 = H, R^5 = SO_3H, R^6 = Cl, R^7 = HO,

R^8 = H, X = O, R^9 = H

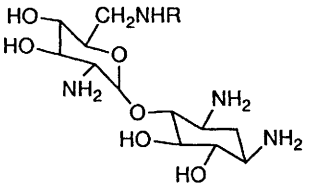


(80)



(81) R = CH_2O—H—Ala-Glu—OH

(82) R = CH_2CO—Ala-D-Glu—NH_2



(83) R = H—Phe, H—Ala, H—Lys

similar to aridicin. ^{1}H Nmr revealed that the antibiotic exists in two conformations in d_6DMSO solution, with a *cis* to *trans* amide isomerisation being implicated. That this happens in (79) and not for aridicin can be due to the charge dipole interaction involving the aryl sulfate ester. Complete ^{1}H and ^{13}C nmr assignments have been reported[131] for eremomycin and confirm the similarities with vancomycin. Dimerisation of eremomycin was observed both in d_6DMSO/CCl_4 and D_2O solutions, and complexation with Ac-D-Ala-D-Ala was also demonstrated.

4.2 *Other Glycopeptides*

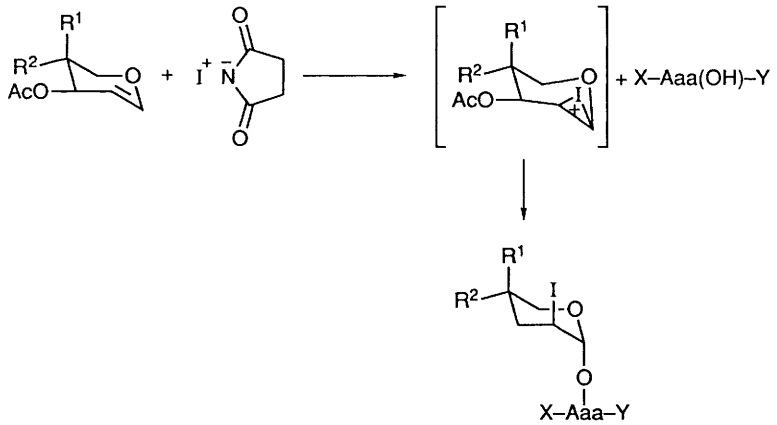
This frontier between two major series of natural products is currently being very actively researched, especially in synthetic methodology. In the synthesis[132] of the basic glycopeptide structure of C-mycosides (80), the first residue (alaninol) was linked using a bromorhamnopyranoside, followed by coupling with Ac-D-Phe-D-aThr-OH using DCC/HOBt. Using suitably protected peptides it has been possible[133] to use the cesium salt of 2,3-dideoxy-β-D-manno-2-octulosonic acid without protection of the hydroxyls to give conjugates (81) and (82). Both of these conjugates were cleaved by bacterial enzymes. In contrast to this all the functional groups in neamine (83) had to be blocked before the amino-acids were introduced[134] *via* the mixed anhydride or active ester methodology. Glycosidation of amino acid and peptide derivatives of Ser, Thr and HOPro takes place *via* an interesting iodinium intermediate using N-iodosuccinimide as summarised[135] in Scheme 7. Raney nickel is required for de-iodination of the final product but the yield is high and with excellent anomeric purity. The poor reactivity of Ser/Thr hydroxyl groups can be improved[136] significantly by using Schiff bases and then reacting the 'activated' hydroxyls depicted in (84) with electrophiles (E^+) such as glycosyl bromides using AgOTfl/CH_2Cl_2 a variant of the Koenigs-Knorr reaction.

Glycosylation of resin-bound serine peptides with the serine OH left free in an Fmoc-based protocol, can be carried out[137] using oxazoline derivatives such as (85) obtained by reacting 2-acetamide-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose with trimethyl silyl trifluoromethanesulfonate. Crude 1-glycosylamines prepared from satd. aq. NH_4HCO_3 and reducing sugars have been coupled[138] successfully to Fmoc-Asp(OPFp)-OBu^t in DMF/water. These Fmoc-L-Asn-N-β-glycosides were then used in solid phase protocols. A speculation[139] that N-Fmoc-derivaties of Ser, Thr, Cys should be glycosylated by sugar 1,2-*trans* acetates has been proved possible in the formation of (86) from components summarised in Scheme 8. A derivative of T-cell stimulating peptide, α-D-Galp(1→4)β-D-Galp-1-S$(CH_2)_2$CO-Asp^{52}-Tyr-Gly-Ile-Leu-

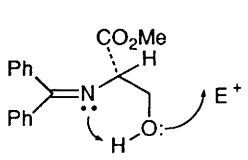
Gln-Ile-Asn-Ser-Arg[16]-NH_2 was synthesied to illustrate the application of the methodology.

To synthesise[140] H-Val-(GalNAc-α)Thr-His-Pro-Gly-Tyr-OH the original choice was to use a peracetylated *O*-galactosaminyl threonine derivative, Z-Val-[GalNAc(Ac_3)-α]Thr-OH and couple it with H-His-Pro-Gly-Tyr(Bzl)-OBzl using the BOP/HOBt coupling agent. However a glycopeptide resulting from racemisation of the Thr residue to D-allo-Thr was isolated in 20% yield. This racemisation was prevented by stepwise elongation using Fmoc[GalNAc($Ac)_3$-α]Thr-OH with the C-terminal tetrapeptide, followed by Z-Val-OH. The glyco-hexapeptide appears to be a competitive inhibitor of the binding of the FDC-6 monoclonal antibody to the oncofetal fibronectin. The tetrapeptide morphiceptin (H-Tyr-Pro-Phe-Pro-NH_2) has high opioid activity, so the effect of replacing the Pro residue with glucopyranosyl- or galactopyranosyl-hydroxyprolyl has been monitored[141]. However the new derivatives showed a distinct decrease in biological activity with respect to morphiceptin. Glycoconjugates based on the enkephalins (87) and (88) have been prepared[142] using published methodology and show significantly higher anti-viral activity than the parent enkephalin. Glycosylated and phosphorylated versions of 4-Val-Val-Glu-Asp-Glu-Gly-Cys-Thr-Asn-Leu-Ser-Gly-Phe-NH_2(VF13) and H-Glu-Lys-Ala-Tyr-Thr-Ile-Phe-Asn-Lys-Thr-Leu-Met-NH_2(GM12) have been prepared[143] and compared to the native peptides by FTIR and cd. Incorporation of one sugar unit into either peptide resulted in a high probability of a type I (III) β-turn. In contrast, phosphorylation of VF13 caused distorted conformation of the peptide backbone. The results could be beneficial in determining peptide antigen structure and function. Isomers of the *N*-triglycosyl dipeptide (89) have been prepared[144] as models for a derivative present in the glomerular basement membrane. Key step in the synthesis was condensation of the dipeptide with a protected triglycosylamine in the presence of *O,O*-diethylcyanophosphonate followed by deprotection. *Cis*- and *trans*-Proline rotamers could be separated on silica gel and reversed phase hplc during deprotection. The linkage in the fragment (90) of the variant specific surface glycoprotein membrane anchor from *Trypanosoma brucei*, has been made[145] by coupling of Z-Lys(Z)-Asp(OBn)-$NHCH_2CH_2OH$ to the dissaccharide unit which already had a -$P(OBu)N(CHMe_2)_2$ group attached. In situ oxidation was carried out with peracetic acid. A novel method[146] for specific glycosylation of cysteine residues has been tested on glutathione which could be glycosylated successfully at Cys using (91).

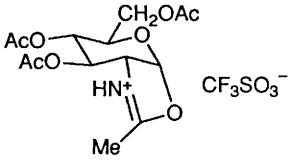
Synthesis and biological activities of acylated derivatives (the acyl group ranging fron n = 0,6,8,12,14 and 16 in $Me(CH_2)_nCO$) of some peptidoglycan monomers have been reported[147], and lipophilic diesters



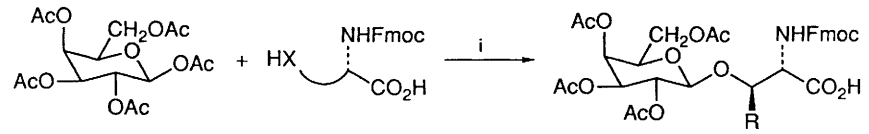
Scheme 7



(84)



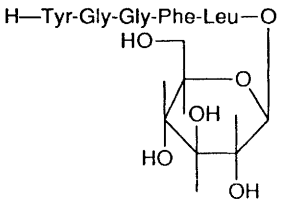
(85)



Reagents: i, $BF_3 \cdot Et_2O/SnCl_2$

(86)

Scheme 8



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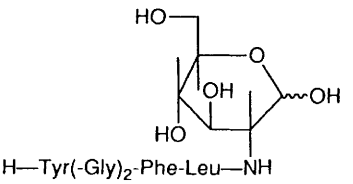
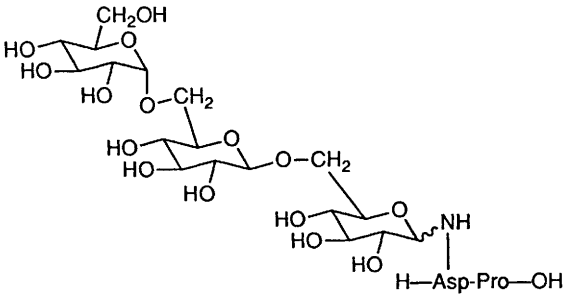
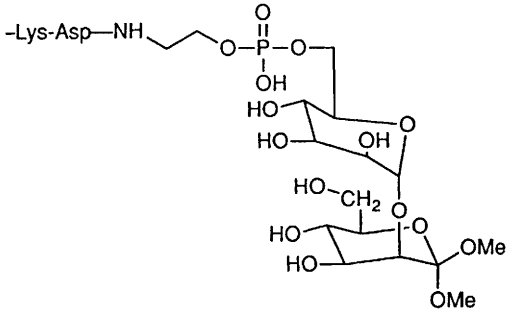


(88)



(89)



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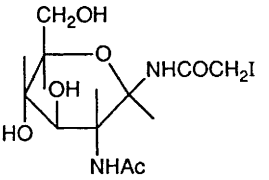
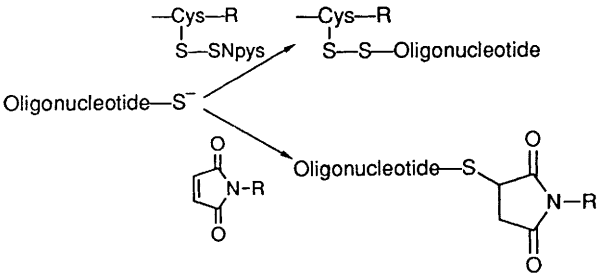
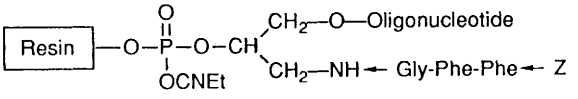


(91)



R = -Ala-Ala-Pro-Lys-Lys-Lys-Arg-Lys-Val—$CONH_2$

Scheme 9



(92)

(e.g., n-heptadecyl, n -dodecyl and n-octyl) of *N*-acetylmuramoyl-L-Ala-D-GluOH have been prepared[148] *via* esterification of the glutamyl residue prior to coupling. Glycopeptides of glycyrrhizic acid[149] have also been made by coupling a variety of amino acid methyl esters *via* the tris(N-hydroxysuccinimido)glycoside esters.

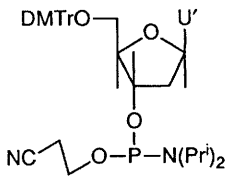
Fast Atom Bombardment (FAB) Mass Spectrometry has been applied[150] to *O*-linked *N*-acetylglucosamine-bearing glycopeptides for the first time. Technique involved propionylating the peptides/glycopeptides using a mixture of trifluoroacetic anhydride/propionic acid prior to FAB analysis. Results showed that the majority of the peptides (from Tn glycophorin) were substituted with a single GlcNAc residue. FAB/MS and gas-phase sequencing strategies show promise for the identification of sites of glycosylation in peptides.

4.3 *Oligonucleotide Peptide Conjugates*

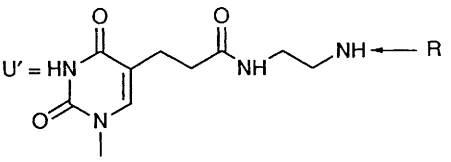
Oligonucleotide-peptide hybrid molecules containing a nuclear transport signal have been synthesised[151] either by direct linkage to a cysteine residue or *via* a succinimide link as summarised in Scheme 9. A branched modified linker as in (92) has been used[152] to link the carboxy end of an oligonucleotide. In a study[153] aimed at assessing the altered transport and reactivity which may be provided by Lys or His residues being attached to oligonucleotides, a phosphoramidite derivative (93) has been used. The Lys (or His) residues were added to the nucleoside basic side chain either using the Fmoc-strategy or trifluoroacetylation. The attached derivatives proved to be compatible with the oligonucleotide solid phase synthetic strategies.

4.4 *Lipopeptides and related Conjugates*

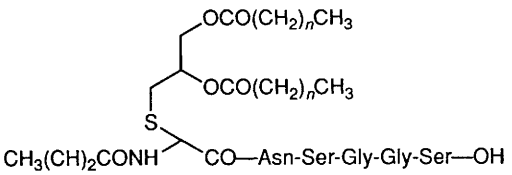
The role of peptide leukotrienes as highly potent and selective antagonists of pharmaceutical interest has been the subject of a perspective review[154]. An immunoactive peptide NS1279 isolated from *Streptomyces* sp has been identified[155] as the *S*-[2,3-bis(palmitoyloxy)propyl]-N^{α}-palmitoyl derivative (94) from chemical/physical data and a total synthesis. The same N-terminal lipo-cysteine derivative has been used to synthesise[156] peptide segments such as N^{α}-palmitoyl-S-[2,3-bis(palmitoyloxy-(2*RS*)-propyl]-(*R*)-Cys-Ser-$(Glu)_4$-OH. This peptide and its analogue $(Pam)_3$Cys-Ser-$(Lys)_4$-OH proved to be potent macrophage and B-cell activators, and non-toxic, non-pyrogenic immune adjuvants in combination with, or linked to, antigens and haptens. The increasing interest in these conjugates has justified[157] a detailed look at useful derivatives for their solid phase synthesis. Fmoc-*S*-(2,3-dihydroxypropyl)-L-Cys-OH, and Fmoc-*S*-[2,3-bis(palmitoyloxy)propyl]-L-Cys-OH have been pre-



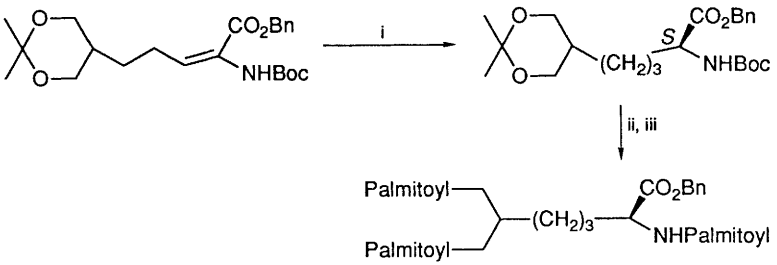
(93)



R = -Lys(Boc)Fmoc or -Lys(Tfa)Tfa.

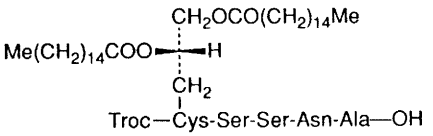


(94) n = 14, palmitoyl.

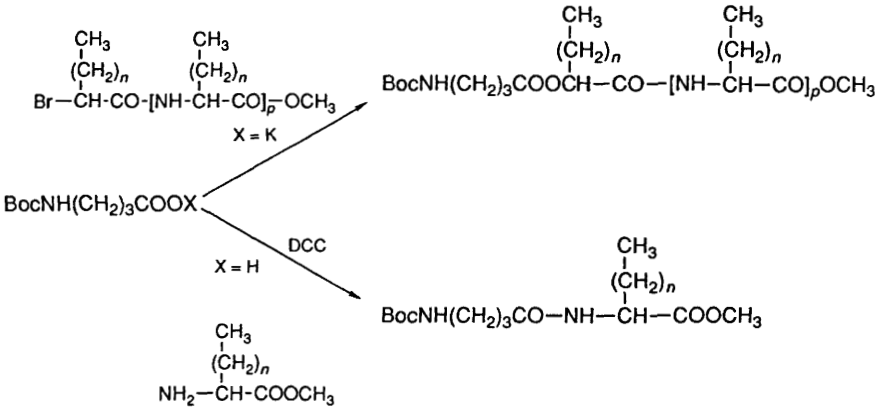


Reagents: i, H_2/(*RR*)-DI-PAMP Rh-catalyst; ii, HCl/H_2O/dioxan;
iii, palmitoyl chloride/pyridine/CH_2Cl_2

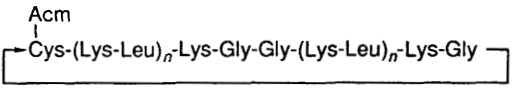
Scheme 10



(95) Troc = –COOCH$_2$CCl$_3$

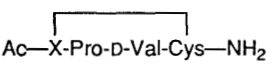


Scheme 11

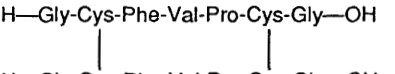


n = 1 or 2 Acm = acetamidomethyl

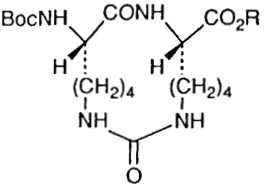
(96)



(97) X = L-Cys
(98) X = penicillamine



(99)



(100) R = Me, CMe_3

pared in high diastereomeric purity for this purpose. Tripalmitoyldihydroxy α-amino acids have been synthesised[158] from didehydroprecursors using chiral rhodium catalysts as summarised in Scheme 10.

Optically active lipopeptide analogues using chiral glycerol derivatives have been synthesised[159] and tested. In the series investigated, e.g., (95), and smaller fragments, derivatives from (*R*)-glycerol showed higher mitogenic activities than the (*S*)-series, while the Troc derivatives had increased mitogenic activity. A series of γ-aminobutyric acid (GABA) ester conjugates have been synthesised[160], *via* the routes summarised in Scheme 11. The conjugate size varied from 1 to 3 units while the alkyl side chains ranged from 5 to 17 C atoms.

5 Miscellaneous Examples

Annually, there are a few papers that do not exactly fit into the sub-paragraphs adopted for the Chapter. Yet they contribute extra information to the subject matter over all.

A range of cyclopeptides (96) with alternating hydrophobic and basic hydrophilic residues have been synthesised[161] using the BOP reagent for the cyclisation step at a C-terminal glycine. Cyclopeptides (96) mimic two adjacent strands of the β-sheet, and markedly increase the rate of hydrolysis of oligoribonucleotides. Nmr and cd studies[162] have been undertaken on the cyclic disulfides (97) and (98) to determine any differences in conformation due to the different side-chain substitution. Both peptides formed a type II β-turn, but the dihedral angle of the disulfide bridge proved to be different although the chirality is the same. Semiselective TOCSY/NOESY experiments using a pulse that excites the complete amide region, has been used[163] to clarify ambiguity raised by nOe data in symmetrical dimer peptides such as (99). Macrocyclic dilysine derivatives (100) have been formed[164] from protected dilysyl precursors using 1,1′-carbonyldiimidazole under mild conditions.

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5
β-Lactam Antibiotic Chemistry

BY C.H. FRYDRYCH

1 Introduction

Interest in β-lactam antibiotics and in the parent azetidinone ring remains high. Last year saw a significant increase in the number of publications relevant to this review. Many of the extra publications relate to structure-activity relationships in cephalosporins and to the synthesis of numerous azetidinones for biological evaluation. Many of these publications are listed in the Appendix.

The section headings which follow are identical to those used in Volumes 22 and 23.

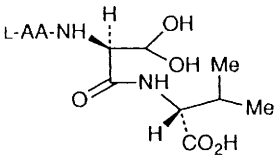
A large number of reviews have been published this year. An article has appeared detailing the discovery of β-lactam and β-lactam-like antibiotics from bacteria.[1] Two general reviews have appeared on β-lactam biosynthesis.[2,3] An article reviews the penicillin antibiotic Piperacillin.[4] The modification of cephalosporins at C-3 has been reviewed.[5] A review covers penem antibiotics[6] and another the non-traditional β-lactams, namely carbapenems, penems and monocyclics.[7] An article on the rhodium (II) catalysed reactions of diazo-carbonyl compounds includes numerous β-lactam examples.[8] Syntheses of 3-aminoazetidinones have been reviewed,[9] a further publication covers the asymmetric synthesis of azetidinones.[10] The stereochemistry of electrophilic substitutions in β-lactams is the subject of a review.[11] An article has appeared covering the preparation of monobactams.[12] Two general reviews cover the design of new β-lactam antibiotics[13] and some aspects of their industrial synthesis.[14] Finally, a review of γ-lactam analogues of β-lactam antibiotics has appeared.[15]

2 New natural products

No reports of new β-lactam containing natural products have been found in the 1991 literature.

3 Biosynthesis

The past year has been one of consolidation in biosynthetic studies. A number of substantial full papers have appeared collating previously



(1)

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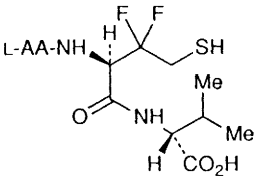

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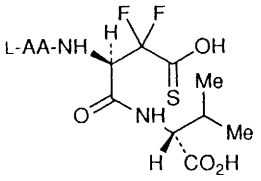

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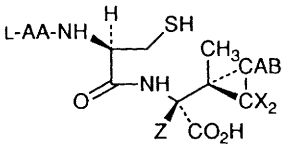
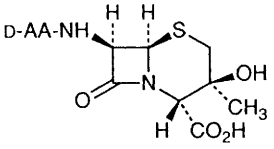
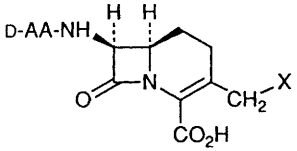


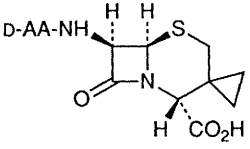
(4) Z, X, A, B = H, D



(5)



(6) X = H
(7) X = OH



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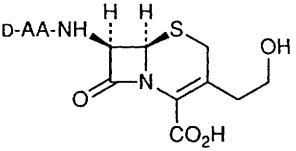

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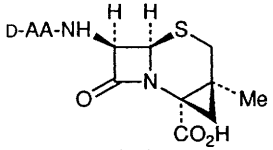

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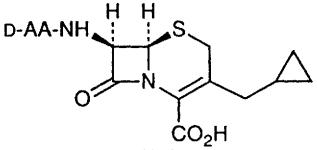

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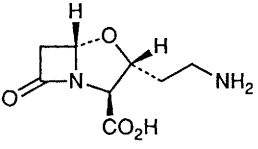

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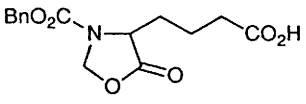

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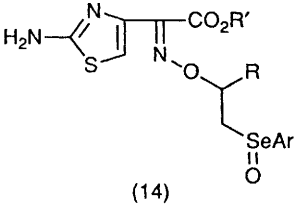


(14)

communicated results and providing full experimental details (*vide infra*). Accompanying mechanistic discussions draw together explanations of the way in which various products arise to give a unified hypothesis on the biosynthesis of penicillins by isopenicillin N synthetase (IPNS).

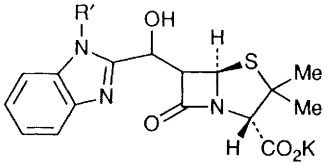
Full details have appeared on studies with δ-L-α-aminoadipoyl-L-[3-^{13}C]cysteinyl-D-[3-^{2}H] valine which resulted in isolation of the shunt metabolite (**1**), supporting the intermediacy of a monocyclic β-lactam.[16] The synthesis and enzyme incubation of tripeptides containing D-propargyl-glycine, D-cyanoalanine and D-[4,4,5,5-$^{2}H_4$] norvaline, each in turn replacing the usual valine, are detailed in a full paper. A total of four new penicillins were isolated (β-ethyl, α-acetylenic and α- and β-nitrile).[17] The preparation[18] and enzyme incubation results[19] of δ-L-α-aminoadipoyl-L-cysteinyl-D-allylglycine and eight deuterated analogues have been reported in full. Incubation of δ-(L-α-aminoadipoyl)-L-(3,3-difluorohomocysteinyl)-D-valine (**2**) with IPNS has resulted in a new mode of reactivity for the latter, in which both equivalents of the dioxygen molecule (usually consumed in penicillin formation) are utilised in the oxidation of a single carbon to form thiocarboxylic acid (**3**).[20] Experiments with tripeptides in which valine is replaced by cyclopropylglycine derivatives (**4**) have been extended to include deuterated analogues. The stereochemical results observed support a stepwise insertion-homolysis mechanism for carbon-sulfur bond formation in second ring-closure in penicillin biosynthesis.[21] A detailed study has appeared concerning the functional role of the two cysteinyl residues (106 and 255) present in IPNS from *Cephalosporium acremonium*. Neither was found to be absolutely essential for bond making or breaking events during catalysis.[22] The enzyme cephalosporin 7α-hydroxylase from *Streptomyces clavuligerus* has been purified and characterised.[23] Full details have appeared on the incubation of penicillin N with deacetoxy/deacetylcephalosporin C synthase (DAOC/ DAC synthase) from *C.acremonium* CO728, including isolation of the cepham metabolite (**5**). A mechanistic interpretation is offered which is consistent with all of the observed results.[24] Incubation of the 3-methylcarbacephalosporin (**6**) with DAOC/DAC synthase gave the 3-hydroxymethyl derivative (**7**) mimicking the conversion of DAOC to DAC by the same enzyme.[25] The 3-spirocyclopropylcephem (**8**) is a substrate for DAOC/ DAC synthase, giving rise to the 3-(2-hydroxyethyl) derivative (**9**); two possible mechanisms for this transformation are offered. The cyclopropyl cephems (**10**) and (**11**) fail to act as substrates for the same enzyme.[26] A number of publications detail the preparation of various penicillins by incubation of the enzymes phenylacetyl coenzyme A (CoA) ligase from *Pseudomonas putida* and acyl-CoA:6-aminopenicillanic acid acyltransferase from *Penicillium chrysogenum* with CoA, ATP,

Mg^{2+}, dithiothreitol, 6-aminopenicillanic acid and the corresponding side-chain precursor.[27,28,29] A very detailed study using a very active and very stable penicillin G acylase-agarose derivative has resulted in optimal conditions for the industrial scale preparation of penicillin G (as a model for other antibiotics) on an industrial scale.[30] Substantial accounts, with full experimental details have appeared from two research groups concerning the biosynthesis of clavulanic acid. Each highlights the isolation of dihydroclavaminic acid (**12**), an intermediate in the conversion of proclavaminic acid to clavaminic acid (see also Volume 23). The results support a mechanism in which cyclisation precedes oxidation.[31,32,33,34]

4 Penicillins and cephalosporins

There have been no reports on the total synthesis of penicillins this year. A synthesis of penicillin N used the protected aminoadipic acid derivative (**13**) in a coupling with the benzyl ester of 6-aminopenicillanic acid (6-APA). Deprotection to penicillin N was achieved in a single hydrogenation reaction.[35] A full account of the introduction of 6α-substituents in penicillins highlights the synthetic advantages of the succinimidoxy leaving group.[36] The preparation of penicillins with O-vinyl oxime side-chains has been achieved by selenoxide elimination from the intermediate (**14**).[37] The 6-(benzimidazolyl)hydroxymethyl penicillanic acids (**15**) have been prepared from 6,6-dibromopenicillanates by aldol condensation and debromination with a tin hydride. The (*6R,8S*) isomers are potent antibacterial agents and β-lactamase inhibitors.[38] A synthesis of 2,2-bis(monosubstituted)methyl penam sulfones (**16**) relies upon functionalisation of the β-methyl group, ring opening to give (**17**), followed by concomitant ring-closure-functionalisation. The first-introduced substituent eventually finds itself in the α-methyl group.[39] A synthesis of 2-exomethylene penam (**18**) involves intramolecular addition of sulfur onto an allene (**20**) derived from the isopropenyl unit of a secopenicillin. Compound (**18**) provides an entry into penems *via* 2-oxopenam (**19**), and to 2β-substituted penams by Michael addition on the sulfoxide of (**18**).[40] The (2,3)-α-methylene penicillanic acid sulfone (**21**) has been prepared, biological evaluation revealed it to have cephalosporin-like activity in accord with a prediction based on the spacial orientation of its β-carboxy group.[41] A cobalt (I) mediated insertion-expansion-β-elimination of iodomethylpenams and iodocephams provides an efficient route to 3-exomethylene cephalosporins (**22**).[42]

Moving on to cephalosporin chemistry, an asymmetric synthesis of 7-amino-7-methylcephems from chiral α-fluorocarbamoylaminoesters uses S-(-)-ethyl lactate as a chiral auxiliary. The route parallels a synthesis



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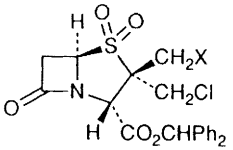

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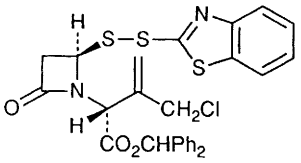

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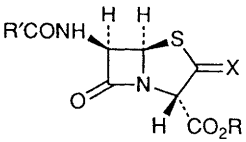
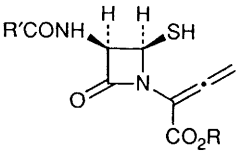


(18) X = CH_2
(19) X = O



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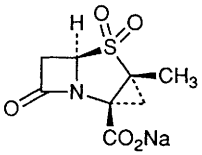

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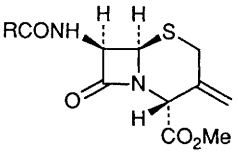

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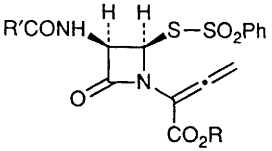

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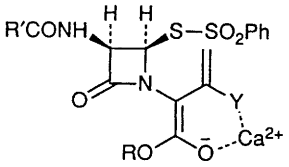

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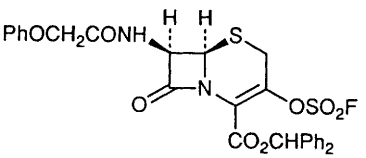

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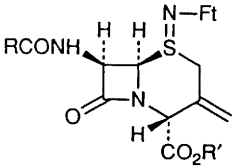
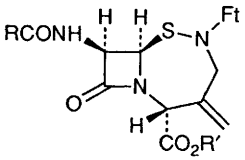


(26) Ft = phthalimido



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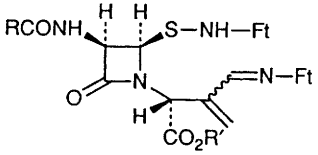
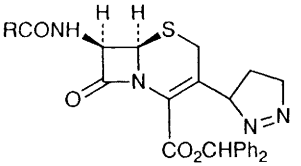


(28)

previously reported for racemic compounds (see Volume 22).[43] A synthesis of 3-norcephalosporins from (**23**) involves addition of nucleophiles to the allene to give (**24**) followed by attack on sulfur by the terminal carbon achieving closure to the six-membered ring. The addition of a metal salt such as calcium chloride is essential to allow ring-closure to compete with unproductive protonation of (**24**).[44] A convenient and high yielding reduction of cephalosporin sulfoxides involves treatment with trimethylsilyl iodide in dichloromethane.[45] Removal of various acid-labile ester protecting groups from cephalosporins has been accomplished by treatment with small quantities of strong acid in phenol.[46] The use of dibromoethyl esters and related carbamates as protecting groups is possible as a result of their extremely mild removal by treatment with cobalt (I) phthalocyanin anion.[47] The fluorosulfonyl group has been developed as an inexpensive replacement for triflate in palladium acetate mediated C-3 coupling reactions of (**25**).[48] The C-3 carbon chain elongation of cephalosporins has been achieved by a bimetal redox-promoted allylation of 3-formylcephems.[49] A [2,3] sigmatropic shift of sulfilimines (**26**) derived from reaction of 3-exomethylene cephams with phthalimidonitrene gives the 1,2,6-thiadiazepine azetidinones (**27**). Addition of a second phthalimidonitrene moiety results in ring-opening to (**28**).[50] A study of the regioselectivity of cycloaddition reactions of 3-vinylcephalosporins with diazomethane has appeared. With an unsubstituted vinyl group the 1-pyrazoline (**29**) is formed; the regioselectivity is reversed when one or more electron withdrawing substituents are present providing (**30**) or (**31**) respectively.[51] The cephalosporin phosphorane (**32**) reacts with α-halogeno ketones to give new tricyclic cephalosporins (**33**), oxidation with m-chloroperbenzoic acid (m-CPBA) results in formation of the α-sulfoxide as the major isomer, rationalised as a result of increased steric bulk on the β-face.[52] 3-(α-chlorovinyl) cephalosporins (**34**) have been prepared from the 3-acetyl derivatives by reaction with triphenylphosphine and carbon tetrachloride.[53] Full details have appeared of the reaction of cephem ester anions with Michael acceptors to give C-4 disubstituted Δ2-cephems such as (**35**). Oxidation to the sulfoxide followed by ester hydrolysis decarboxylation, and sulfoxide reduction gives novel C-4 substituted Δ3-cephems (**36**).[54] Reaction of the *p*-nitrobenzyl ester of 4-hydroxymethyl cephem (**37**) with bromine in methanol gave a mixture of isomers of adduct (**38**) together with both isomers of the spiro orthoester (**39**).[55] A study of substituent effects in the reactions of 2-methylenecephems (**40**) with diazoalkanes, revealed that groups at C-3, C-4 and C-7 exert little influence on the reaction. Sulfoxides react more quickly than the corresponding sulfides and substituted diazoalkanes react more slowly than diazomethane.[56,57] A one-pot conversion of 7-



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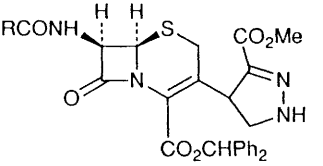

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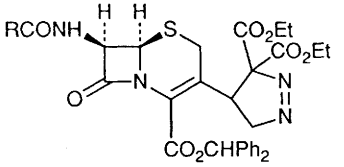

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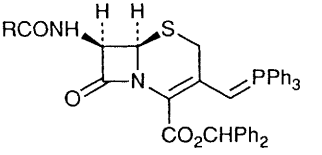

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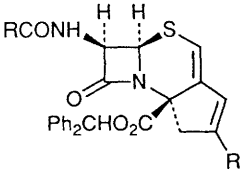

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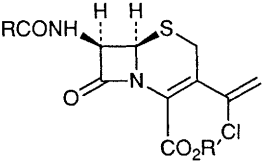
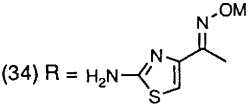
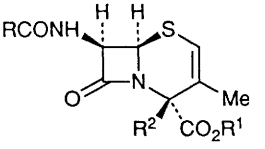
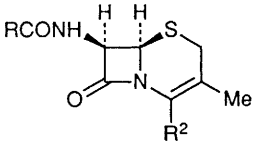


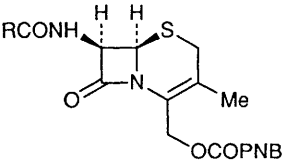




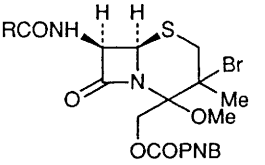
(35) R^2 = CH_2CH_2CN, CH_2CH_2COMe, $CH_2CH_2CO_2Me$, $C(CO_2Me)=CHCO_2Me$



(36) R^2 = CH_2CH_2CN, CH_2CH_2COMe, $CH_2CH_2CO_2Me$, $C(CO_2Me)=CHCO_2Me$



(37) PNB = *p*-nitrobenzyl



(38)

aminocephalosporanic acid (7-ACA) to 7α-formamido ACA *via* quinone methide (**41**) uses 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) for the crucial oxidation. The method is amenable to large-scale reactions.[58] Direct conversion of 7β-amino-3-deacetoxycephalosporanic acid (**42**) to the corresponding 7-methoxy derivatives (**43**) was achieved by reaction with poorly nucleophilic acids in methanol. A 6:1 mixture of α- and β-isomers was obtained.[59] Alkylation of 6α-methoxy cephem acid chlorides at sulfide or sulfone level with Grignard reagents, stannanes and cuprates gave C-4 ketones. Further functionalisation gave the 2- and 3-substituted derivatives (**44**) and (**45**) which are potent inhibitors of human leukocyte elastase.[60]

5 Clavulanic acid and oxapenams

The antifungal β-lactam (**46**) has been prepared from chiral 4-acetoxy-3-phthalimido azetidinone, derived in turn from 6-APA. Displacement at C-4 followed by functional group manipulation including deamination provided (**47**) which subsequently underwent ring closure using potassium carbonate in dimethylformamide.[61]

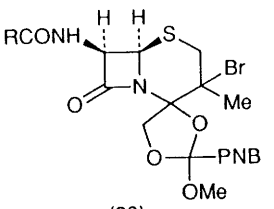
6 Penems

The 6α-bromopenems (**48**) have been synthesised from 6α-bromo-6-(1-hydroxyethyl)penicillanic acid derivatives by ring-opening, S-acylation and ozonolysis to give (**49**) which underwent phosphite mediated reductive condensation to give (**48**). Reductive debromination of the latter gave poor yields of the corresponding 6α-(1-hydroxyethyl)penems. Further examination of the synthetic route indicated that debromination was more efficient at the azetidinone stage.[62] Irradiation of *trans* penem esters (**50**) with pyrex-filtered ultra violet light resulted in isomerisation to mixtures of *cis* and *trans* penems together with variable amounts of the corresponding thiazole (**51**). The isomerisation is proposed as occurring *via* a puckered diradical which prefers to reclose to the *cis* isomer.[63]

7 Carbapenems, carbacephems and related systems

The scope of this section remains as defined in Volume 19, Section 8 should be consulted for the synthesis and chemistry of azetidinone precursors of carbapenems.

The carbacyclic analogue of clavam antifungal (**46**), namely (**52**) has been prepared from the same chiral 4-acetoxy-3-phthalimido azetidinone used in the synthesis of (**46**). Reaction with an allylsilane and oxidative



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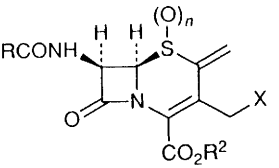
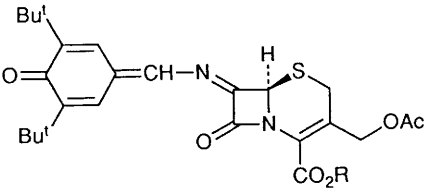


(40) n = 0,1



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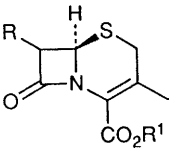
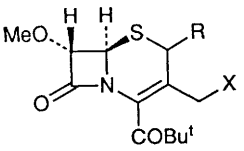
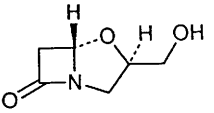


(42) R = β-NH_2
(43) R = α- or β-MeO



(44) R = α-SHet, X = H
(45) R = H, X = SHet
Het = heterocycle



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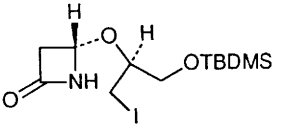

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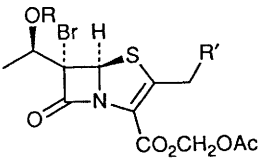

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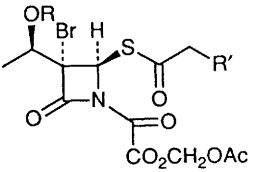

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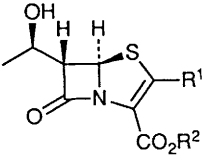

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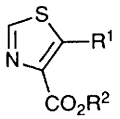

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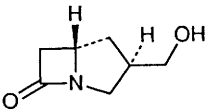

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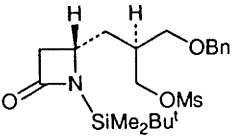

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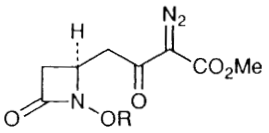
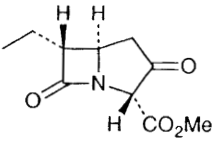


(54) a, R = Bn; b, R = Me
c, R = CH_2OCH_3



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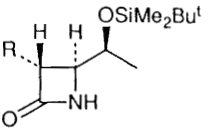
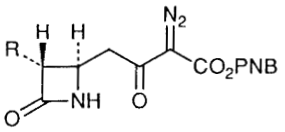
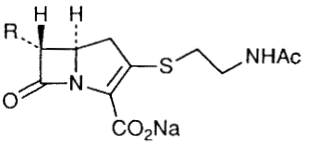


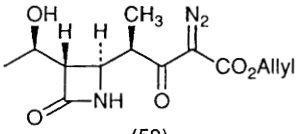
(56) a, R = Et; b, R = Pr^i



(57) a, R = Et; b, R = Pr^i



(58) a, R = Et; b, R = Pr^i



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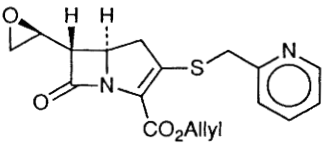

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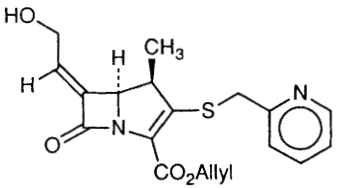

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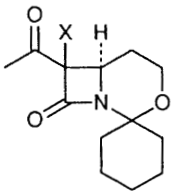
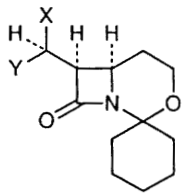
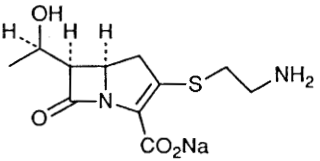


(62) a, X = Cl; b, X = SR



(63) a, X = OH, Y = Me;
b, X = Me, Y = OH



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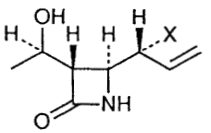


(65) a, X = H; b, X = OH

hydroboration was followed by stepwise deamination to (**53**). Conversion of tosylate to iodide was followed by N-desilylation and ring closure to (**52**).[64] The scope of the previously reported (see Volume 23) direct ring-closure of (**54a**) to a 2-oxocarbapenam has been extended. In addition to the N-benzyloxy derivatives (**54a**) the reaction also works with N-methoxy (**54b**) and N-methoxymethoxy (**54c**) substituents. Incorporation of a 3α-substituent provides a synthesis of (**55**), a known intermediate for the synthesis of the natural carbapenem PS-5.[65] The use of chiral N-trimethylsilyl imines based on (S) -lactaldehyde has been extended to the synthesis of advanced intermediates for carbapenems (+)-PS-5 and (+)-PS-6. Reaction with t-butyl butanoate and isovalerate respectively gives azetidinones (**56a**) and (**56b**) in a fully stereocontrolled reaction. Conversion to 4-acetoxy derivatives and C-4 displacement provided (**57a**) and (**57b**). Rhodium catalysed ring closure and further manipulation provided (+)-PS-5 (**58a**) and (+)-PS-6 (**58b**).[66] A further extension was the use of mandelaldehyde rather than lactaldehyde. Extensive manipulation of the 3-(1-hydroxyethyl) substituent of carbapenem intermediate (**59**) gave the corresponding epoxide derivative. Cyclisation and C-2 functionalisation provided (**60**). Epoxide ring-opening with DBU and a catalytic quantity of zinc chloride gave the asparenomycin analogue (**61**) which was successfully deprotected.[67] Full details have appeared on the use of 7-chloro and 7-thioalkyl (aryl) ketones (**62a**) and (**62b**) for the preparation of olivanic acid (cis carbapenem) intermediates (**63a**) and (**63b**) respectively by ketone reduction and tin hydride removal of the 7-substituent. The intermediate (**63a**) was further utilised in the synthesis of (±)-6-epi thienamycin (**64**).[68] Allylic oxidation of the carbapenem intermediate (**65a**) provided the α-hydroxy derivative (**65b**). Further manipulation gave 1α-acyloxy olivanic acid analogues (**66a,b**).[69] Full details have been published of the synthesis of 6-(substituted methylene) carbapenems such as (**67**) from 6-APA (see also Volume 22).[70]

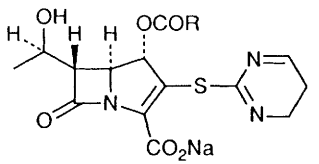
There is only a single publication this year dealing with the chemistry of carbacephalosporins. A convenient one-pot transesterification of intermediate (**68a**) to (**68b**) used a phase transfer catalyst to effect both hydrolysis and re-esterification.[71]

8 Azetidinones

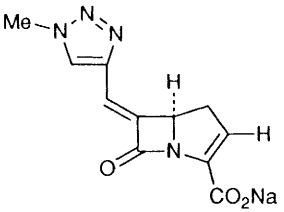
Syntheses are mentioned first, according to the bond(s) created in the ring-forming step.

8.1 *Reactions in which one bond is formed*

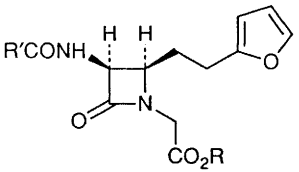
8.1.1 *1-2 bond-forming reactions.*- This section includes 'two-step' [2+2]



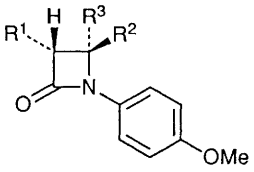
(66) a, R = Me; b, R = Ph



(67)



(68) a, R = Me;
b, R = (4-substituted) benzyl



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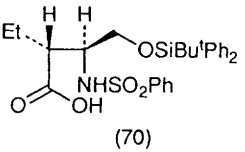

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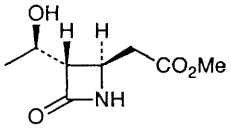

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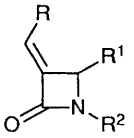

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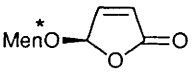
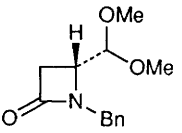


(73) Men = menthyl



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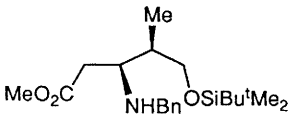

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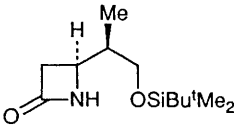

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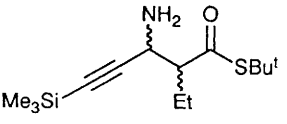

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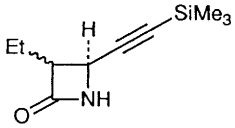

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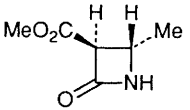

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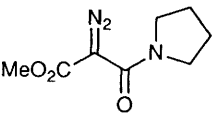
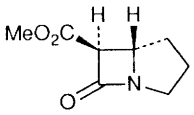


(80)

additions where an intermediate β-amino-acid or ester was actually isolated. The use of various phosphorus reagents for the cyclisation of β-amino acids has been reported. N,N-bis(2-oxo-3-oxazolidinyl)phosphorodiamidic chloride gives good yields of azetidinones with a wide variety of substituents.[72] A second saccharin derivative (see also Volume 23) has been shown to be effective in this reaction.[73] Cyclisation with phenyl phosphorodichloridate in acetonitrile gives N-aryl azetidinones (**69**).[74] The use of triphenylphosphine/hexachloroethane/triethylamine/acetonitrile achieves ring closure to a variety of β-lactams.[75] The intermediate (**70**), prepared from L-glutamic acid, was cyclised with dicyclohexylcarbodiimide (DCC) and 4-pyrrolidinopyridine.[76] The carbapenem intermediate (**71**) has been prepared from the corresponding β-amino acid by reaction with mesyl chloride/ sodium hydrogen carbonate.[77] α-Alkylidene β-lactams (**72**) have been prepared from α-alkylidene-β-amino acids by reaction with mesyl chloride/potassium hydrogen carbonate and a phase transfer catalyst.[78] Amine addition to the 5(R)-menthyloxy-2-[*5H*]-furanone (**73**) followed by ring-opening to a β-amino acid and ring closure with 2-chloro-1-methylpyridinium iodide gives (**74**) having 92% ee.[79] tert-Butylmagnesium chloride induced cyclisation of β-amino esters (**75**) gave the 1β-methylcarbapenem intermediate (**76**).[80] A similar reaction of thioesters (**77**) provided (**78**), intermediates in the synthesis of (±)-PS-5 and (±)-6-epi-PS-5.[81] A further example used lithium hexamethyldisilazide in a cyclisation providing (**79**).[82]

8.1.2 *3,4 bond forming reactions*.- The rhodium diacetate induced insertion reaction of simple amides of diazomalonic acid gives β-lactams. Of particular interest is the reaction of (**80**) to give the carbapenam (**81**).[83] A detailed study of a similar reaction of diazoacetoacetamides such as (**82**) revealed that insertion resulted in mixtures of β- and γ-lactams with a strong preference for four-membered ring formation.[84] Free radical cyclisations of bromides (**83**) upon treatment with tributyltin hydride are favoured by increasing the bulk of the N-substituent; the N-methyl derivative (83; R′ = Me) reacted mainly by simple reductive debromination.[85] A full account has appeared on the photochemical cycloaddition reactions of N-(α,β-unsaturated carbonyl)thioamides (**84**) which provide the unusual bicyclic β-lactams (**85**) by a [2 + 2] process.[86]

8.1.3 *1,4 bond-forming reactions*.- Full details have now appeared on the silicon-induced Pummerer-type rearrangement of β-amido sulfoxides (**86**) to 4-phenylthio azetidinones (**87a**). Oxidation to the sulfinyl derivative (**87b**) allowed displacement by a ketene silyl acetal providing carbapenem intermediate (**87c**).[87] Cyclisation of β-hydroxyamide (**88**) using triphenyl-



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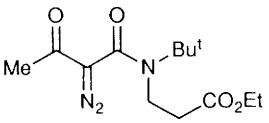

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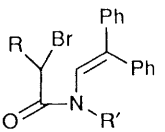

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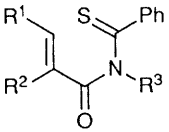

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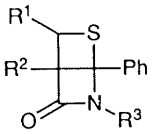

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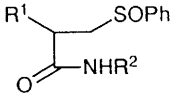

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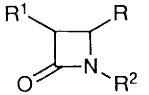
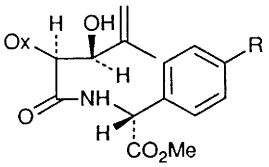


(87) a, R = SPh;
b, R = SOPh;
c, R = CH_2CO_2Me



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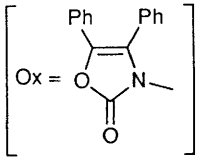
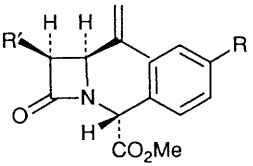
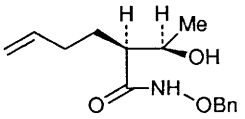




(89) a, R′ = Ox;
b, R′ = PhCONH



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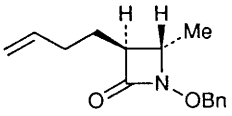

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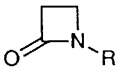

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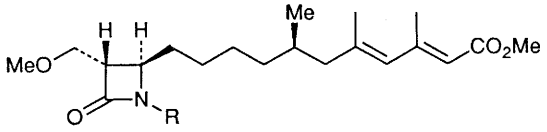

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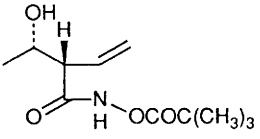


(94)

phosphine and di-tert-butylazodicarboxylate gave azetidinone (**89a**). Ozonolysis achieved cleavage of the isopropylidene and oxazolin-2-one moieties and induced a Chapman rearrangement resulting in isolation of (**89b**).[88] Cyclisation of β-hydroxy hydroxamate (**90**) with triphenylphosphine and diethylazodicarboxylate gave 3,4-*trans* azetidinone (**91**).[89] 3,4-Unsubstituted β-lactams (**92**) have been prepared from β-bromo propionamides by reaction with potassium hydroxide and a variety of phase-transfer catalysts in organic solvents.[90] The β-lactam analogue (**93**), of a natural β-lactone HMG CoA synthetase inhibitor, has been prepared using a very similar procedure.[91] Oxidative cyclisation of the β,γ-unsaturated hydroxamate (**94**) was achieved by treatment with bromine and potassium carbonate, providing the 4-bromomethyl derivative (**95**).[92]

8.1.4 *2,3 bond-forming reactions.*- The highly functionalised carbamylcobalt salophen complex (**96**) undergoes thermal homolytic cleavage with concomitant ring closure in a 4-*exo*-trig mode to give, after dehydrocobaltation, the *trans*-azetidinone (**97**) an intermediate for the synthesis of (±)-thienamycin.[93]

8.2 *Reactions in which two bonds are formed*

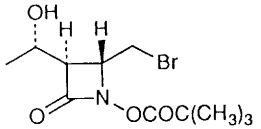
This sub-section includes formal [3 + 1] and [2 + 2] additions which may be concerted or stepwise under the conditions used.

8.2.1 *[3 + 1] additions*

8.2.1.1 *1-2 and 2-3 bond formation.*- Rhodium catalysed silylcarbonylation of propargylamine derivatives (**98**) gives α-silylmethylene-β-lactams (**99**) in a one-pot coupling reaction. The reaction works well when both R^1 and R^2 are alkyl; if one or both of R^1 and R^2 are H then the main or sole product is a silylated propenal.[94] The palladium-catalysed carbonylation of 4-amino-2-alkynyl carbonates (**100**) gives α-vinylidene-β-lactams (**101**) and/or α-alkynyl-β-lactams (**102**) depending upon the reaction conditions.[95] Aziridines (**103**) have been converted to β-lactams (**104**) by palladium (O)/carbon monoxide mediated ring opening, carbonylation and ring closure.[96]

8.2.2 *[2 + 2] additions*

8.2.2.1 *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 appeared on the synthesis of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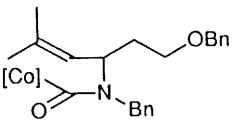
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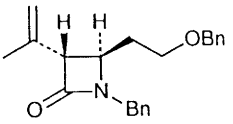
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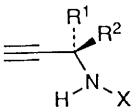
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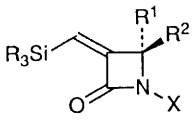
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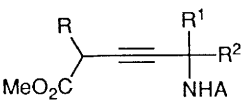
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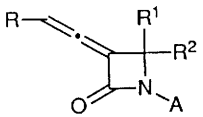
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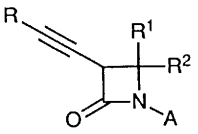
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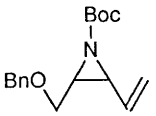
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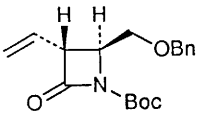
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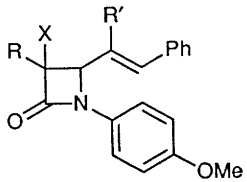
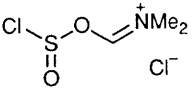

(105) a, R = ArS; b, R = ArSO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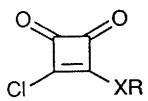
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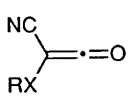
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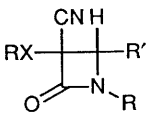
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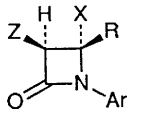
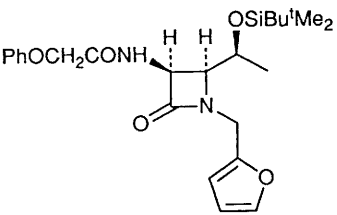

(110) a, X = SR^1;
b, X = H
Z = N_3, Ft, PhO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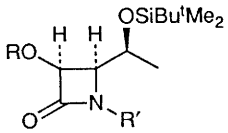
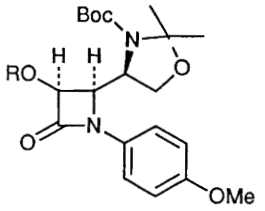

(112) R = Alkyl, Acyl

α-arylthio β-lactams (**105a**) by reaction of β-(arylthio)alkanoyl chlorides and cinnamaldehyde imines. Desulfuration subsequently provided 3-alkyl β-lactams in a highly stereoselective manner. α-Alkylidene β-lactams were prepared by thermal elimination of the α-phenylsulfinyl derivatives (**105b**).[97] N-{[(Chlorosulfinyl)oxy]methylene}-N-methylmethanaminium chloride (**106**) is an efficient dehydrating agent for the preparation of ketenes from carboxylic acids.[98] A modification of an established procedure for ketene preparation replaces triethylamine by tripropylamine, giving a substantial improvement in chemical yields.[99] Treatment of squaric acid derivatives (**107**) with sodium azide gives captodative cyanoketenes (**108**) which react with imines to give 3,3-disubstituted azetidinones (**109**).[100] The reaction of alkylthioimidates with ketenes (or their equivalents) gives *trans* 3,4,4-trisubstituted azetidinones (**110a**). Reductive desulfuration gives *cis*-derivatives (**110b**).[101] Numerous examples of asymmetric [2 + 2] cycloadditions have appeared. Imines derived from homochiral lactaldehyde have been used in cycloadditions with a Dane salt to give, after hydrolysis and N-acylation, the *cis* 3-amido-4-alkyl β-lactam (**111**).[102] The same imines also react with acyloxy[103] and aryloxy[102] ketenes to give (**112**). Imines derived from N,O-diprotected L-serinal react with acid chlorides in the presence of triethylamine to give *cis* β-lactams (**113**) with complete diastereoselection.[104] The chiral 4-alkoxycarbonyl azetidinone (**114**), obtained by [2 + 2] cycloaddition of the corresponding imine with an α,β-unsaturated acid chloride, undergoes C-3 epimerisation during hydrolysis of the chiral ester. Subsequent oxymercuration-reduction of the vinyl group and oxidative decarboxylation with lead tetraacetate gave the carbapenem intermediate (**115**).[105] Reaction of a galactosamine derived imine with aryloxy acetyl chlorides provided β-lactam (**116**). Hydrolysis and N-benzoylation gave the N-benzoyl-(*2S,3R*)-3-phenylisoserine (**117**), the enantiomer of a unit for the side-chain of anti-cancer agent taxol.[106] The ketene derived from D-glucose derivative (**118**) undergoes cycloaddition with a cinnamaldehyde imine providing 3-hydroxy β-lactam (**119**) with 70% ee after hydrolytic removal of the carbohydrate moiety.[107] The synthesis of 4-formyl azetidinones by reaction of the bis-imine (**120**) with acid chlorides followed by hydrolysis (see also Volume 22) has been extended to provide 3-monosubstituted derivatives (**121a,b**).[108] The conversion of a 4-formyl azetidinone to an imine similar to (**121a**) was followed by [2 + 2] cycloaddition to give the bis-azetidinone (**122**).[109] A detailed study of the cycloaddition of (N-alkyl-N-phenylamino) ketenes with imines indicated that the size and electronic nature of the imine substituent are the main factors which determine the stereochemistry of the β-lactam products. Small N-substituents favour *cis*-isomers while bulky substituents give *trans*



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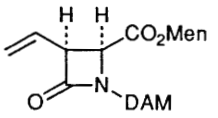
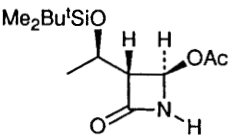


(114)
Men = D-Menthyl
DAM = di-*p*-anisylmethyl



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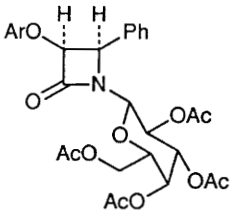

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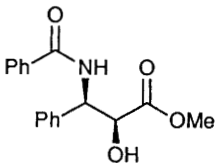

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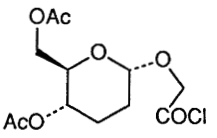

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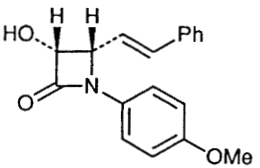

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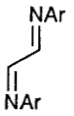

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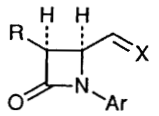
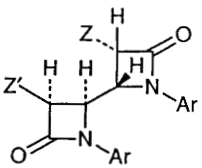


(121) a, X = NAr;
b, X = O



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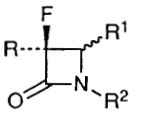
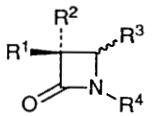


(123) a, C-4βR^1;
b, C-4αR^1

(124) a, R' = $SiMe_2Bu^t$;
b, R' = $SnMe_3$



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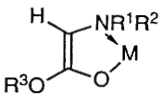
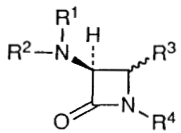
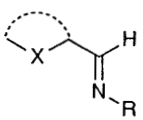


(126) a, M = $AlR^3{}_2$;
b, M = ZnX



(127) a, C-4 α R^3;
b, C-4 β R^3



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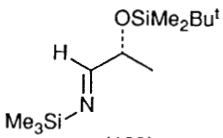
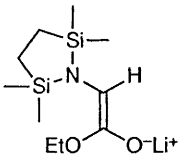


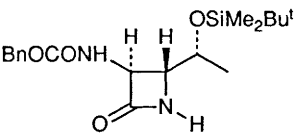
(129)

isomers.[110] A substantial publication compares the stereoselectivity dependence in β-lactam formation on the method of ketene generation, either acid chloride/triethylamine or photolysis of chromium-carbene complexes.[111]

Moving now to the ester enolate plus imine variant, a study of the preparation of 3-fluoro azetidinones (**123**) revealed that the use of an ester enolate gave mixtures of (**123a**) and (**123b**) while the corresponding ketene gave only (**123a**).[112] A new synthesis of N-silylimines (**124a**) occurs *via* the trimethylstannyl imine (**124b**), prepared from aldehydes and *tris*-trimethylstannyl amine. Reaction with ester enolates gave N-silyl azetidinones.[113] The reaction of titanium enolates of 2-pyridylthioesters with imines gives mixtures of β-lactams (**125**).[114] A detailed account of the synthesis, characterisation and synthetic application of the α-amino dialkylaluminium ester enolates (**126a**) reveals that their reaction with simple imines affords *trans* 3-amino azetidinones (**127a**). The stereoselectivity is explained in terms of highly ordered transition states, constructed from Z-aluminium enolates and *E*-imines.[115] The same authors have reported lithium and zinc ester enolates (**126b**); the former react only with activated imines to give *cis* 3-amino azetidinones (**127b**) while the latter have more general reactivity and provide *trans* isomers (**127a**).[116] The same lithium and zinc enolates have been reacted with imines (**128**) having donor heteroatoms in their C-substituents. The presence of a nitrogen donor atom gives better stereoselectivity than either oxygen or sulfur substituents.[117] The reaction of homochiral lactaldehyde with lithium hexamethyldisilazide gives the chiral silylimine (**129**). Reaction with the lithium enolate (**130**) gives the *trans* azetidinone (**131**) after N-deprotection-reacylation. The use of other metal counterions, in both imine and enolate formation, gives mixtures of *cis* and *trans* isomers.[118] Reaction of the chiral imine (**132**) with ester enolates gives the C-4 β-substituted β-lactam (**133a**) (>84% S) with lithium, sodium and zinc counterions, and the α-substituted isomer (**133b**) (>89% R) with titanium enolates.[119] The homochiral chromium-complexed imines (**134**) undergo cycloaddition with ester enolates to give β-lactams with an enantiomeric excess >98%; there was little selectivity for *cis* or *trans* isomers.[120] Reaction of the chiral glycoaldehyde imine (**135**) with lithium ester enolates gives a mixture of *cis* and *trans* 3-amino azetidinones (**136**) with the *trans* compound as the major isomer.[121] Minimal problems were caused by the enolisable nature of the imine. The chiral ester enolates (**137**) react with N-trimethylsilylimines to give the *cis* 3-hydroxy β-lactam (**138**), ring opening of which allows preparation of N-benzoyl (*2R,3S*)-3-phenylisoserine, the C-13 side-chain of anti-cancer agent taxol.[122] The reaction of thioesters with imines promoted by the chiral diazaborolidine



(130)



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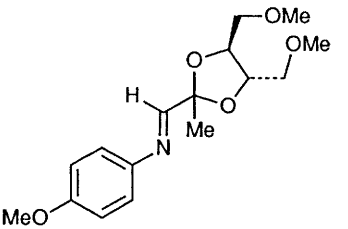

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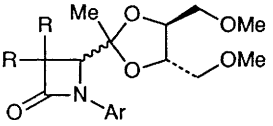
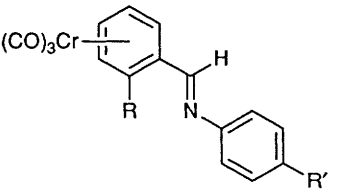


(133) a, C-4 β isomer;
b, C-4 α isomer



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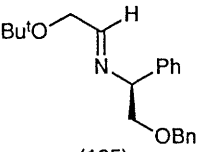

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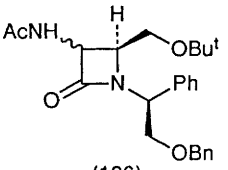

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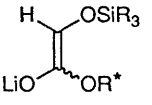

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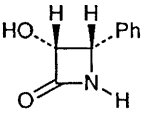

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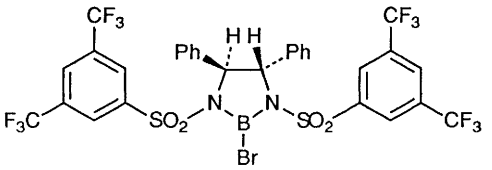

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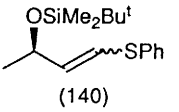

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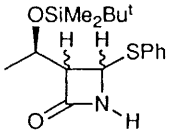
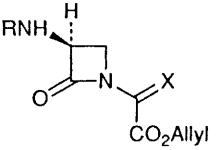
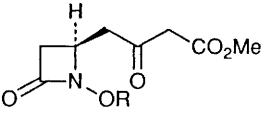


(141)



(142) a, X = PPh_3;
b, X = CHR



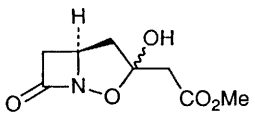
(143) a, R = Bn; b, R = H

(**139**) and triethylamine afford β-lactams with high diastereo- and enantioselectivity.[123]

8.2.2.2 *1-4 and 2-3 bond formation.*- Coupling of chlorosulfonyl isocyanate with the separable chiral E- and Z- vinyl sulfides (**140**) gives mixtures of the two possible *trans* azetidinone isomers (**141**). The ratio of isomers varies on the sulfide isomer used and on the reaction solvent.[124]

8.3 *Chemistry of azetidinones*

This section will deal with reactions at each of the azetidinone N-1 to C-4 positions. Wittig reactions of the phosphorane (**142a**) with various aldehydes having substituents found at C-3 in cephalosporins, gave the N-(2-substituted-1-carboxy)vinyl azetidinones (**142b**). The low level of biological activity observed was taken as evidence that structural elements play an important role in determining biological potency, in addition to any electronic factors present.[125] Deprotection of the N-benzyloxy azetidinone (**143a**) gives the hemiketal (**144**) in equilibrium with the N-hydroxy derivative (**143b**). Further intramolecular reaction of the β-hydroxy epimer of (**144**) results in rearrangement to the bicyclo[3.2.1] system (**145**).[126] Moving on to chemistry involving the C-3 position, oxidation of the 3-hydroxy β-lactam to the 3-oxo derivative was followed by ring expansion to the N-carboxyanhydride (**146**) with m-CPBA. The use of the latter to acylate an enolate gave the γ-amino-β-keto esters (**147a**) which were successfully reduced to γ-amino-β-hydroxy esters (**147b**).[127] Ozonolysis of the bicyclic azetidinone (**148**) and the cyclopropylcarbapenam (**149**) failed to give the expected α-oxo derivatives but provided instead the α-aminoacid-N-carboxyanhydrides (**150**) and (**151**).[128] A 3-acetyl azetidinone reacts with tert-butyldimethylsilyl triflate to give the kinetic enol ether (**152a**). Ozonolysis results in the corresponding silyl ester (**152b**). Reduction with tetrabutylammonium borohydride provides the 3-(hydroxymethyl) derivative (**153**) which was progressed to a 6-(hydroxymethyl)-1β-methyl carbapenem.[129] The acylation of a single enantiomer of the racemic *cis*-3-amino β-lactam (**154a**) using Penicillin G amidase from *E.coli* allows the isolation of optically pure (*3S,4R*) azetidinones (**154b,c**). The latter are key intermediates in the synthesis of carbacephalosporin antibiotic Loracarbef.[130] Reaction of the anion of a 3-nitro azetidinone with 2-halomethyl-5-nitroimidazoles results in C-3 alkylation and loss of nitrous acid to give the novel 3-alkylidene azetidinone (**155**). The reaction was applied more generally to nitrolactams of various ring sizes.[131] The scope of the phthalimido protecting group has been extended by reaction with pyrrolidine. The resulting o-pyrrolidinocarbonylbenzamide (OPCB) is base/nucleophile



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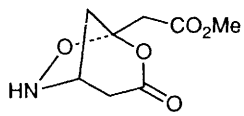

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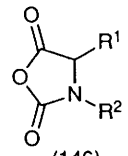
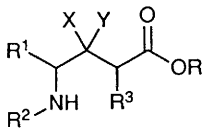
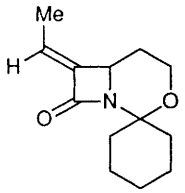


(146)



(147) a, XY = O;
b, X = OH, Y = H



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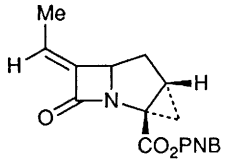

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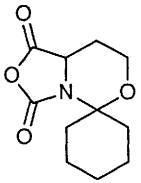

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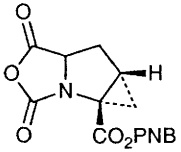

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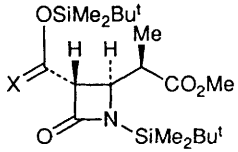
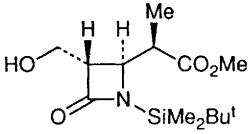
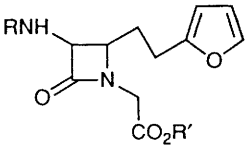


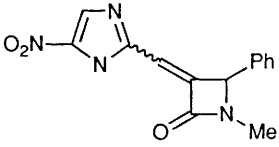
(152) a, X = CH_2; b, X = O



(153)



(154) a, R = H; b, R = $PhOCH_2CO$;
c, R = $PhCH_2CO$



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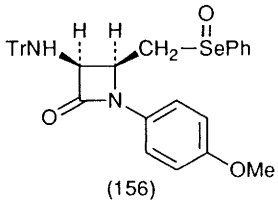

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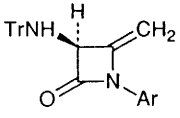

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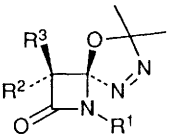
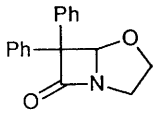


(158) a, R^1 = CH_2CH_2OH

stable, in contrast with phthalimido itself. The OPCB group conveniently reverts to phthalimido on treatment with acid. Most of the examples given of this technique are on 3-phthalimido β-lactams.[132] As is usual, the largest number of publications concern various reactions at C-4 of the β-lactam. Thermal elimination of the 4-selenomethyl derivative (**156**) provides the corresponding 4-methylene azetidinone (**157**).[133] Thermolysis of the spiro-fused β-lactam oxadiazolines (**158**) gives β-lactam-4-ylidenes, reactive carbenes which can be trapped by both inter- and intramolecular reactions. Of particular interest is the isolation of the oxapenam (**159**) from reaction of the N-(2-hydroxyethyl) derivative (**158a**).[134] Three publications have appeared on the synthesis of 4-acetoxy azetidinones. Kolbe-type electrolysis of 4-carboxy azetidinones (**160a,b,c**) in acetic acid/acetonitrile/sodium acetate gives 4-acetoxy azetidinones (**161a,b,c**), presumably by decarboxylation to the azetidinium ion (**162**) and capture by acetate.[135] The osmium-catalysed oxidation of β-lactams with peroxides in acetic acid gives 4-acetoxy β-lactams similar to (**161a,b,c**); the reaction also provides 4,4-disubstituted derivatives (**163a,b**). Peracetic acid gives the best results, methyl ethyl ketone peroxide, mCPBA PhIO and $PhI(OAc)_2$ also give reasonable results. Osmium trichloride is the most effective catalyst, a number of cobalt complexes can also be used although their catalytic activity is lower.[136] The same authors have reported the ruthenium trichloride catalysed reaction of β-lactams in the presence of acetaldehyde and molecule oxygen in an acid and ethyl acetate under buffered conditions gives the corresponding 4-acyloxy β-lactams (**164**) in good yields.[137] Compounds (**161a,b**) and (**163a,b**) were also prepared by this method. The N-(Z)-crotyldimethylsilyl azetidinone (**165**) undergoes a novel stereocontrolled intramolecular Sakurai-type reaction upon treatment with trimethylsilyl triflate. The product (**166a**) can be N-desilylated to give the much-used 1-methylcarbapenem intermediate (**166b**).[138] A number of publications have appeared detailing further advances in nucleophilic displacements at azetidinone C-4. The Lewis acid catalysed reaction of allyltriphenyltin reagents with 4-acetoxyazetidinones (**167a**) gives the corresponding 4-allyl azetidinones (**167b**) which were converted to 1α- and 1β-methylcarbapenems.[139] The reaction of 4-acetoxy azetidinone (**161b**) with crotyl halides and zinc dust gives 4-allyl derivatives similar to (**166b**).[140]

The reaction of 4-acetoxy derivatives with silylmethylmagnesium chlorides gives 4-(silylmethyl) β-lactams (**168**). The same compounds could also be prepared by cycloaddition of allylsilanes with chlorosulfonyl isocyanate.[141] (See also Volume 23). The substitution reactions of 4-heterosubstituted azetidinones with 1,2- and 1,3-disilyl-2-butenes in the presence of Lewis acids have been reported. The primary products



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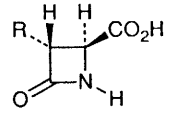
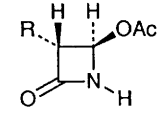


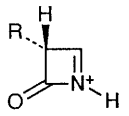
(160) a, R = H;

b,  ; c, 

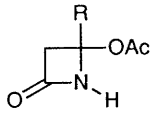


(161) a, R = H;

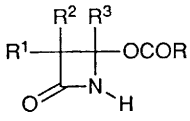
b,  ; c, 



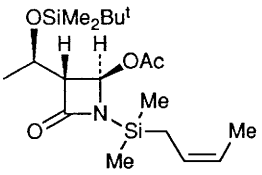
(162)



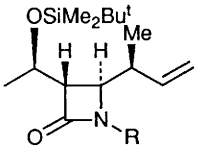
(163) a, R = Me;
b, R = CO_2Me



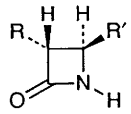
(164)



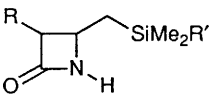
(165)



(166) a, R = $SiMe_2OAc$;
b, R = H

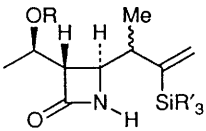


(167) a, R′ = OAc;
b, R′ = CH(Me)CH=CH_2



(168)

R = H, Br



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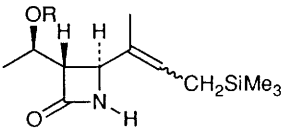

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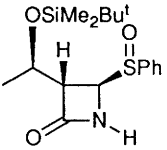

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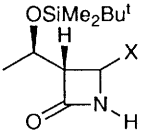
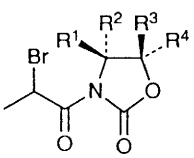
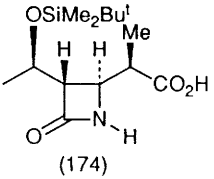


(172) X = SR, SCOR,
OCOR, N_3, NHAc, Het



(173)



(174)

(**169**) and (**170**) respectively can be desilylated to 4-(1-methylallyl) derivatives similar to (**167b**).[142] The zinc iodide mediated reaction of 4-sulfinyl azetidinone (**171**) with silylated heteronucleophiles gives a wide range of *trans* 4-hetero function substituted azetidinones (**172**) in high yields.[143] Reformatsky reaction of the bromo-oxazolidinone (**173**) gives a zinc enolate which reacts with 4-acetoxy β-lactam (**161b**) to give, after hydrolysis, the carbapenem intermediate (**174**) and its 1-methyl isomer. The best diastereoselectivity (95β:5α) is obtained with sterically crowded oxazolidinones.[144] The stereoselective hydroboration oxidation of 4-isopropenyl azetidinones (**175a,b**) gives the 4-(1β-methyl-2-hydroxyethyl) derivative (**176a,b**). The same publication also reports the stereoselective hydrogenation of the related (**177**) to give the corresponding 1β-methyl derivative.[145]

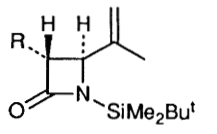
8.4 *Further uses of azetidinones*

Full details have now appeared on the reduction of β-lactams and bis-β-lactams to the corresponding azetidines and bis-azetidines using aluminium-based reducing agents. Subsequent hydrolysis gives diamines, amino alcohols, polyamino alcohols and polyamino ethers in good yields.[146] The 3-chloromethyl azetidinones (**178**) rearrangement to azetidine-3-carboxylic acids (**179**) in good yield upon treatment with alkoxides. An alternative ring opening-ring closure procedure was also identified.[147]

9 Major structural variants

As usual, systems retaining a β-lactam ring will be dealt with first. A novel insertion reaction of the diazo-substituted azetidinones (**180**), catalysed by rhodium (II) acetate, gives the 3-oxacephem sulfone (**181**). Each separate isomer at the hydroxyacetate centre gives rise to a mixture of both possible isomers α- to the sulfone moiety. The reaction works with both the free hydroxy derivative and directly with its tetrahydropyranyl protected counterpart.[148]

Reaction of the 4-acetoxyazetidinone (**161b**) with a dithiostannyl ester failed to give the expected dithioketene acetal, providing instead the bis-azetidinone (**182**). Reaction with sulfurdiphthalimide generated sulfenamide (**183**). Treatment with triphenylphosphine did not provide the expected 2-thioxo penam, leading instead to the novel fused 4,5-bicycle (**184**). These compounds are potent inhibitors of human leukocyte elastase.[149] A [2+2] cycloaddition of the (protected-amino) ketene (**185**) with 1*H*-1,2-diazepines gives mixtures of the *trans* β-lactams (**186**).[150] Reaction of a 1-allyl-4-styryl β-lactam with sulfur dichloride gives a



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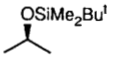
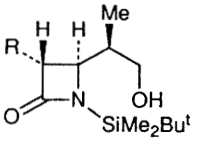
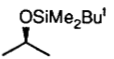
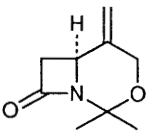
b, R = 



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



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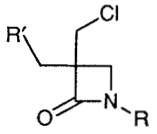

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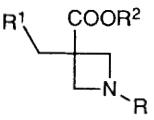

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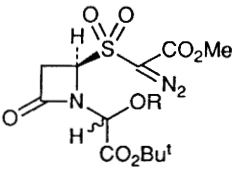

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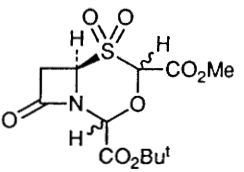

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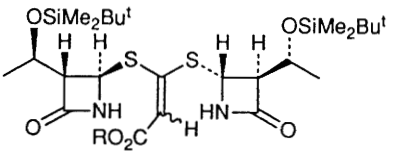

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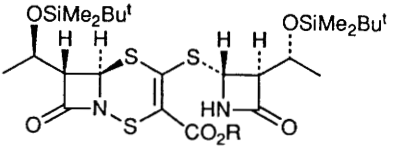

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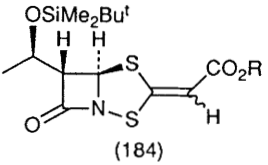

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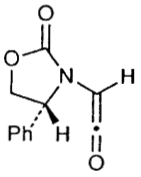

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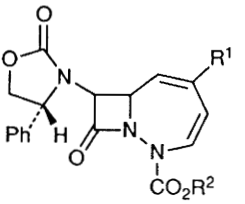

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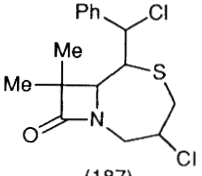

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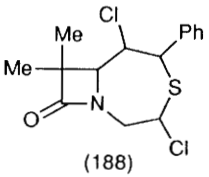
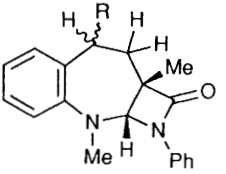


(188)



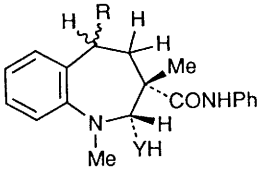
(189)

mixture of the products (**187**) and (**188**).[151] The tricyclic β-lactam (**189**), prepared by a [2+2] cycloaddition involving phenyl isocyanate, reacts with protic reagents to give products of type (**190**) and/or (**191**).[152] Sensitised irradiation of the 2-pyridone (**192**) gives the tricylic product (**193**). Thermal isomerisation then affords the tricyclic β-lactam (**194**).[153] Tributyltin hydride mediated radical cyclisations of α-chloroacetamides (**195a,b**) gives the corresponding 4-spiro azetidinones (**196a,b**).[154] Cycloaddition reactions of the 1,3-thiazines (**197**) with acid chlorides in the presence of base gave the corresponding tricyclic cephams (**198**).[155]

Moving on now to non β-lactam derivatives, ring closure of 2-chloroacyl hydrazines (**199**) gives the 4,4-disubstituted 1,2-diazetidin-4-ones (**200**).[156] An alternative synthesis of the 4-substituted 1,2-thiazetidine-1,1-dioxides (**201a**) involves acylation and reduction of the C-4 unsubstituted parent compound (**201b**).[157] The lactivicin analogue (**202**) has been synthesised from phenoxyacetyl-(L)-O-aminoserine and diallylsquarate *via* (**203**).[158] In a reaction analogous to that observed with β-lactams, the homopenam sulfoxide (**204**) undergoes ring expansion to the homocephem (**205**) upon reaction with pyridine in dichloroethane.[159] The bicyclic tetrahydropyridazinones (**206**), homologues of antibacterially active bicyclic pyrazolidinones, have been synthesised in a manner analogous to that used for the latter. Cycloaddition of the *in situ* generated azomethine imine (**207**) with alkynes or vinyl sulfones was successful. An alternative method using an intramolecular Horner-Emmons reaction also provided a further example.[160] Condensation of various vinylogous urethanes with acrylates, haloacrylates and propiolic acid has provided bicyclic dihydropyridone and pyridone analogues of cephalosporins, carbapenams, penicillins and bisnorpenicillins characterised by the partial structure (**208**).[161]

10 Mechanistic studies, mode of action and degradation

As in previous years this section will encompass general mechanistic studies, interactions of β-lactams with enzymes, molecular graphics and mechanisms and products of degradation of β-lactams. A ^{13}C cross polarisation magic angle spinning (CP-MAS) study of frozen aqueous solutions of penicillins revealed resonances from two distinct thiazolidine ring conformers. The relative intensities of the resonances correlated well with previous estimates of the populations of the different conformers in liquid aqueous solutions.[162] Evidence for two different mechanisms of azetidinone formation, operating simultaneously in a single reaction, was found in a ^1H NMR study. The reaction of phenylacetyl chloride with benzal-anilines in d_7-DMF gave rise to proton signals assigned to two



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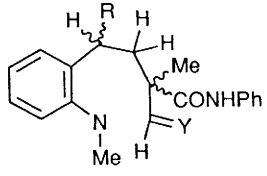

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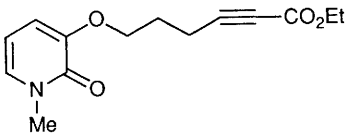

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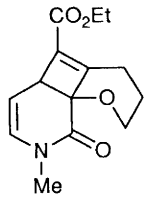

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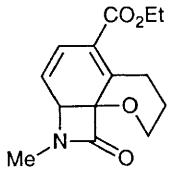

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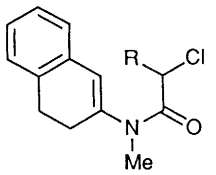
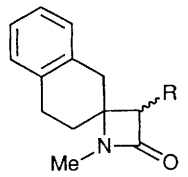
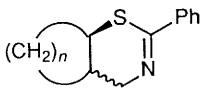


(195) a, R = H; b, R = Me



(196) a, R = H; b, R = Me



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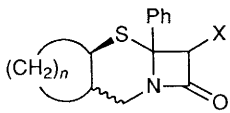

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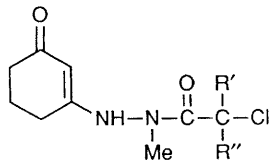

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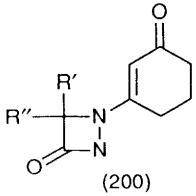

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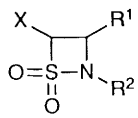
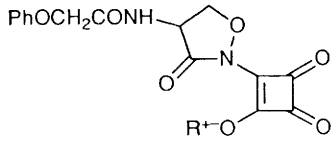


(201) a, X = R—CH(OH)—;
b, X = H



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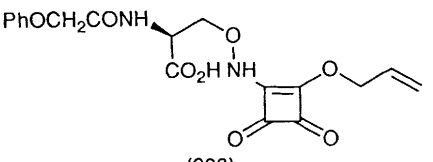

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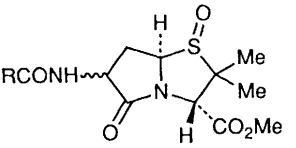

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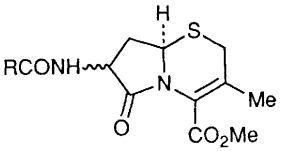

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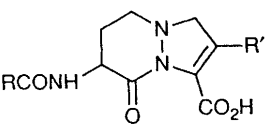

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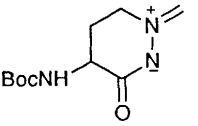
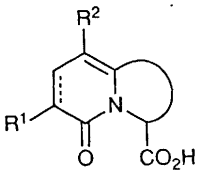
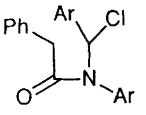


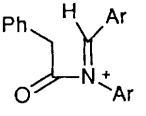
(207)



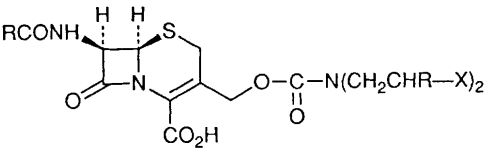
(208)



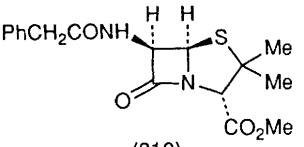
(209)



(210)



(211) X = OH, Cl, Br



(212)

different intermediates, namely a 2-phenyl-*N*-(α-chlorobenzyl)acetanilidine (**209**) and a nitrogen charged adduct (**210**).[163] A series of cephalosporin carbamates (**211**) has been synthesised for antibody directed enzyme prodrug therapy (ADEPT). The theory is that a β-lactamase conjugated to a tumour specific antibody would hydrolyse the cephalosporin releasing the cytotoxic nitrogen mustard to kill the tumour cells.[164] Porcine liver esterase selectively catalyses the hydrolysis of the β-lactam ring of the penicillin methyl ester (**212**) without hydrolysing the methyl ester.[165] A detailed study of the interaction of penicillin and cephalosporin C3(C4)-esters and amides with a number of penicillin recognising enzymes indicated that an ionic group in that position is necessary for effective acylation of these enzymes. Discrepencies were found between the observed activities of the various esters and amides and their expected activities based on intrinsic electronic and geometric properties.[166] The geometries of several cephalosporins have been determined by MINDO/3, MNDO and AM1 semiempiracal calculation methods. Of the three methods used, MINDO/3 provided the best estimations of geometric values, while MNDO reproduced the pyramidal character of the β-lactam nitrogen with greatest accuracy; AM1 yielded an intermediate solution.[167] AM1 calculations have also been used on a series of bicyclic azetidinones and 1,3-diazetidin-2-ones. Bicyclic diazetidinones are predicted to be stable systems with a highly electrophilic carbonyl group.[168] Conformational analysis of various β- and α-lactams identified a binding motif common to a number of derivatives which successfully acylate the active site serine of penicillin-binding proteins (PBP). The motif consists of the central scissile amide bond flanked by a carbonyl unit on one side and a carboxylate on the other.[169]

Moving now to degradation and hydrolysis of β-lactams, a system composed of 2-amino-2-hydroxymethylpropane-1,3-diol (Tris) and Zn^{2+} simulates the catalytic action of serine β-lactamases and is able to cause the degradation of a number of cephalosporins.[170] A detailed study has identified two stable complexes of sodium amoxicillin with copper (II) ion in methanolic media. The molar ratios are 1:1 and 2:1 for the two complexes.[171] The hydrolysis of benzylpenicillin is catalysed by alkoxide ions and other oxygen bases. Catalysis occurs by a nucleophilic pathway and the intermediate ester can be detected in some cases. The conclusion is reached that the high rate of reaction of penicillin with the enzyme active site serine in PBP's is not due to the intrinsic activity of the penicillin but is the result of favourable non-binding interactions at the active site which lower the activation energy of the acylation reaction.[172] Full details have now appeared on the highly complex processes involved in ring-opening, both β-lactam and thiazolidine, in penicillins, their esters

and amides. Different dependencies on the pH of the aqueous solution were taken as evidence for three different mechanisms operating over the various pH ranges.[173,174]

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*
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