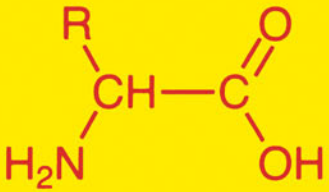

SPECIALIST PERIODICAL REPORTS

AMINO ACIDS, PEPTIDES AND PROTEINS

VOLUME 30

SENIOR REPORTER J.S. DAVIES

ROYAL SOCIETY OF CHEMISTRY

A Specialist Periodical Report

Amino Acids, Peptides and Proteins

Volume 30

A Review of the Literature Published during 1997

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RS•C
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Preface

The literature surveyed for this Volume updates the coverage of the World's peptide/protein literature to the end of 1997 and will be published 30 years to the date of publication of the first Volume. In the last ten of those years the annual number of total syntheses of peptides has doubled to approximately 2000 (statistics from the Institute of Protein Research Foundation, Osaka, Japan). Dietrich Brandenburg at the 1st International Peptide Symposium, at Kyoto Japan (*'Peptide Science-Present and Future'* ed. Y. Shimonishi, publ. Kluwer Academic) reported that searching Medline for 1997 gave him 20,000 entries. This enormous field of activity has therefore provided my colleagues reporting in this Volume with the unenviable task of compiling what they believe to be the significant developments in their particular topics. Thirty years on, in this series of Specialist Reports it is still difficult to assess whether the Chapter headings and their sub-divisions have kept abreast with the current vogues in the peptide literature. However, in a survey of the submissions to the 25th European Peptide Symposium (*'Peptides 1998'*, eds. S. Bajusz and F. Hudecz, publ. Akademia Kiado, Budapest), it was shown that the papers distributed themselves amongst the topics: Structure - Activity Studies, Bioactive Peptides (38%); Synthesis and Combinatorial Chemistry (28%); Structure Elucidation and Analysis of Peptides (23%); Peptide- and Protein-Drugs, Present Status and Perspectives (11%).

I hope readers will see that all four of these latter topics have been adequately covered by our four Reporters (G. C. Barrett, D. T. Elmore, A. Dutta and J.S. Davies) in the early Chapters of this Volume. Incidentally, Barrett and Elmore have also found time to write a student text on *Amino Acids and Peptides* (Cambridge University Press) during the harvesting of material for the current Chapters. The annual review of Current Trends in Protein Research is not included this year but will appear as coverage of 1997/98 literature next year. However, the biennial treatment of β-Lactam Chemistry has seen Christopher Schofield and a colleague return to the Reporters' rostrum. The continuing interest in trying to overcome antibiotic resistance keeps the latter subject area very buoyant in spite of the annual prognosis of its imminent demise by the 'super bugs'. While computer searches have eased the burden of gathering the material for Chapters, its collation and formatting to readable Reports is still intellectually demanding and time-consuming. I can only offer my most sincere thanks to the current Reporters for their continuing willingness to subject themselves to such a thankless task.

Again, the bringing together of the variety of manuscripts, and their conversion to a readable printer's format between these yellow covers, has been the patient

task of Janet Freshwater and her crew at RSC publications. Sincere thanks to everyone.

John S. Davies
University of Wales, Swansea

Contents

Abbreviations

The abbreviations for amino acids and their use in the formation of derivatives follow in general the 1983 Recommendations of the IUB-IUPAC Joint Commission, which were reprinted as an Appendix in Volume 16 of this series. These are also published in:

Eur. J. Biochem., 1984, **138**, 9–37: *Int. J. Pept. Protein Res.*, 1984, **24**, after p.84; and *J. Biol. Chem.*, 1985, **260**, 1442.

A complete listing of the single-letter codes for amino acids appeared in the Abbreviations section of Volume 24 of these Reports, together with structures for the closely related BOP family of coupling reagents.

Chapter authors have been encouraged annually to include new abbreviations in their texts. With the ever increasing diversification in structures, lists of unusual abbreviations are periodically compiled. Some examples are listed below.

Abo	2-azabicyclo[2.2.2]octane-3-carboxylic acid
Abu	α-aminobutyric acid
A_2bu	2,4-diaminobutyric acid
ACCA	4-aminocyclohexanecarboxylic acid
εAhx	6-aminohexanoic acid
Aib	α-aminioisobutyric acid
Aic	2-aminoindane-2-carboxylic acid
A_2pr	2,3-diaminopropionic acid
Atc	2-aminotetralin-2-carboxylic acid
Ava	5-aminopentanoic acid
Aze	azetidine-2-carboxylic acid
Cha	3-cyclohexylalanine
Cpg	α-cyclopentylglycine
Cpp	1-mercaptocyclohexaneacetic acid, *or* β-mercapto-β,β-cyclopentamethylenepropionic acid, *or* Pmp (*below*)
*c*PzACAla	*cis*-3-(4-pyrazinylcarbonylaminocyclohexyl)alanine
Dab	2,4-diaminobutyric acid
Dap	2,3-diaminopropionic acid
Dbf	3-(2-dibenzofuranyl)alanine
Dip	3,3-diphenylalanine
Dph	α,α-diphenylglycine
Dpr	2,3-diaminopropionic acid
Gly(Ph)	phenylglycine
Har	homoarginine
Hib	α-hydroxyisobutyric acid

Hyp	*trans*-4-hydroxyproline
Iva	isovaline
Mpt	*trans*-4-mercaptoproline
1-Nal	3-(1-naphthyl)alanine
2-Nal	3-(2-naphthyl)alanine
Nap	β-(1′-naphthyl)alanine
Oic	octahydroindolecarboxylic acid
Opt	*O*-phenyltyrosine
3-Pal	3-(pyridyl)alanine
Pen	penicillamine
Phg	phenylglycine
Pip	pipecolic acid
Pmp	β,β-pentamethylene-β-mercaptopropionic acid, *or* Cpp (*above*)
Qal	3-(3-quinolyl)alanine
Qua	quinoline-2-carboxamide
Sar	sarcosine
Thi	β-thienylalanine
Tic	1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid

1
Amino Acids

BY GRAHAM C. BARRETT

1 Introduction

The science of amino acids described here is based mainly on the literature of 1997. Criteria used for structuring this Chapter in all preceding Volumes of this Specialist Periodical Report have been used again for defining the papers chosen for citation here.

Thus, advances in the chemistry of the amino acids, and biological aspects impinging on their chemistry, have been the preoccupation for this Chapter, with routine aspects being excluded from consideration. Even so, the year-on-year increase in the number of papers eligible for inclusion here has continued, and has required a certain amount of restraint and retrenchment in the layout of this Chapter, with merging of some Sections.

Most of the papers cited here are the rewards of scanning the major Journals, and of scanning *Chemical Abstracts* (Issue 10 of Volume 126, up to and including Issue 9 of Volume 128).

2 Textbooks and Reviews

Monographs providing detailed coverage of enantioselective synthesis of β-amino acids[1] and protein sequence determination[2] have appeared; the latter contains a range of chapters relevant to the analysis of amino acids.[3] A text[4] that is aimed at advanced undergraduate and postgraduate students will also assist those active in amino acid and peptide research in chemical, biochemical, pharmaceutical and related research areas.

The biochemistry of L-arginine, and the context of this amino acid in biology, has been reviewed.[5] A survey of methods for the enantioselective synthesis of chiral drugs[6] also covers amino acid synthesis protocols. The occurrence of D-amino acids in free form (particularly D-serine and D-aspartic acid in human brain[7]) and in naturally-occurring peptides[8] has been reviewed, and the literature dealing with hypusine (a post-translationally modified L-lysine derivative) has been surveyed.[9] Other reviews cover the biosynthesis and metabolism of those amino acids (isoleucine, threonine, methionine and lysine) that derive from aspartic acid in higher plants,[10] the amino acid composition of bacterial and mammalian cells,[11] and the natural provenance of dihydroxyprolines.[12]

Amino Acids, Peptides and Proteins, Volume 30
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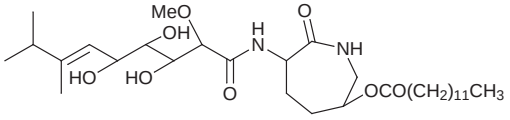
3 Naturally Occurring Amino Acids

3.1 Occurrence of Known Amino Acids – Leaving until later (Section 5.6) some citations that were traditionally placed here (*e.g.,* papers describing unusual results from preparative-scale isolation of amino acids from mixtures), the non-routine literature describes three further N^G,N^G-permethyl arginines in ribonucleoprotein,[13] N-acetyl-L-aspartic acid and N-acetyl-L-histidine as components of the vertebrate nervous system[14] and in the eye lens of goldfish and rats,[15] N-(17-hydroxylinolenoyl)-L-glutamine, known as volicitin, in a secretion of the caterpillars of beet armyworm that attracts predators,[16] four new N-acyl 2-methylene-β-alanine methyl esters (hurghamides A – D, from a Red Sea sponge *Hippospongia*),[17] and five new bengamides (*e.g.* 1) from the New Caledonian sponge *Jaspis carteri*.[18] Further information has been provided on S-methyl-L-methionine salts, with the suggestion that this widely-distributed cellular species (vitamin U) probably acts to diminish lipid peroxidation and monoamine oxidase activity.[19] New data on the neurotoxicity of domoic acid have been reported.[20]

Norvaline has been incorporated into leucine positions in recombinant human haemoglobin expressed in *Escherichia coli*, probably through mis-aminoacylation of $tRNA^{Leu}$ (norleucine is misincorporated in similar circumstances in place of methionine).[21] The tryptophan residue in the cardioexcitatory tripeptide amide H-Asn-Trp-Phe-NH_2 from *Aplysia kurodai* heart tissue is of the D-configuration (the all-L tripeptide amide is much less physiologically active).[22]

A continuing fascination is the occurrence of common amino acids in extraterrestrial samples, and the implications of the report[23] that small excesses of L-amino acids have been found in the Murchison meteorite have been considered.[24] The result is confirmed independently, and stable isotope analysis



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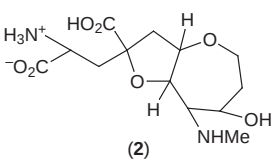

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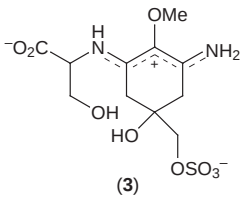
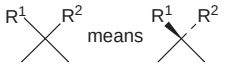


(**3**)

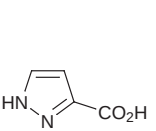
Three-dimensional features of molecules are depicted throughout this chapter as follows: Horizontally-ranged atoms and bonds, and ring atoms, are to be understood as being in the plane of the paper; substituent atoms and groups attached to these are to be understood to be ABOVE the page if ranged LEFTWARDS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



indicates that the amino acids are not terrestrial contaminants.[25] The amino acids involved include 2-amino-2,3-dimethylpentanoic acid (α-methylisoleucine; the 'L-enantiomers' among the four possible stereoisomers exist in 7.0 and 9.1% excess, respectively). However, other amino acids are present as racemates (aminoisobutyric acid, norvaline, isovaline and α-methylnorvaline). This might be interpreted to show that what Bada calls 'asymmetric influences' were at work on organic reactions occurring in prebiotic times.

The delivery of amino acids and other extraterrestrial compounds was an incidental feature of the catastrophe that wiped out the dinosaurs and most other species in the Cretaceous – Tertiary era. The same result is implied in the theory that is increasingly gaining support: the encounter of Earth with a giant molecular cloud (which better explains the lowered oxygen levels seen in amber-entombed contemporary air samples and lack of amino acids carrying oxygenated functional groups).[26]

3.2 New Naturally Occurring Amino Acids – The cis-fused hexahydro[3,2-b]pyran (2) that is reminiscent of domoic acid in its neurotoxic effects is a new dysherbaine from the Micronesian sponge *Dysidea herbacea*.[27] One of two palythines (3 and homologue CHMeOH in place of CH_2OH), new UV-B absorbing amino acids of the mycosporin family extracted from a reef-building coral *Stylophora pistillata,*[28] is the sulfate ester of one of the compounds present in *Pocillopora eydouxi*.[29] New mycosporin-like amino acids have been found in the Antarctic sea urchin *Sterechinus neumayeri*.[30] The first report of pyrazoles as natural products (4; and its 4-methyl homologue) concerns the sponge *Tedania anhelans*.[31]



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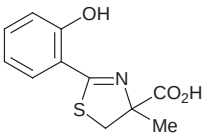
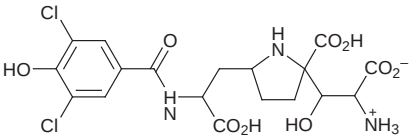


(**5**)

4-Methylaeruginoic acid (5) is a new cytotoxic imino acid from *Streptomyces* KCTC 9303.[32] *Eupenicillium shearii* PF1191 produces kaitocephalin (6; information on stereochemical features not yet available), a novel glutamate receptor antagonist that is a potent suppressor of kainate toxicity.[33] 1-Amino-3-methylcyclobutanecarboxylic acid has been identified in seeds of *Atelia glazioviana Baillon*, though without information on its stereochemistry.[34]

Cycasindene (7; see also Ref. 974) and cycasthioamide (8) have been found, together with eight known 'non-protein amino acids', in seeds of *Cycas revoluta* Thunb.[35] Fruiting bodies of *Clavulinopsis helvola* contain cis-DL-2-amino-3(cis),5-hexadienoic acid.[36] Root bark of *Calotropis gigantea* produces giganticine (9) which functions as an insect antifeedant.[37]

3.3 New Amino Acids from Hydrolysates – *Dysidea herbacea* contains (10), composed of two unusual α-amino acids, and is accompanied by a closely related



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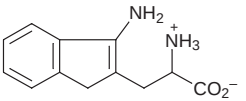

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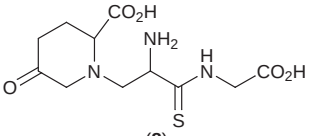

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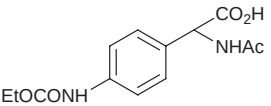
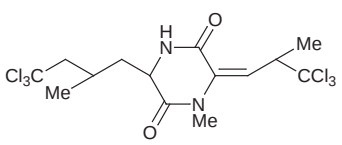


(**9**)

dioxopiperazine ($CHCl_2$ in place of CCl_3),[38] also found as the bis-N-methyl homologue dysamide D (10, NMe in place of NH, >$CHCH_2$- in place of >C=CH-), in *Dysidea fragilis*.[39] (-)-Phenylahistin, (11), from *Aspergillus ustus*, is a prenylated dehydrohistidine derivative.[40] A review[41] covers the identification of β-(methylthio)aspartic acid as a novel post-translationally modified amino acid in ribosomal protein S12 from *E. coli*.

Oscillaginin B, a tetrapeptide from the freshwater toxic cyanobacterium *Oscillatoria agardhii* contains the new amino acid, 3-amino-10-chloro-2-hydroxydecanoic acid.[42] Fischerellin B [(3R,5S)-3-methyl-5(E)-pentadec-5-ene-7,9-diynyl)pyrrolidin-2-one], a new algicide from the cyanobacterium *Fischerella muscicola,* is the lactam of a δ-amino acid.[43]



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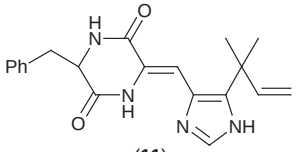


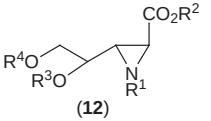
(**11**)

4 Chemical Synthesis and Resolution of Amino Acids

General reviews of amino acid synthesis are located in the appropriate subsections of this Chapter. More specific reviews relate to preparations of coded α-amino acids labelled with stable isotopes (^{2}H, ^{13}C, ^{15}N, and ^{18}O).[44] Examples throughout this Chapter describe preparations and uses of amino acids labelled with ^{2}H (Refs. 51, 221, 539, 543, 553, 566, 576, 584, 586, 806, 967), ^{11}C (Refs.113, 162, 805, 839), ^{13}C (Refs. 219, 221, 229, 442, 443, 538, 586, 781), ^{15}N (Refs. 228, 229, 442, 444, 547, 966), ^{17}O (Ref. 586), ^{18}F (Refs. 162, 260, 261, 913), ^{35}S (Ref. 893), ^{77}Se (Ref. 832) and iodine isotopes (Ref. 910). Preparations of aminoboronic and aminophosphonic acids are likewise scattered through Section 4, rather than grouped together as in recent previous Volumes.

4.1 General Methods for the Synthesis of α-Amino Acids, including Enantioselective Synthesis – *4.1.1 Amination of Alkanoic Acid Derivatives by Amines and Amine-related Reagents*. – These processes provide reliable routes to α-amino acids in many cases. They are illustrated in their simplest form in the conversion of chiral α-bromoacrylates into cis- and trans-1H-aziridinecarboxylates (12) through Michael-type reactions with ammonia,[45] and in the synthesis of methyl aziridine-2-carboxylate from methyl 3-(2,2,2-trimethylhydrazino)propionate bromide through N-N-bond cleavage;[46] in the reaction of dehydroascorbic acid with cyanate to give the amino acid carbamate (13) present in *Solanum tuberosum*;[47] and for the rhodium(II) acetate-catalysed decomposition of diazoacetates in the presence of compounds containing N-H groups [α-phenyl diazoacetate or $PhC(N_2)P(O)(OMe)_2$ giving N-substituted phenylglycines or corresponding phosphonates respectively].[48] 2-(N-Trifluoroacetylamino)alkanoic acids are formed from trifluoroacetamide and a 2-bromoalkanoate in the presence of a base, using phase-transfer catalysis.[49]

Some simple nitrogen species that are suitable for the task are indicated in these preceding examples. Azidolysis is also convenient, an interesting example starting with α-alkenyl N-Boc oxazolidines and leading *via* an epoxy-bromocyclocarbocation (formed by reaction with NBS) to β-aminoalkanols through azidolysis, and completed through routine elaboration.[50] Epoxidation of E-but-2-en-1-ol with tert-BuO_2H using L-(+)-di-isopropyl tartrate – titanium isopropoxide, followed by C^2H_3Li-LiI opening, mesylation, and azidolysis are the main steps in a synthesis of (2S,3S)-4,4,4-[$^2H_3$]valine,[51] and mesylate displacement by azide is also featured in a route to (2S,1′S,2′S)-2-(carboxycyclopropyl)glycine (see also Ref 271).[52] The L-lysine keto-amide derivative $BocNH(CH_2)_4CH(NH_2)CO$-$CONHPh$[53] has been prepared similarly by oxirane ring-opening azidolysis, and nucleophilic opening of the epoxide formed from 4-$TBSO\text{-}C_6H_4CH(OH)$-$CH{=}CH_2$ is the crucial step in a synthesis of (2S,3R)-β-hydroxytyrosine.[54] A new procedure for the reductive transformation of azido esters into N-Boc-amino acid derivatives using $Pd(OH)_2$-C, $EtSi_3H$ and Boc_2O in ethanol has been outlined.[55] Mitsunobu azidolysis of the homochiral secondary alcohol $TolS(O)CH_2$-$CH(OH)CH_2F$ followed by sulfoxide cleavage through non-oxidative Pummerer rearrangement gives 3-fluoro-D-alanine.[56]



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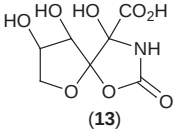

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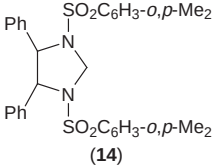


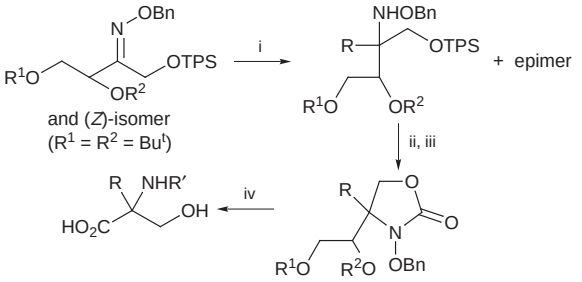
(**14**)

(Ethoxycarbonyl)nitrene, produced through photolysis *in situ* of ethyl azidoformate, reacts with β-silylated silyl ketene acetals $RCH(SiMe_2Ph)CH{=}C$-$(OMe)OSiMe_2Bu^t$ to give preferentially anti-β-silylated α-N-(ethoxycarbonylamino) esters.[57] Full details are available[58] describing the preparation of

N-substituted 3-alkyl-aspartic acids (Vol. 29, p. 13) through conjugate addition of amines to fumaric acid under catalysis by β-methylaspartase.

Enantioselective electrophilic amination by di-tert-butyl azodicarboxylate (S:R-ratios ranging from 90:10 to 95:5) of an achiral N-acyloxazolidin-2-one (*cf.* Scheme 5; H in place of Ph and R), is efficiently catalysed by (14), prepared from the bis-sulfonamide and dimethylmagnesium.[59] The use of this amination reagent, applied to preparation of α-amino-β-hydroxy acids from β-hydroxyester enolates, has been reviewed.[60] Palladium(0)-catalysed allylic amination of homochiral allyl acetates by simple amines, followed by oxidation, gives arylglycines and glutamic acid derivatives.[61]

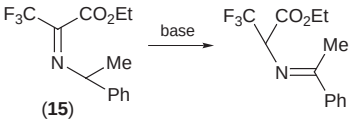
Oxime ethers of 2-furyl ketones $BzlON{=}CR^1R^2$ (R^2 = 2-furyl) undergo enantioselective alkylation with a homochiral boron complex, the furan moiety providing the carboxy group in the final stage of a novel α-amino acid synthesis (a route whose expense may be justified in certain circumstances).[62] A more straightforward method (see also Ref. 879) uses an O-benzyloxime (α-alkylation using an organolithium compound leading to αα-dialkylglycines; Scheme 1).[63]



Reagents: i, RLi; ii, TBAF; iii, carbonyldi-imidazole; iv, routine processing of 1,2-diol

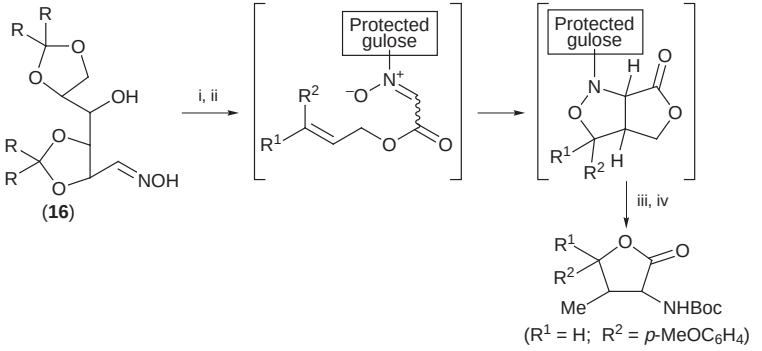
Scheme 1

The (R)-O-(1-phenylbutyl) ether of cinnamaldoxime has provided the substrate for alkylation using an organolithium compound in a diastereoselective synthesis of α-amino acids.[64] A proton shift induced by NEt_3 in the homochiral imine (15) starts a route to βββ-trifluoro-L-alanine.[65] Nitrones formed from aldoximes (*e.g.* from the protected L-gulose oxime, 16 in Scheme 2) have served in a synthesis of the N-terminal component of Nikkomycin Bz.[66]



(**15**)

A common feature of many of the preceding examples is their dependence on a supply of halogeno-acids and analogues, and a route to α-halogeno-amides from αα-dicyanoepoxides by reaction with a tertiary amine hydrohalide is notable.[67] Mitsunobu condensation of $Me_3Si(CH_2)_2SO_2NHCO_2Bu^t$ and a chiral cyano-



Reagents: i, methyl glyoxylate hemiacetal, toluene, reflux; ii, (*E*)-*p*-methoxycinnamyl alcohol; iii, $Mo(CO)_6$, 1% HCl-MeCN; iv, MsCl then NaI, $Bu^n{}_3SnH$ after $NH_2 \rightarrow NHBoc$

Scheme 2

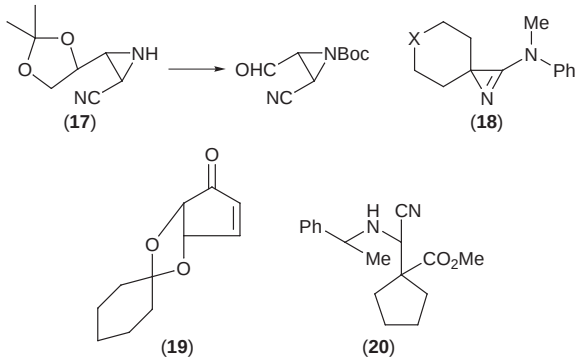
hydrin provides protected α-amino nitriles that are readily converted into α-amino acids.[68]

Electrophilic amination of chiral amide cuprates [from RCH_2COX (X = chiral amide moiety) with nBuLi/CuCN] by lithium tert-butyl N-tosyloxycarbamate illustrates further the improving prospects for carbamates as amination reagents in amino acid synthesis.[69] Benzyl carbamate serves in a route to 1-(Z-amino)-2-arylmethyl phosphinates ZNHCHArP(O)(Ph)R through condensation with ArCHO and dichlorophenylphosphine with acetyl chloride,[70,71] and in an equivalent route to phosphonates using an alkoxydichlorophosphine;[72] phenyl α-(Z-amino)benzyl phosphonates ZNHCHArP(O)(OH)OPh are obtained similarly.[73]

N-(Arene- or methanesulfonyl)aziridinecarboxylates formed as above (see also Refs. 281, 282) can undergo PdL_4-catalysed isomerization (L = ligand) as detailed in a study of five sets of four stereoisomers.[74] The expected higher stability of chiral alkyl (2E)-4,5-cis-(2E)-products, compared with their isomers, was established in this study. β-Erythro-substituted aspartic acids can be obtained through stereospecific nucleophilic ring-opening of dimethyl aziridine-2,3-dicarboxylates,[75] and hydrogenolysis of an aziridine to give (2S,3S)-(-)-3-methylphenylalanine has been described (Ref. 281). tert-Butyl (2R,3R)-2-cyano-3-formylaziridine-1-carboxylate has been obtained from the glyceraldehyde acetonide (17).[76] Further examples of ring-opening of 2H-azirin-3-amines (18; formed from N-methylanilides using $LiNPr^i{}_2$/DPPCl), *e.g.* with PhCOSH, leading to heterocyclic α-amino acid derivatives, have been reported (*cf.* Vol. 29, pp. 6, 22).[77]

Both syn- and anti-β-methyl-L-phenylalanines have been prepared starting from (2S,3S)-2,3-epoxy-3-phenylpropan-1-ol, ring-opening with $Me_2CuCNLi_2$, then mesylation and azidolysis being followed by routine functional group development.[78]

The classical Strecker and Bucherer-Bergs syntheses are also amination processes, illustrated for the former in preparations of (R)-N-Boc-3,5-dichloro-4-methoxyphenylglycine,[79] and for the latter with syntheses of '3-phosphonocyclo-

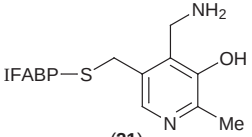


(**17**) (**18**) (**19**) (**20**)

butyl amino acids' (*i.e.* 1-amino-3-diethylphosphonocyclobutane-1-carboxylates) from the corresponding cyclobutanone.[80] (+)-2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (a potent and selective Group 2 mGluR agonist) has been prepared by cyclopropanation of cyclopentenone (19) and Bucherer-Bergs synthesis.[81] 4-Aminocyclohexanones have been converted into N,N′-Boc-hydantoins by the Bucherer-Bergs procedure followed by treatment with Boc_2O,[82] also the basis of preparations of 1-aminocycloalkanecarboxylic acids (see also Section 4.4) and α-methyl-(4-carboxyphenyl)glycine.[83]

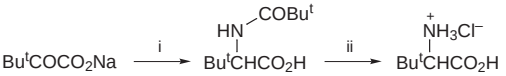
The asymmetric Strecker synthesis has been illustrated in an intramolecular version[84] for syntheses of both enantiomers of α-benzyl and α-carboxymethyl-serine, and for an improved synthesis of 'L-cyclopentylaspartic acid' [(S)-1-(2α-aminocarboxymethyl)-1-carboxycyclopentane] *via* the (S)-α-methylbenzylamino nitrile (20) on a large scale.[85]

The amination by pyridoxamine of an α-keto acid is a classical biogenetic route to amino acids, a fact that has stimulated a search for a laboratory equivalent, seen in the generation of glutamic acid using the pyridine reagent (21) covalently bound to the cysteine residue (Cys-60) of intestinal fatty acid-binding protein IFABP (*cf.* Vol. 29, p.13).[86] The amination rate is 62 times faster than that effected by pyridoxamine itself. Amination of α-keto-acids has also been illustrated for tert-leucine with an adaptation of the Leuckart reaction (Scheme 3),[87] and for syntheses of vinylglycines using a modified Mannich reaction (Scheme 4)[88] and α-aryl and α-heteroarylglycines.[89]



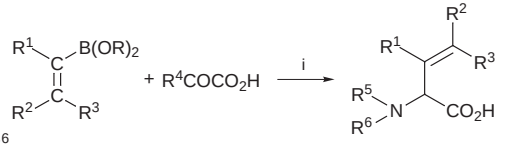
(**21**)

'Multicomponent reactions' employing amination reactions, used in α-amino acid synthesis (the Strecker synthesis, and Ugi and amidocarbonylation routes, amongst others covered later in this Section), have been reviewed.[90]



Reagents: i, $NH_4^+HCO_2^-$/HCO_2H, reflux; ii, 6M HCl, reflux

Scheme 3



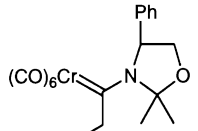
Reagent: i, R^5NHR^6

Scheme 4

The catalytic asymmetric aminohydroxylation of alkenes developed by Sharpless and co-workers (used in a synthesis of phenylisoserine;[91] see Vol. 29, p. 46 and Section 4.15, Refs. 400-403) has been reviewed;[92] this route provides aminoalkanols that are readily converted into α-amino acids, as illustrated with a synthesis of (S)-1-naphthylglycine *via* a homochiral 2,3-dihydroxyalkylamine.[93] Another enantioselective approach giving 3-amino-1,2-diols starts with a glyceraldehyde-derived α-alkoxynitrone (a relative of 16, see Scheme 2), and its arylation with a Grignard reagent in the presence of $ZnBr_2$ and Et_2AlCl.[94]

4.1.2 Carboxylation of Alkylamines and Imines, and Related Methods – Addition of 2-lithiofuran or 2-lithiothiazole to a sugar nitrone is essentially a carboxylation process as in the examples at the end of the preceding section;[95] this approach has been reviewed.[96] Attack of the 3-alkoxy-1-cyanopropene carbanion on a chloroformate is another hidden example of carboxylation,[97] and the classic route is illustrated by carboxylation by CO_2 after lithiation of a benzylamine. This leads to (R)-phenylglycines when (-)-sparteine is part of the reagent system (see also Ref. 329).[98] Use of (-)-sparteine – lithium carbanion pairs in enantioselective synthesis has been reviewed.[99]

A further example of carboxylation *via* chromium carbene complexes (Vol. 28, pp. 7, 15) employing a homochiral N-alkyloxazolidine has been published, illustrating alkylation (22; R = H to R = allyl, *etc*) and photolysis in the presence of a phenol to give the corresponding aryl ester.[100]



(22)

Achiral N-(mesitylsulfonyl)imines add homochiral α-bromovinyl-lithium species to give α-amino acid methyl esters with better than 95% e.e., after ozonolysis in methanol: MesN=CHR → MesNHCHRCBr=CR^1R^2 →

$MesNHCHRCO_2Me$.[101] The asymmetric synthesis of amines $NH_2CHR^1R^3$ by nucleophilic 1,2-addition of metal reagents R^3ML to imines $R^1CH{=}NR^2$ has been reviewed.[102] Equivalent syntheses of amino phosphonic acids include addition of hypophosphorous acid H_3PO_2 to an aldoxime, and mild oxidation of the resulting aminophosphinic acid.[103] An asymmetric synthesis of α-aminophosphonic esters has exploited the addition of a metal phosphite to the chiral sulfinamide TolS(O)N=CHAr.[104]

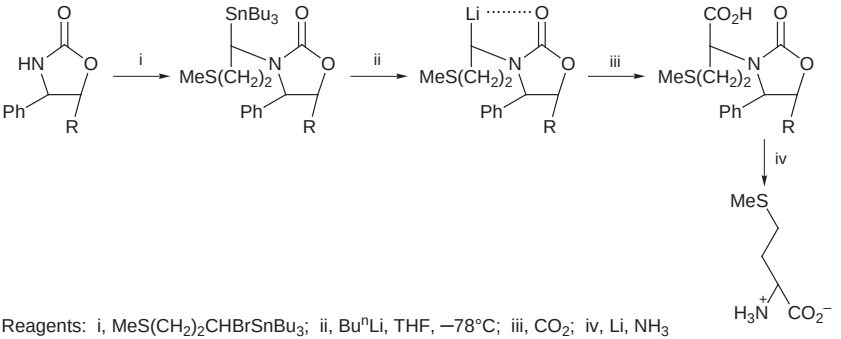
Conversion of a phthalimidoketene PhtNCR=C=O into the corresponding ester through addition to (R)-pantolactone generates a new chiral centre with good e.e. favouring the L amino acid;[105] this opens up a new deracemization protocol for N-phthaloyl-DL-amino acids.

The conversion of β-amino acids into α-amino acids [β-lactams give N-carboxylic amino acid anhydrides (Vol. 29, pp.23, 74) through NaOCl/TEMPO oxidation] has always been recognised to be limited in scope, since it is dependent on the supply of homochiral starting materials of known stereochemistry that are stable to the reaction conditions, but a further application, a tert-leucine synthesis, shows that good yields are obtainable.[106]

4.1.3 Use of Chiral Auxiliaries in Amino Acid Synthesis – Under this heading, established methods are included in which a homochiral grouping (N-acyl, ester, or aminoacylamide, *etc*) is released for re-use, in principle, at the end of an α-amino acid synthesis. Evans' oxazolidinones fall in this category, and their applications have been reviewed.[107] Typical examples include its use *via* azides, for syntheses of the four stereoisomers of β-methyl-3-(2′-naphthyl)alanine,[108] β-isopropylphenylalanine,[109] β-isopropyltyrosine,[110] and β-isopropyl-2′,6′-dimethyltyrosines[111] in the same approach already described in numerous papers from the Hruby group; the same method is used for the synthesis of 3-aminocarbonylmethylprolines (as glutamine mimetics).[112] Untypical examples include a route to L-methionine (Scheme 5) that can be completed in less that 40 minutes, so is potentially useful for the production of α-amino acids labelled in their carboxy group with ^{11}C.[113] A resin-tethered oxazolidinone has been used for α-hydroxy acid synthesis.[114] The indeno-oxazolidine (23) contributes efficient diastereoselection to the Wittig rearrangement of its N-allyloxyacetyl derivative, the resulting allylic α-hydroxy acid being converted into an amino acid through azidolysis and ensuing manipulations.[115] A route to L-proline involves allylation of the enolate of (S)-1-benzyloxycarbonyl-2-tert-butyl-3-methyl-1,3-imidazolin-4-one, hydroboration of the C=C bond and cyclization.[116] Double alkylation with (Z)-3-chloro-2-chloromethylprop-1-ene gives (24) and (25) from which (-)-baikiain and (-)-4-methyleneproline respectively were obtained in high yield.[117]

The related (4R,5S)-imidazolin-2-one may be N-acryloylated through an improved protocol employing DABCO as base.[118] Diastereoselective ring expansion of an N-(N′-acylaziridin)oyl moiety linked to this homochiral imidazolin-2-one, to give an oxazoline, is the basis of a route that provides homochiral β-hydroxy-α-amino acids.[119]

Oppolzer's camphorsultam continues to be used, *e.g.* for syntheses of enantiomers of amino acids bearing o- and p-carboranyl substituents in side-chains,[120] of

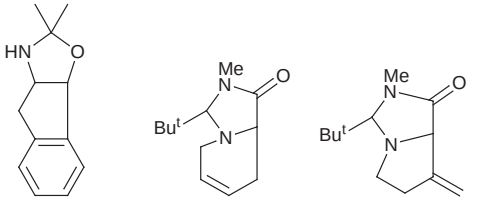


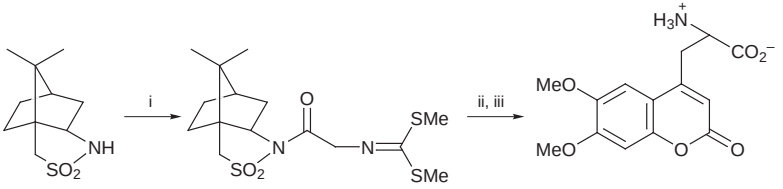
Reagents: i, $MeS(CH_2)_2CHBrSnBu_3$; ii, Bu^nLi, THF, −78°C; iii, CO_2; iv, Li, NH_3

Scheme 5

L-2,3,5,6-tetrafluoro-4-(phosphonomethyl)phenylalanine and L-4-(phosphonodifluoromethyl)phenylalanine,[121] and L-(6,7-dimethoxy-4-coumaryl)alanine (Scheme 6).[122] In a typical application, the sultam formed from glyoxylic acid oxime ether RON=CHCOX* (X* = sultam chiral auxiliary linked through N) undergoes highly diastereoselective radical addition ($RI/Bu_3SnH/Et_3B$) to give D-α-amino acids after standard functional group modifications.[123]

Details have been provided for routes employing ψ-ephedrine;[124] it is N-acylated in high yield and enolates of the resulting amides undergo highly diastereoselective alkylation (as described in Vol. 29, p. 22). Decagram quantities of L-prenylglycine (needed for conversion into Seebach's oxazolidinone) have been prepared using ψ-ephedrine glycinamide in this cost-effective route.[125] The early Seebach methodology based on an L-proline-derived auxiliary, given the label 'self-regulation of chirality', has been reviewed.[126]



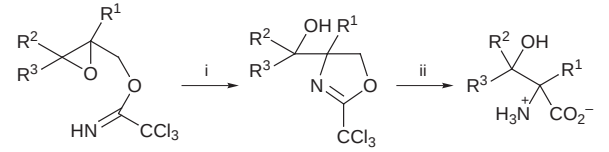


Reagent: i, $(MeS)_2C{=}NCH_2CO_2H$, unspecified coupling protocol; ii, BuLi, then 4-chloromethyl-6,7-dimethoxycoumarin; iii, H_3O^+, then aq. LiOH, then neutralisation

Scheme 6

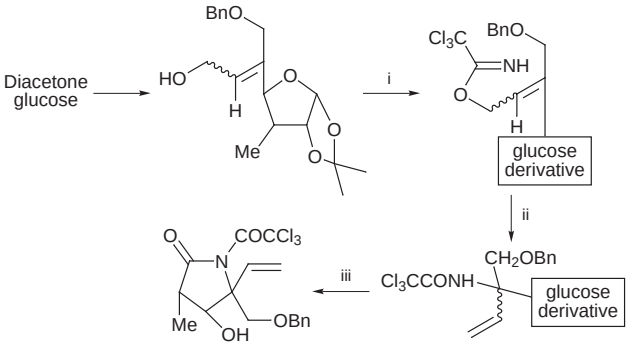
4.1.4 Use of Rearrangements Generating a Carbon–Nitrogen Bond – Rearrangement of trichloroacetimidates provides the stereochemical security associated with an electrocyclization process, and a clear example (Scheme 7) illustrates the preparation of α-substituted serines in good yields.[127] A new synthesis of (+)-myriocin illustrates the value of this rearrangement in routes to αα-disubstituted glycines.[128] A synthesis of (+)-lactacystin (Scheme 8) starts from D-glucose so as to generate the correct stereochemistry in the α-substituted α-amino acid moiety[129] (routes to this compound published in 1992-1994 differ in principle, since they all start from another amino acid).

A process used for the synthesis of α-allyl α-amino acids involves α-allylation of a homochiral α-cyanoalkanoate $R^1CH(CN)CO_2R^2$ followed by Curtius rearrangement with preservation of the initial stereochemistry.[130]



Reagents: i, Lewis acid, Et_2AlCl; ii, hydrolysis, then $CH_2OH \rightarrow CO_2H$

Scheme 7

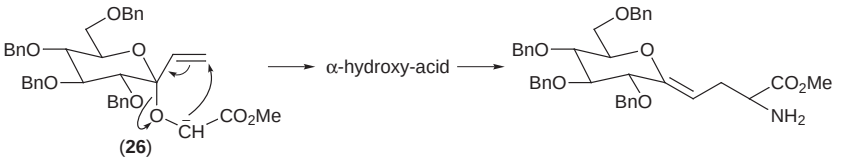


Reagents: i, NaH, then Cl_3CCN; ii, 140 °C, toluene; iii, H_3O^+ then aq. $NaIO_4$, then CrO_3–acetone

Scheme 8

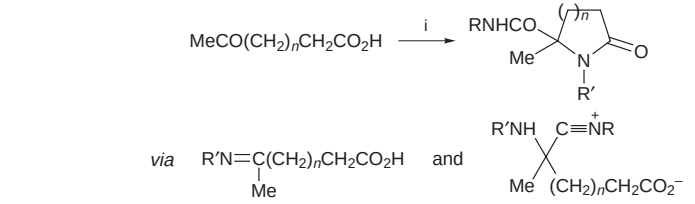
4.1.5 Other Rearrangements - [2,3]-Wittig rearrangement of a 1-vinylglycoside (26) is the essential step in a synthesis of a β-glycosidylalanine (obtained through amination of the intermediate hydroxyacid), useful as a C-analogue of β-D-glucopyranosylserine.[131]

4.1.6 Amidocarbonylation and Related Processes – The scope of this approach, reviewed recently,[132] is seen in a route from an alkene $RCH{=}CH_2$ with acetamide and CO to give α-(N-acetylamino)alkanoic acids $RCH_2CH_2CH(NHAc)CO_2H$ (R = C_8, C_{10}, C_{12}); the sodium salts have commercial uses since they yield viscous



aqueous solutions.[133] N-Acetylphenylalanines are formed from the aldehyde with acetamide and CO in a reducing atmosphere (H_2-DIPHOS with a cobalt carbonyl catalyst).[134] Palladium complexes catalyse the condensation of aldehydes and amides with carbon monoxide to give N-acylamino acids in good yields (99% for the synthesis of valine).[135]

The scope of the Ugi 'four-component condensation' in combinatorial synthesis, leading to amino acid derivatives (see Vol. 29, p.7), has been extended.[136] The same chemistry (without the combinatorial context) has been used for β-lactam synthesis,[137] and includes an intramolecular three-component version (Scheme 9) suitable for the synthesis of 7- and 8-membered lactams.[138] A so-called Ugi 'five-component condensation' uses an alkanol, an amine, an aldehyde, an isocyanide, and CO_2, COS, or CS_2 as oxidized carbon source, and leads to N-protected α-amino acid amides $R^1O_2CNR^2CHR^3CONHR^4$.[139]



Scheme 9

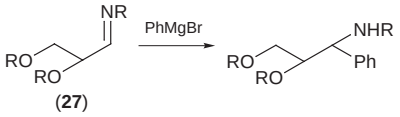
4.1.7 From Glycine Derivatives – The most familiar textbook example under this heading concerns alkylation of diethyl acetamidomalonate, $AcNHCH(CO_2Et)_2$, although conducting this approach with simpler glycine derivatives is now more common. It has been used for syntheses of β-(2-anthraquinolinyl)alanine,[140] 4-fluoro-3-nitrophenylalanine and its 3-fluoro-4-nitro-isomer (see also Ref. 326),[141] and DL-3-(2-furyl)-alanine.[142]

The new chiral atropisomeric αα-disubstituted glycine, α-(1,1′-binaphthylmethyl)-α-methylglycine and its biphenylmethyl analogue, have been prepared from a Schiff base of glycine tert-butyl ester, $4\text{-}Cl\text{-}C_6H_4\text{-}C{=}NCH_2CO_2Bu^t$.[143] Corresponding synthesis of a biphenyl-based amino acid has been reported.[144] The 1,1-bis(alkylthio)methylideneglycine esters $(RS)_2C{=}NCHR^1R^2$, have a long history of service in this context, recent acylation studies revealing their further possibilities in synthesis of α-alkyl-β-hydroxy-α-amino acids[145,146] (see also Scheme 6). The α-aminonitrile derivative $Ph_2C{=}NCH_2CN$ has been used for the

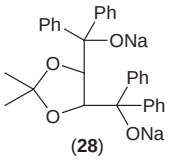
synthesis of 2-amino-6-hydroxyalk-4-enoic acids,[147] and as substrate for the preparation of γ-substituted vinyl phosphonates through Pd(0)-catalysed alkylation by CH_2=CHCH(OCO_2Me)P(O)($OR)_2$ (also used for preparation of carboxylates in an analogous route).[148] Alkylation of Ph_2C=NCH_2CO_2Me by fluoroalkyl bromides RCHFCH_2Br gives α-amino-γ-fluoroalkanoic acids,[149] while double alkylation of PhCH=NCH_2CO_2Et (at the α-carbon and at nitrogen) gives 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid derivatives when an o-xylylene dibromide is used.[150] Enantioselective alkylation can be effected (67 – 94% e.e.) using a *Cinchona* alkaloid-derived quaternary ammonium salt as phase transfer catalyst[151] or by conducting the alkylation in a homochiral reverse micelle medium.[152]

Esterification of these glycine Schiff bases to solid supports gives substrates that can be αα-di-alkylated in the usual way,[153] and mono-α-alkylated by unactivated alkyl halides.[154]

Numerous further examples of applications of glycine Schiff base synthons appear in this year's literature, as they have in all preceding Volumes, and for use in the preparation of aminodiols through addition of phenylmagnesium bromide to (27) with total diastereoselectivity leading to either (R)- or (S)-phenylglycine depending on the reaction conditions;[155] the same synthon has been used to prepare (2R)-4-oxopipecolic acid from the Danishefsky diene (see also Ref. 290) and L-vinylglycine using vinylmagnesium bromide,[156] and L- or D-trans-β-hydroxypipecolic acid through addition to 2-furyl TBS ether.[157] The long-established alkylation of the nickel(II) complex of an L- or D-prolyl-N-aryl-ideneglycine Schiff base (*cf.* Vol. 29, p. 15) has been used for the synthesis of L- and D-ββ-diphenylalanines,[158] (2S,4R)-4-methylglutamic acid [through Michael addition to methyl methacrylate catalysed by the chiral phosphine (4R,5R)-TADDOL, (28)],[159] (2S,3S)-3-trifluoromethylpyroglutamic acid,[160] and 3-per-fluoroalkyl-2,3-diamino acids.[161] Careful kinetic studies have shown that a five-minute alkylation by a substituted benzyl halide using acetone as solvent leads to satisfactory product yield and good diastereoselectivity, so the process is therefore suitable for the preparation of amino acids labelled with short-lived radio-isotopes (specifically, [β-^{11}C]-L-DOPA and [6-^{18}F]-L-DOPA).[162]



(**27**)

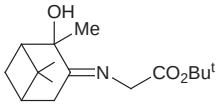


(**28**)

The homochiral Schiff base (29) is well suited to asymmetric amino acid synthesis, alkylation using ICH_2CHMeO$SiMe_3$ and standard elaboration giving L-γ-hydroxynorvaline.[163] The equivalent camphorsulfonamide Schiff base can be alkylated with representative halides and usually gives (S)-α-amino acids [but benzyl halides give (R)-products] and e.e.s are only moderate.[164]

An unusual glycine-related imine, MeC(OEt)=NCH($CO_2Me)_2$, *i.e.* an imidate,

has been used in β-hydroxy-α-amino acid synthesis through its propensity to undergo cycloaddition to aldehydes to give oxazolines.[165] An isocyanoacetic acid derivative is at first sight the prototypical glycine imine, but although this is oversimplifying its structure, it behaves as an imine with aldehydes to give oxazolines (and thence to α-amino-β-hydroxyacids).[166] Methyl isocyanoacetate forms oxazolines through reaction with ketones, but its diastereoselectivity is a more subtle detail that has been probed to establish the control exerted that is by transition metal catalysts and base.[167] Isocyanoacetates undergo double alkylation by a quinoxalylxylylene dibromide under solid-liquid phase-transfer catalysis, to give (30),[168] a protocol applied to other 1,2-bis(bromomethyl)arenes.[169] p-Boronophenylalanine has been prepared from ethyl isocyanoacetate.[170]



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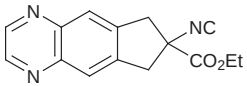
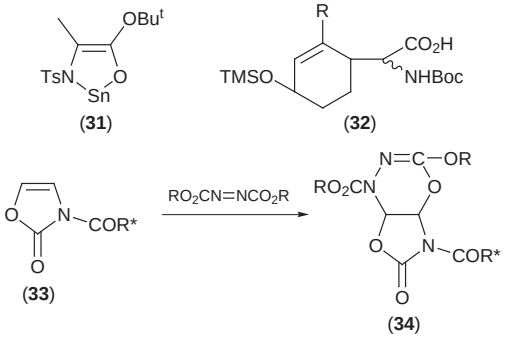


(**30**)

Protected α-bromoglycine offers access to nucleophilic attack; thus, anionic organotransition metal compounds react with α-bromoglycines to open up access to new organometallic amino acids.[171] Related glycine derivatives behave as nucleophilic synthons in standard amino acid syntheses, and α-acetoxyglycine deserves to be more widely used; thus a reasonable level of deracemization accompanies $Pd(OAc)_2$/(+)-BINAP-catalysed alkylation of its N-diphenylmethylidene ester $Ph_2C{=}NCH(OAc)CO_2Me$ by malonate anions to give β-carboxy-L-aspartates.[172] Organozinc reagents Ar_2Zn react with the protected acetoxyglycine in an efficient α-arylglycine synthesis,[173] and α-triazolylglycines are formed from α-amino-α-azidoglycine esters through cycloaddition of alkynes.[174] α-Hydroxyglycine is referred to in Refs. 88, 89. The new α-(toluene-p-sulfonyl)glycine derivative, $BocNMeCHTsCO_2Et$, is amenable to α-allylation, followed by reductive removal of the toluene-p-sulfonyl group (Mg/MeOH).[175] The phosphonate $BocNHCH[P(O)(OMe)_2]CO_2Me$ condenses with a homochiral aldehyde, illustrated for an efficient synthesis of C-galactosyl-L-serine using tetra-O-acetyl-D-galactopyranosylacetaldehyde, and Rh[DuPHOS]-catalysed hydrogenation of the intermediate αβ-dehydroamino acid.[176]

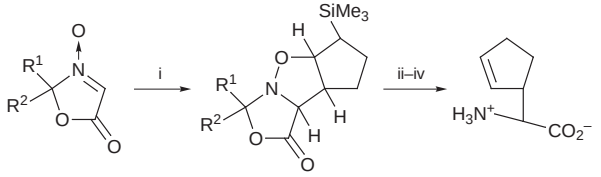
Phase-transfer catalysed alkylation of glycine derivatives,[177] and the wider range of alkylation, Michael addition, and aldolization of enolates,[178] have been reviewed. Deprotonation of N-(toluene-p-sulfonylamino)alkanoates with an excess of LDA and subsequent transmetallation with tin chloride probably results in the formation of the chelated enolate (31), which readily undergoes aldolization and gives trihydroxypipecolates (*alias* 'azasugars'), through addition to a protected aldotetrose followed by Mitsunobu ring closure.[179]

Claisen rearrangement of glycine enolates of homochiral diols provides an unusual example of a well-established process, leading to the cyclohexenylglycine (32) with chiral centres located with known configuration.[180] Enolates from $Ph_2C{=}NCH_2CO_2Me$ or $Bn_2NCH_2CO_2Bu^t$ and LDA have also been employed in



a synthesis of α-glycosyl α-amino acids (*cf* Ref. 131) through addition to an α-D-ribohexofuranos-3-ulose.[181]

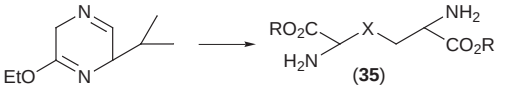
Oxazolones continue to hold their place as glycine derivatives that are amenable to C-alkylation (*cf* Ref. 920), illustrated by reaction of 4-ethoxycarbonyl-2-phenyl-oxazol-5(4H)-one with aryl-lead triacetates and with (E)-styryl-lead triacetates, to give α-aryl- and α-vinylglycines.[182,183] N-[cis-2-Alkoxy-1-apocamphanecarbonyl]oxazol-2-ones (33) undergo [4 + 2]-cycloaddition with dialkyl azodicarboxylates to give (34) and its isomer, from which α-amino acids and α-amino aldehydes are obtained by ring-opening and functional group manipulation.[184] Oxazol-4-one N-oxides formed from homochiral cyclic ketones through cycloaddition to nitrosoketene represent a distant relative of glycine that is a willing partner in cycloaddition reactions, leading to cyclopentenylglycines for example (Scheme 10).[185]



Reagents: i, 1-trimethylsilylcyclopent-2-ene, BF_3–Et_2O; ii, aq. $NaHCO_3$, Amberlite IRC–50; iii, H_2, Pd–C; iv, BF_3–Et_2O, Dowex 50W–X4

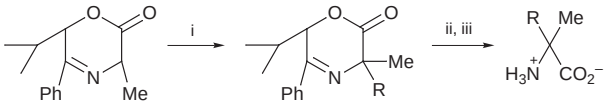
Scheme 10

Homochiral 2,5-diethoxy-3-isopropylpiperazine, now the favoured substrate for the Schollkopf amino acid synthesis, amounts to another hidden form of a glycine Schiff base. Further examples of bis(amino acid)s have been synthesized, *e.g.* diaminosuberic acid and analogues [35; X = -CH=CH- or -CH(OH)-CH(OH)-],[186] analogues with X = (Z)- or (E)-CR=CH-,[187] and with X = -CH=CH-,[188] and (35; X = substituted thiophen groupings),[189] typically using diiodoalkanes and -arenes with Pd-CuI for alkylating the Schollkopf synthon. (2S,9R)-2,9-Diaminododecanedioic acid has been prepared (using the higher order bislactim ether lithium cyanocuprate for creation of the first chiral centre,



(**35**)

and using Evans oxazolidinone methodology to introduce the second),[190] and similar manipulation of (S)-4-benzyloxazolidin-2-one has provided cyclopropane bis(glycine)s (35; X = cyclopropyl).[191] Use of the Schollkopf synthon provides D-(4α-cyanophenyl)alanine,[192] and 5-, 6-, or 7-membered ring 1-aminocycloalkane-carboxylic acids,[193] 1-amino-2-hydroxycyclopent-3-enoic acid,[194] also other 1-aminocycloalkene-1-carboxylic acids[195] through ruthenium(II)-catalysed ring-closing metathesis with alkenes. Alkylation by a brominated 3-methyl-6-methoxyindole to give the appropriate tryptophan is a key step in an enantiospecific total synthesis of tryprostatin A,[196] and a broad range of brominated tryptophans has been prepared in this way,[197] also 6-methoxytryptophans.[198] Face-selective 1,6-addition to 1E,3E-butadienylphosphonates opens up access to 2,3-anti-4E-2-amino-6-phosphonohexenoic acid derivatives.[199]

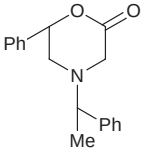


Reagents: i, K_2CO_3, RBr, TBAB, MeCN, r.t., or allylic carbonate/[$Pd(PPh_3)_4$]-dppe; ii, 6M HCl, 150 °C; iii, propylene oxide/EtOH

Scheme 11

Homochiral oxazin-2-ones, *e.g.* the (3R,6R)-3-methyloxazinone shown in Scheme 11, although little used so far for analogous asymmetric syntheses, have been shown to undergo diastereoselective alkylation to give (R)-α-substituted alanines.[200] Further uses for the related (5R)-phenyloxazin-3-one include cycloadditions of derived 1,3-oxazolium-4-olates to give enantiopure αβ-dihydroxy acids.[201] (3R,5R)-3,5-Diphenylmorpholinone undergoes Michael addition to methyl acrylate and its homologues, to give 3-substituted-2-phenylprolines after processing of the adduct,[202] and N-Z-4,5-diphenyl-tetrahydro-oxazin-2-one has been used in a synthesis of L-m-tyrosine.[203] The N-[(R)-1-phenylethyl] tetrahydro-oxazin-2-one (36) has been used in syntheses of α-substituted phenylglycines.[204]

The synthesis of methyl esters of acid-sensitive or highly-hindered α-amino acids from 1-Boc-2-tert-butyl-4-methoxy-2,5-dihydroimidazoles (37) succeeds in part because work-up following their alkylation calls only for mild conditions.[205]



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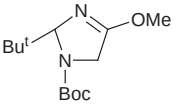

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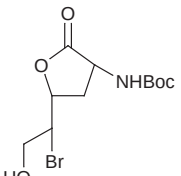
4.1.8 From 'Dehydro-amino acid' Derivatives – Theoretical aspects [molecular orbital calculations providing evidence for rhodium(I)/N-alkenylamide/phosphine complexes for homogeneous catalysis of the hydrogenation of N-acylaminoacrylates[206]] and synthesis objectives [asymmetric hydrogenation of 1-(formamido)alkenyl phosphonates[207]] represent interests that have been pursued for many years; the general topic has been reviewed.[208] The last-mentioned study employs (S)-BINAP-ruthenium(II) compounds that are typical of the highly-enantioselective catalysts on which attention is currently focussed. A new C_2-symmetric biphosphine, [2,2]PHANEPHOS, has been proposed for Rh-catalysed hydrogenations leading to 91 – 99.6% e.e. when applied to 2-aminoacrylates,[209] while the well-established Rh-DIPAMP system is favoured for synthesis of homochiral ferrocene-bridged bis(alanine).[210] A novel chiral Rh catalyst involving a bicyclo[3.2.0]heptane has been advocated and used in a synthesis of D-phenylalanines with high e.e.[211] Asymmetric hydrogenation [Rh/(R,R)-EtDuPHOS] of αγ-dienamide esters $R^1CH{=}CR^2CH{=}C(NHAc)CO_2Me$ formed by Suzuki cross-coupling and Horner-Emmons reactions gives the L-α-amino acid $R^1CH{=}CR^2CH_2CH(NHAc)CO_2Me$; for R^1 = $TBSOCH_2$, R^2 = H, a route to (+)-bulgecinine has been opened up *via* (38).[212]



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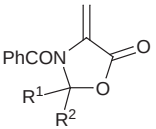

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Diastereoselective Michael addition of azomethine ylides derived from $Ph_2C{=}NCH_2CO_2R$ (or from the corresponding Schiff base formed between camphor and glycine tert-butyl ester) to the 4-methylene oxazolidin-5-one (39) has been used in a synthesis of all four stereoisomers of 4-benzamidopyroglutamic acid,[213] and the same intermediate leads through PPh_3-catalysed cycloaddition to allenes, to 1-amino-2- and -3-carboxycyclopent-2- and -3-ene-1-carboxylic acids, of interest as conformationally-restricted L-glutamic acid analogues.[214] A simpler alkylation procedure, $CrCl_3$-Fe-catalysed reaction of a perfluoroalkyl iodide with methyl α-acetamidoacrylate, gives corresponding α-aminoalkanoates.[215]

4.2 Synthesis of Protein Amino Acids and other Naturally Occurring α-Amino Acids – As an extension of the preceding Section, this concentrates on synthesis targets that either require modification of general synthesis methods, or require an individually tailored synthesis strategy.

The literature describing enzymic synthesis of common protein amino acids is already substantial, and is augmented in the current literature by accounts of production scale methods for L-cysteine (immobilized *Pseudomonas* M-38),[216] L-aspartic acid (immobilized *Brevibacterium flavum* and *E. coli* using ammonium

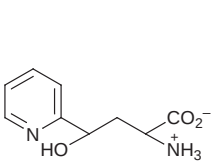
fumarate *via* L-malic acid),[217] L-lysine (*Corynebacterium glutamicum*),[218] L-[3-^{13}C]serine from [^{13}C]formaldehyde using L-serine hydroxymethyltransferase and tetrahydrofolate,[219] hyperproduction of L-threonine (modified *E. coli* that shows impaired threonine uptake),[220] L-[3-^{13}C]- and -[3-^{2}H]phenylalanine and leucine (*Brevibacterium methylicum* in $^{13}C^{2}H_3OH$ – $^{2}H_2O$),[221] L-tryptophan (genetically-modified *E. coli* that shows elevated tryptophan synthetase activity),[222] and a novel approach to L-β-aryl-α-alanines using red yeast cells (*Rhodotorula rubra* and *Rhodotorula glutinis*) with ammonia and a trans-β-arylacrylic acid.[223] An aminoacylase from *Bacillus thermoglucosidius* converts α-(chloracetamido)cinnamic acid into phenylpyruvic acid, which is a substrate for phenylalanine dehydrogenase and accounts for the production of L-phenylalanine by this organism.[224] Close relatives of the protein amino acids include S-adenosyl-L-methionine, prepared on a large scale from methionine using an *E. coli* strain.[225]

α-Keto-acids are substrates for the production of D-glutamic acid, D-phenylalanine, and D-tyrosine, based on a D-amino acid transferase/alanine racemase/L-alanine dehydrogenase/formate dehydrogenase system.[226] Reductive amination of α-keto-acids catalysed by leucine or phenylalanine dehydrogenase can give L- or D-amino acids together with L-α-hydroxy acids.[227] Homologation of ethyl (S)-lactate and development to $MeCH(OMOM)COCO_2H$ gives a substrate that is converted into the [^{15}N]-L-threonine derivative using leucine dehydrogenase, and to the allothreonine analogue from the appropriate precursor.[228] [1,2-$^{13}C_2$; ^{15}N]-L-Serine is formed through the action of serine hydroxymethyltransferase on the labelled glycine, and tryptophan synthase leads to the labelled L-tryptophan.[229] Recombinant D- and L-threonine aldolases effect the conversion of aliphatic aldehydes into erythro-β-hydroxy-α-amino acids, and aromatic aldehydes into their threo-analogues (syntheses of 3-hydroxyleucine, γ-benzyloxy- and γ-benzyloxymethylthreonines, and polyoxamic acid, are notable).[230]

Synthesis of (40), the N-terminal amino acid of Nikkomycins K_x and K_z, has employed a pyruvate aldolase-catalysed condensation of 2-pyridinecarboxaldehyde, pyruvic acid, and CO_2.[231]

Uses of transaminases, and of asymmetric hydrogenation of acylamidocinnamic acid derivatives, in commercial scale synthesis of non-natural amino acids, have been reviewed.[232] Reviews of the production of amino acids by methanol-utilizing bacteria[233] and more general production methods,[234] and of enzymic hydroxy-L-proline production,[235] have appeared, and also a review of enzymic synthesis methods used in an Edinburgh laboratory,[236] and more general review coverage,[237] including a broad general survey of the enzymology of amino acid production.[238] Access to this largely biotechnological field, for which only representative citations are given here, is facilitated by *Chemical Abstracts, Section 16: Fermentation and Bioindustrial Chemistry*.

A synthesis of D- and L-enantiomers of threonine and also their allo-isomers is based on an ingenious BF_3-catalysed hetero-Diels-Alder reaction of acetaldehyde with the azadiene (41) and its epimers.[239] Synthesis *via* DL-trans-4,5-dihydro-5-(4-methoxyphenyl)-4-methylisoxazoline-5-carboxylic acid, involving an acylase resolution stage, of (2S,3S,4S)-4-(4-acetoxyphenyl)-2-amino-3-methylbutan-4-



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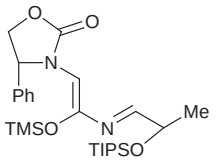
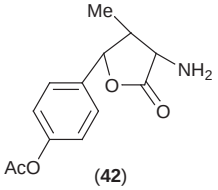


(**41**)

olide (42), a precursor for the unusual amino acid present in Nikkomycin B [also Refs. 66, 824 for other syntheses of Nikkomycin constituents] has been described.[240] Renewed interest in the synthesis of 'MeBmt', the N-methyl-L-threonine derivative that is a component of cyclosporins, is justified if shorter routes can be found that also allow analogues to be targetted; the syn-(2R)-amino-1,3,4-butanetriol derivative (43), accessible from D-isoascorbic acid, has been converted smoothly into the MeBmt precursor (44).[241] For new syntheses by manipulation of threonines, see Ref. 841. The protected component needed for preparing one of the unusual amino acids in microsclerodermins has been synthesized by incorporating a number of strategies newly introduced in this field, namely the use of a nitrone moiety and of a 2-furyl moiety that become the amino and carboxy group, respectively, in the synthesis target (Scheme 12).[242]



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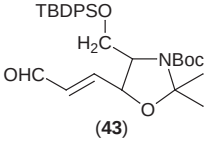

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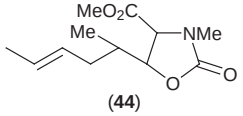

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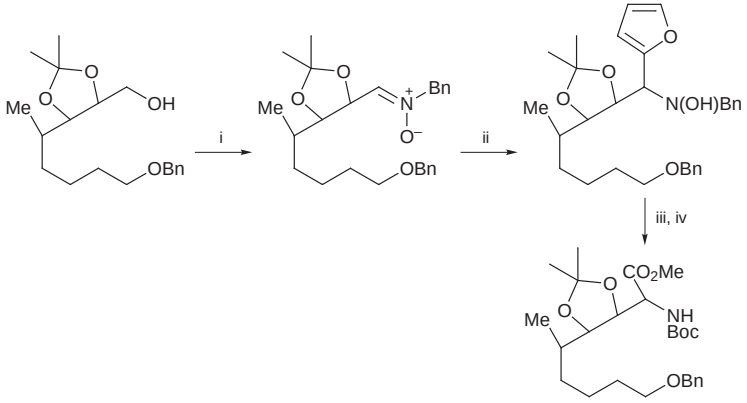


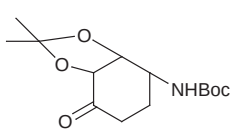
Reagents: i, $(COCl)_2$, DMSO, then BnNHOH; ii, 2-furyl-lithium/Et_2AlCl;
iii, separate epimers, react with $TiCl_3/H_2O$ then Boc_2O; iv, RuO_2, then MeI

Scheme 12

Total synthesis as the means of determining the absolute configuration of radiosumin, shown to be (S), has required the establishment of a synthesis of 2-amino-3-(4-amino-2-cyclohexylidene)propanoic acid and its cyclohexene analogue; the phosphonate $MeON{=}C(CO_2Me)CH_2PO(OMe)_2$ was condensed with the cyclohexanone (45) to give an intermediate from which both target amino acids were obtained.[243]

D- and L-Proline have been prepared starting from D-glucono-1,5-lactone *via* the D-erythrohexonate ester (46) through azide substitution, reductive cyclization into the pyrrolidine, and generation of the carboxy group.[244]

Kainoid synthesis is an area of vigorous exploration, in a search for appropriate methodology leading particularly to neuro-active analogues of these natural proline derivatives. Papers from research groups who have already established their interest in this field cover the introduction of substituents into trans-4-hydroxy-L-proline, giving correctly oriented C-3- and C-4-substituted kainoids.[245] Enamine alkylation for introduction of a C-3 carboxymethyl grouping, conversion into the 4-oxoproline, and subjecting this to Grignard addition or Pd(0)-catalysed cross-coupling to introduce C-4-aryl substituents, gives the useful intermediate (47), whose scope as a starting point for synthetic manipulations has been explored in related studies.[246]



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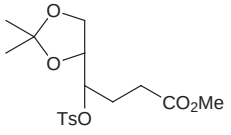

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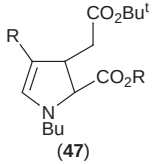

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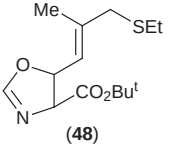
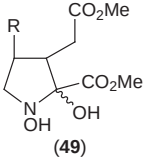
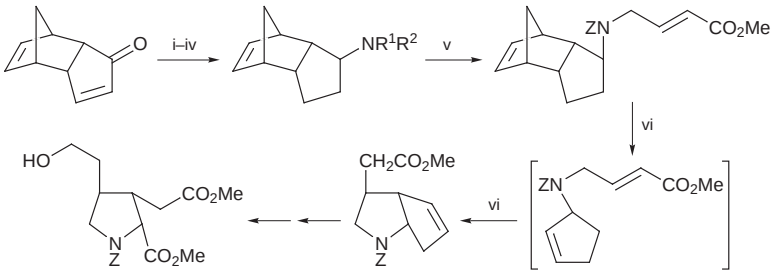


(**48**)



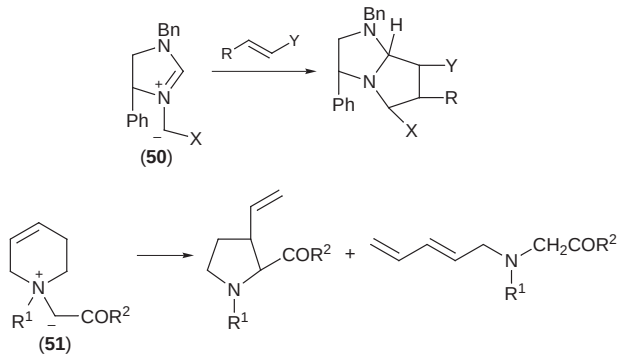
(**49**)

Bachi's proline ring-construction approach [Vol. 29, p. 25; alkylation of tert-butyl isocyanoacetate by 4-(ethylthio)-3-methylbut-2-enal catalysed by 10 mol $(C_6H_{11}NC)_2AuBF_4$/chiral bis(diphenylphosphino)ferrocene] has provided the oxazoline (48) from which (-)-kainic acid was obtained through standard functional group elaboration.[247] A proline ring-construction starting with Mitsunobu alkylation of (S)-$CH_2{=}CH(NHTs)CH_2OCH_2OMe$ with $PhSCH_2CMe{=}CH\text{-}CH_2OH$ and a subsequent thiyl radical addition – cyclization – elimination sequence has also provided (-)-kainic acid.[248] Racemic kainic acid analogues have been obtained through Michael addition of dimethyl α-ketoglutarate to 2-methoxy-β-nitrostyrene, taking advantage of the favourable 14:1 anti:syn mixture of adducts from which the cis-trans target stereochemistry (49) was achieved through reduction of the nitro group followed by cyclization.[249] The route to (-)-kainic acid *via* the Diels-Alder cycloadduct of a (+)-norcamphor synthon (Vol.

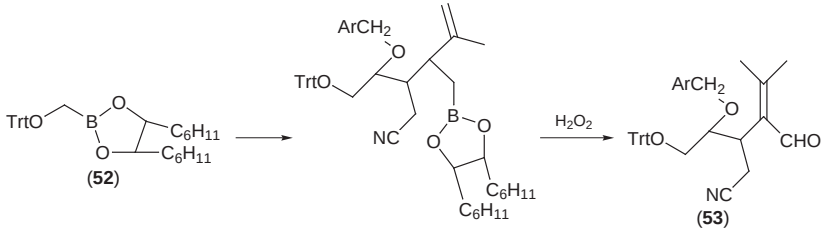


Reagents: i, Zn/AcOH–EtOH, reflux; ii, $NaBH_4$–MeOH; iii, DPPA; iv, $LiAlH_4 \rightarrow R^1 = R^2 = H$; v, $BrCH_2CH{=}CHCO_2Me$ then ZCl/NaH/DMF; vi, PhOPh, reflux 60 min

Scheme 13

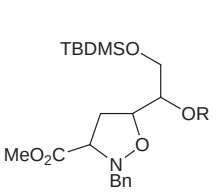


21, p. 15) has been explored further,[250] and a new stereocontrolled route to (-)-kainic acid from the same research group employs concurrent retro-Diels-Alder and intramolecular ene reactions of the optically pure ketodicyclopentadiene shown in Scheme 13.[251] Trisubstituted pyrrolidines including kainoids are accessible from intramolecular cycloadditions of homochiral azomethine ylides (50).[252] [3,2]-Sigmatropic rearrangements of didehydropiperidinium ylides (51) can give acceptable yields of disubstituted proline derivatives as ring-contraction products although the route is seriously devalued by competing elimination.[253] Stepwise additions of dihalogenomethyl-lithium to the homochiral boronic acid ester (52) gave the aldehyde (53) on H_2O_2 cleavage of the C-B bond, rather than the expected sec-kainic acid.[254]

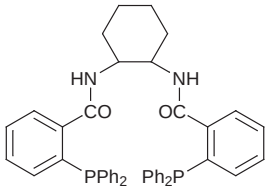


Kainic acid analogues with the isopropenyl moiety replaced by the $CF_3C(N_2)CO$- group should prove valuable probes for tissue studies aimed at mapping kainoid receptors.[255] The same objective has stimulated studies of 1,3-cycloaddition of $PhCH_2N(O)=CHCO_2Me$ to (Z)-(2-X-C_6H_4)$CH=CHCH_2$-CO_2Me for a preparation of oxa-analogues[256] (for aza-analogues, see Ref. 879).

(-)-Bulgecinine has been prepared (see also Section 6.3, Ref. 859) together with three of its isomers from the 1,3-dipolar cycloadduct (54) of N-benzyl α-methoxycarbonyl methanamine N-oxide with the homochiral allylic alcohol (R)-$H_2C=CHCH_2CH(OH)CH_2OSiPh_2Bu^t$.[257]



(**54**)



(**55**)

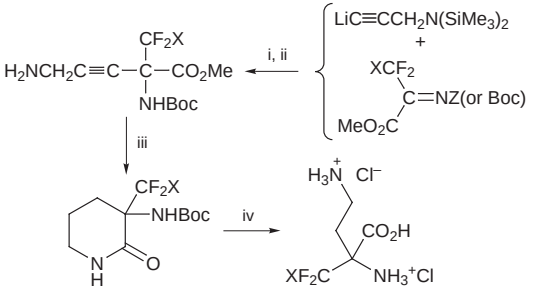
4.3 Synthesis of α-Alkyl-α-Amino Acids – Standard general methods leading to these compounds are reliable, and, apart from the importance of the targets as potential enzyme inhibitors, points of interest are mostly to be found in difficulties in synthesis, associated with steric hindrance. The applications for the synthesis of α-alkyl-α-amino acids, of the Schollkopf, Strecker, and Seebach methods (see examples in Sections 4.1, 4.2) have been reviewed,[258] and a review of the general topic has appeared.[259]

The Seebach imidazolidin-4-one, as its 3,5-dimethyl derivative, has been applied to the synthesis of [^{18}F]fluoro-α-methyl-L-phenylalanines,[260] and 2- and 3-[^{18}F]fluoro-α-methyl-L-tyrosines have been prepared.[261] Alkylation of DL-4-methyl-2-phenyloxazol-5(4H)-one with 3-acetoxycyclohexene is highly stereospecific when catalysed by [(η^3-$C_3H_5PdCl)_2$] in the presence of the chiral ligand (55; 8.7:1 d.e.),[262] applicable also for a synthesis of serine analogues using $PhCH=CHCH_2CH(OAc)_2$ as alkylating agent. The N-(2-cyanopropionyl) derivative of the sultam introduced by Oppolzer undergoes efficient diastereoselective alkylation with methyl bromoacetate to provide (S)-α-methyl-aspartic acid.[263] N-2-Alkenylsultams act as dipolarophiles towards diazomethylsilanes Me_3SiCHN_2, to give α-alkyl-azaprolines.[264]

The direct α-alkylation of an amino acid is rarely used because of the need for full protection, but ethyl N-Boc-N-methyl-L-phenylalaninate meets this criterion and lithium 2,2,6,6-tetramethylpiperidide and methyl iodide at -78°C brings about α-methylation with retention of chirality (82% e.e.).[265] Protected (R)-4-hydroxyphenylglycine similarly gives the α-methyl analogue,[266] and so does L-tryptophan, protected by conversion into bis-N-benzyloxycarbonyl tetrahydropyrrolo[2,3-b]indole-2(S)-carboxylic acid methyl ester.[267] The L-proline-derived

Seebach oxazolidinone undergoes α-alkylation by α,α′-dibromo-m-xylene to provide a novel ligand for a tetraprolinate dirhodium catalyst.[268]

Imines are more easily alkylated, illustrated in a new efficient synthesis of α-difluoromethyl- and α-trifluoromethyl-ornithines (Scheme 14) in which a synthon carrying the latent side-chain of the named amino acid reacts with a halogeno-methylimine.[269] This alternative approach is also needed for the large-scale preparation of α-phenyl-α-amino acids, *viz*. phase-transfer-catalysed α-allylation of N-benzylidene-DL-phenylglycine, then successive resolution using an esterase (but better results were obtained using the *Ochrobactrum anthropi* amidase; see also Ref. 471), hydroboration and Mitsunobu cyclization to give (R)-α-phenyl-proline.[270]

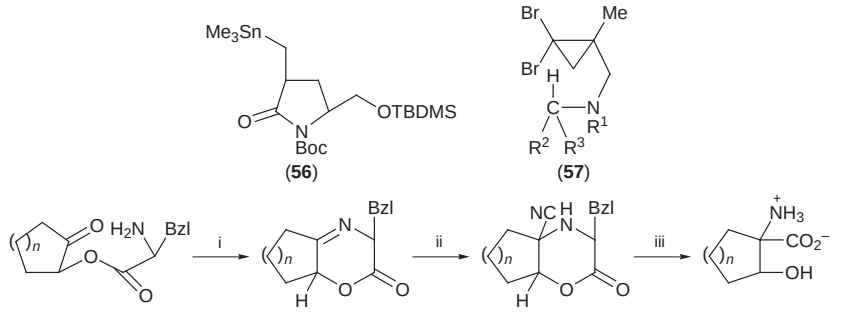


Reagents: i, −78 °C → r.t.; ii, aq. HCl; iii, H_2, Pd/C–MeOH; iv, 6M HCl

Scheme 14

4.4 Synthesis of α-Amino Acids Carrying Alkyl Side-chains, and Cyclic Analogues – The synthesis of close analogues of the aliphatic protein amino acids, as well as alicyclic and saturated heterocyclic examples, is surveyed here (see also Section 6.3); but several studies, mentioned earlier under the heading of general methods of synthesis, also extend to non-protein amino acids.

'Methano-amino acids', perhaps better described as α-cyclopropylglycines and their homologues, are of continuing interest as conformationally-constrained analogues of familiar protein amino acids. Synthesis methodology is straightforward for (2S,1′S,2′S)-(2-carboxycyclopropyl)glycine (oxazolidinone method)[271] and its α-methyl homologue,[272,273] and (S)-2-amino-2-methyl-4-phosphonobutanoic acid, following routes already applied (Vol. 29, p. 29) to the synthesis of these targets as glutamic acid mimics, isotype-selective agonists of metatropic glutamate receptors (see also Refs. 52, 81). (2S,3R,4S)-4,5-Methanoproline and the corresponding 5,6-methanopipecolic acid enantiomer have been prepared by a novel intramolecular cyclopropanation of iminium ions, *e.g*. from L-pyroglutaminol to give the former *via* (56),[274] and by intramolecular insertion into C-H bonds five atoms distant from a tertiary amine function (*cf*. 57).[275] 2-Fluoro-1-aminocyclopropane-1-carboxylic acid has been prepared by cyclopropanation of the fluoroacrylate followed by standard manipulations (ester → NH_2; aryl moiety → CO_2H).[276]



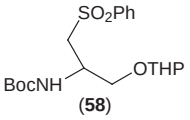
Reagents: i, base; ii, NH_4CN, aq. MeOH; iii, H_2/Pd then hydrolysis

Scheme 15

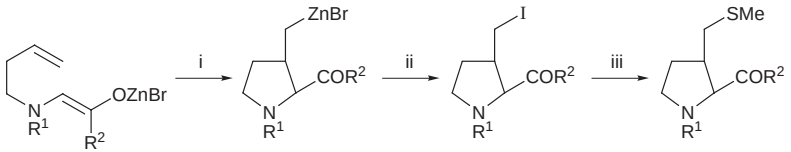
1-Aminocyclobutanecarboxylic acid carrying the 2-[1,7-dicarba-closo-dodecaboran(12)-1-yl]ethyl substituent has been prepared for its potential in neutron capture therapy, using the hydantoin general synthesis applied to the corresponding cyclobutanone.[277] Synthesis of 2-hydroxy-1-aminocycloalkanecarboxylic acids, designed as conformationally-restricted serine analogues, has involved a novel intramolecular Strecker procedure (Scheme 15).[278] Further examples of these types of conformationally-restricted analogues of common amino acids have been prepared by exploiting the dienophilic character of 2-phenyl-4-alkylideneoxazol-5(4H)-ones (Vol. 29, p. 18; *cf* also Ref. 290).[279] 1-Aminocyclohexanecarboxylic acids constructed in this way carry substituents with known stereochemical relationships to each other, as illustrated for the synthesis of (1R,3R,6R)-1-amino-3-hydroxy-6-phenylcyclohexane-1-carboxylic acid and its enantiomer.[280]

Synthesis of (2S,3R)-3,3-disubstituted aziridine-2-carboxylic acids through highly selective syn-addition of MeMgBr to enantiopure ethyl 3-phenyl 2H-azirinecarboxylate,[281] and of dimethyl (R)-2-methylaziridine-1,2-dicarboxylate [from the (R)-epoxide prepared from $MeO_2CNHCH_2CMe{=}CH_2$ through chloroperoxidase-mediated asymmetric epoxidation],[282] has been reported.

trans-3-Substituted prolines have featured in a number of papers in which novel routes are explored. Homochiral sulfone (58) condensed with 2-bromoethyl triflate gives cis- and trans-3-allyl prolines.[283] Cyclization of a zinc enolate with a non-activated alkene favours cis-diastereoisomers (Scheme 16), a route demonstrated in a synthesis of proline analogues of methionine and valine [*e.g.* (2S,3S)- and (2S,3R)-3-(methylsulfanylmethyl)pyrrolidine-2-carboxylic acid].[284] A parallel study building on Normant's preceding work with zinc enolates, introducing zinc-ene-allenes, has also been published.[285] [2 + 2]-Cycloaddition of N-benzyloxycarbonylpyrrolid-2-ene to dichloroketene gives the expected dichlorocyclobutanone, from which 3-substituted prolines were obtained (diazomethane ring expansion or ozonolysis of the derived enol acetate).[286] 4,5-Dehydro-L-proline gives 4,5-disubstituted homologues through this route. C_2-Symmetric pyrrolidine-2,5-dicarboxylates and -2,3,4,5-tetracarboxylates have been prepared through 1,3-dipolar cycloadditions to azomethine ylides.[287]



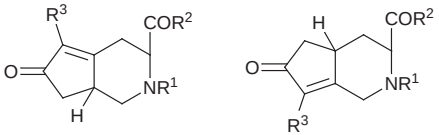
(**58**)



Reagents: i, −90 °C → r.t.; ii, I_2; iii, MeSNa, DMF (R^1 = benzyl, with R^2 = ethoxy, menthyloxy, phenylmenthyloxy, and camphorsultam; R^1 = (–)-α-methylbenzyl, with R^2 = ethoxy)

Scheme 16

3-Phenyl-4,5-benzoprolines (more correctly, cis- and trans-3-phenylindoline 2-carboxamides) have been prepared from indoles as representatives of conformationally constrained phenylalanines, the route being easily generalized to provide correspondingly constrained analogues of other protein amino acids.[288]



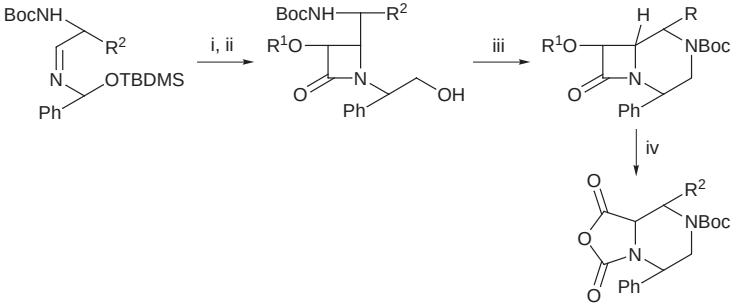
(**59**) (**60**)

Isomeric pipecolic acids (59) and (60) have been prepared through intramolecular Pauson-Khand cyclization,[289] and a Diels-Alder route (*cf.* also Ref. 290) involving the Danishefsky diene leads from the D-glyceraldehyde-derived imine $PhCH_2N{=}CHCH(OCH_2Ph)CH_2OCH_2Ph$ to (2R)-4-oxopipecolic acid (see also Ref. 156).[290] Intramolecular addition of allylsilanes to iminium salts $CH_2{=}N^+CHRCH_2OH\ X^-$ formed between a homochiral β-aminoalkanol and glyoxal starts a route to 3,4-functionalized pipecolic acids.[291] A more traditional route starting from 3-hydroxypyridine-2-carboxylic acid or quinolinic anhydride gives corresponding substituted pipecolic acids in racemic form.[292]

Piperazine-2-carboxylic acids are reached through a lengthy route starting from an acyclic N-Boc-α-amino imines (Scheme 17), including a β-lactam → N-aminocarboxylic anhydride stage (*cf.* Ref. 106).[293]

4.5 Models for Prebiotic Synthesis of Amino Acids – Theories of prebiotic amino acid synthesis have been reviewed,[294] and a limited description of this chemistry has been published.[295]

Irradiation (254 nm) of a propene – ammonia mixture in dry and aqueous environments would have been thought to generate amino acid mixtures, based on more than forty years of similar studies, but we are told that the four nucleic acid bases adenine, guanine, thymidine and uracil are formed.[296] Recent examples



Reagents: i, R^1OCH_2COCl, NEt_3; ii, TBAF; iii, MsCl, Et_3N then TFA, then Boc_2O; iv, NaOCl, TEMPO or P_2O_5–DMSO followed by *m*-CPBA

Scheme 17

of the expected outcome include the formation of amino acids from $CH_4 - NH_4X - H_2O$ at 260-325 °C to simulate undersea thermal vent conditions;[297] some amino acids decompose under these conditions, but it has been reasoned[298] that the particular environment in hydrothermal vents protects amino acids and that laboratory models are misleading, since glycine, alanine and glutamic acid have been found in an outflow from the Okinawa Trough. Amino acids are formed from $CO - N_2 - H_2$ in a magneto-plasma dynamic arc jet,[299] and from $CH_4 - N_2 - CO - H_2O$ under spark discharge or irradiation with high energy particles.[300] The last-mentioned study, intended to simulate primitive planetary environments, shows that high yields of amino acids, as well as uracil and imidazole, are formed through the irradiation process, suggesting that cosmic-ray induced synthesis was important in this context in prebiotic times.

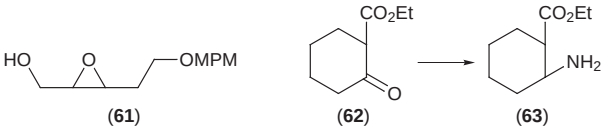
4.6 Synthesis of α-(ω-Halogeno-alkyl)-α-Amino Acids – Alkylation of a homochiral imidazolidin-4-one (*cf*. Section 4.1) has formed the basis of a synthesis of (2S,3S)-4-fluorothreonine.[301] 5,5,5,5′,5′,5′-Hexafluoro-L-leucine has been prepared starting from hexafluoroacetone, giving $(CF_3)_2CHCH_2COCO_2Et$ with ethyl bromopyruvate, baker's yeast reduction introducing the required homochirality preceding routine aminolysis.[302] (-)-4,4,4,4′,4′,4′-Hexafluoro-D-valine has been prepared from $(CF_3)_2C{=}CHCO_2Bn$ through Michael addition of (R)-$PhCHMeNH_2$ as the crucial step, incidentally correcting an earlier assignment of configuration to the (-)-isomer obtained in this way.[303]

These illustrate uses of standard asymmetric synthesis methods, and the modest diastereoselectivity sometimes achieved; in a further example, condensation of 1-bromo-2-fluoroalkenes with glycine ester imines derived from R-(+)-camphor has given seven homologous α-fluoroalkyl-α-amino acids, (R)-(-)-2-amino-4-fluorobutanoate being obtained in 32% e.e.[304] Preparations of 3-fluoroalanine (Ref. 56) and 3,3,3-trifluoroalanine (Ref. 65) are discussed elsewhere.

4.7 Synthesis of α-(ω-Hydroxyalkyl)-α-Amino Acids – Reviews include the use of aldonolactones in the asymmetric synthesis of hydroxyamino acids,[305] and routes to O-glycosyl-α-amino acids.[306]

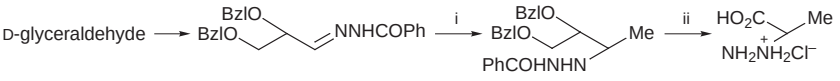
Familiar aldol alkylation and epoxide ring-opening alkylation processes have been used for access to α-amino acids carrying β-hydroxyalkyl side-chains. Thus, the essential step in a synthesis of (2S,3R)-β-hydroxyornithine involves amination of the epoxide (61) with benzyl isocyanate.[307] An alternative approach with hydroxy groups in place from the start is offered by carbohydrate-based synthons, as with the D-glyceraldehyde nitrone (*cf.* Scheme 2; *cf.* Ref. 94) converted into a propargylhydroxylamine with LiC ≡ $CSiMe_3$, thence to the dioxolanylglycine, a protected form of a β-hydroxy-α-amino acid.[308] All stereoisomers of β-hydroxynorvaline have been synthesized, each using an oxazolidinone enantiomer (*cf.* Scheme 5; $BzlOCH_2$ in place of Ph, CH_2CH_2R in place of R; stereochemistry as appropriate to the target stereoisomer).[309]

The net change L-valine → (2S,3S)-γ-hydroxy-L-valine has been accomplished, exploiting 1,2-asymmetric induction occurring in anti-Markovnikov hydrobromination of βγ-dehydro-L-valine.[310] Through this synthesis, identity with the amino acid occurring naturally in leaves and stems of *Kalanchoe diagremonitana* was established.



(**61**) (**62**) (**63**)

4.8 Synthesis of N-Substituted α-Amino Acids – Preparation of N-alkylamino acids has been reviewed.[311] A standard reductive N-alkylation route has provided N^{α}-ω-(Y-alkyl)-α-amino acids (Y = RS, RNH, or HO_2C);[312] where a benzylamine is employed for reductive amination of keto-acid derivatives, hydrogenolysis of the resulting N-benzylamino acid is one of the standard amino acid synthesis protocols, and where (R)-$PhCHMeNH_2$ is used, then useful stereocontrol may be achieved (62) → (63).[313] α-Hydrazino acids feature in a continuing study (a preparation of α-hydrazinopropanoic acid is shown in Scheme 18).[314]



Reagents: i, MeMgBr, $CeCl_3$; ii, successively, Boc_2O, $H_2/Pd(OH)_2$, $RuCl_3/NaIO_4$, then $H_3O^+Cl^-$

Scheme 18

The extraordinary generation of N-propylamides of glycine and alanine from reaction mixtures comprising glucose or ribose with propylamine has been studied further[315] [but the essential basis of this process has already been established (Vol. 29, p. 9].

Interest continues in the preparation of peptides (PNAs; first reported in 1991),[316] made through condensation of (N-aminoethyl)glycines that carry N-(pyrimidin-1-yl)alkanoyl and N-(purin-1-yl)alkanoyl or related groups. A paper describing current work with these compounds advocates Mmt-N-protection.[317] Interactions of PNAs with nucleic acids, and some mimicking of the behaviour of DNA by PNAs, have provided new understanding of certain properties of these biologically-important natural products, and PNAs have been advocated for a role as primordial genetic material.[318]

Monomers needed for this purpose have been synthesized by various means, and a Mitsunobu approach with N-Boc-N-(2-hydroxyethyl)glycine methyl ester as substrate has been disclosed.[319]

4.9 Synthesis of α-Amino Acids Carrying Unsaturated Aliphatic Side-Chains – 'Dehydro-amino acids', *alias* αβ-unsaturated α-amino acids (see also Section 4.1 and Refs. 176, 813), are easily prepared from imines $(MeS)_2C{=}NCH_2CO_2R$ through addition to electron-deficient alkynes,[320] and addition of nucleophiles to conjugated alkynoates (*e.g.*, phthalimide to alkyl propiolates) in the presence of PPh_3 is a practical new alternative.[321] Formation of dehydroamino acids from pyruvic acid through condensation with benzyl carbamate, then N-acylation with bromoacetyl bromide, illustrates a classical approach.[322]

Amination of γ-silylated αβ-unsaturated esters using ethyl N-[(4-nitrobenzenesulfonyl)oxy]-carbamate gives βγ-unsaturated α-amino acid esters, *e.g.* $CH_2{=}CHCMe(CO_2Me)NHCO_2Et$.[323]

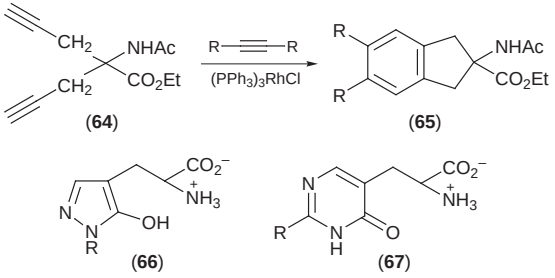
The synthesis of γδ-unsaturated α-amino acids, in ways other than direct allylation of glycines, can involve chelate – enolate Claisen rearrangement of alkenyl esters,[324] or use of unsaturated organozinc reagents with N-(phenylsulfano)imines $PhSN{=}CHCO_2Me$ to give $CH_2{=}CHCH_2CH(NHSPh)CO_2Me$.[325] α,β-γ,δ-Unsaturated amino acids have been described (Ref. 812).

4.10 Synthesis of α-Amino Acids with Aromatic or Heteroaromatic Groupings in Side-chains – Members of the phenylalanine family continue to attract interest through their pharmacological importance, and standard synthesis protocols have been used [4-fluoro-3-nitro-DL-phenylalanine from acetamidomalonate (see also Ref. 141),[326] alkylation of glycine benzophenone imine $Ph_2C{=}NCH_2CO_2Et$ by (4-pinacolylborono)benzyl bromide,[327] and for preparations of N-Boc tyrosine methyl ether carrying various 3′-substituents[328]]. (N-Boc-Aminomethyl)arenes have been elaborated into α-, β-, and γ-aryl amino acids through asymmetric lithiation induced by (-)-sparteine (see also Refs. 95, 96), *via* $Ar^1N(Boc)CH(SnMe_3)Ar^2$.[329] Diphenylalanine derivatives $AcNHCH(CO_2Me)CRPh_2$ and analogues in which the two phenyl groups are linked by aliphatic chains $-(CH_2)_n-$, have attracted interest in view of their 'crowded' side-chains,[330] and conformational constraint has been designed into the general β-arylalanine structure through radical cyclization of N-(o-iodophenyloxymethyl)-N-Boc-dehydroalanine to produce 2,3,4,5-tetrahydro-1H-3-benzazepine-2-carboxylic acid and its benzazocine and benzazonine analogues[331] and 8- and 9-membered ring homologues.[332] Similar objectives lie behind the preparations of 1-amino-1-carboxyindane

5-phosphonic acid and the analogous 3-amino-tetrahydrochroman-3,6-dicarboxylic acid[333] and isoindolinones.[334] A novel approach employing the αα-bis(propargyl)glycine (64), leading to 2-amino-2-carboxyindanes (65), required the discovery of the optimum glycine synthon towards bis(propargyl)ation; that turned out to be ethyl isocyanoacetate.[335]

A constrained tyrosine has been prepared (three-carbon aliphatic chains join one o-position to the β-C, and join the other o-position to the nitrogen atom) for its potential value as a constituent of analogues of biologically-active peptides.[336]

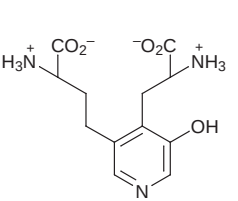
Hydantoin and oxazolone protocols have been applied to the preparation of L-thienylalanines *via* microbial transamination of 2-hydroxy-3-thienylacrylic acid with L-aspartic acid as amino group donor.[337] Bucherer-Bergs synthesis of benz[f]tryptophan from the corresponding formylmethyl N^{in}-allyl benzindole is straightforward.[338] N^{in}-Fmoc-2-Phenyltryptophan has been prepared on a multigram scale from 3-diethylaminoethyl-2-phenylindole through condensation with ethyl nitroacetate,[339] and (7-carboxyindol-3- and -4-yl)glycines have been prepared through alkylation of glycine cation equivalents $BzNHCH(OMe)CO_2Me$ and $Ph_2C{=}NCH(OAc)CO_2Et$.[340]



γ-Tetrazolyl α-aminobutyric acid[341] and related β-heteroaryl alanines (66) and (67) have been prepared, the last-mentioned examples through the 'ring-switching' approach starting from pyroglutamates (Vol. 28, p. 34).[342] The preparation of β-heteroaryl alanines has been reviewed.[343] Analogues of 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (a long-studied neuroactive agent) have been synthesised through established methods (Vol. 29, p.38; Vol. 28, p. 33).[344]

The first total synthesis of the protein cross-linking amino acids deoxypyridinoline and hydroxypyridinoline *via* the 3-hydroxypyridine (68) has provided reliable standards for the development of a diagnostic kit that can be used for monitoring the onset of osteoporosis.[345]

Stimulated by growing interest in PNAs (Section 4.8), the synthesis of N-Fmoc-amino acids carrying a nucleobase in the side-chain has been explored, starting with γ-benzyl Boc-L-glutamate and proceeding *via* the protected 2-amino-4-bromobutanoic acid.[346] A similar approach provides N–Boc-4-(pyrimidin-1-yl)-L-prolines and related γ-amino acid derivatives.[347] ω-(Pyrimidin-4-yl)-α-aminoalkanoic acids, including L-lathyrine, have been prepared through condensation of amidines with alkynyl ketones derived from α-amino acids.[348]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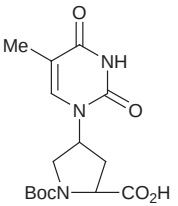

(69)

4.11 Synthesis of α-Amino Acids Carrying Amino Groups, and Related Nitrogen Functional Groups, in Aliphatic Side-chains – Schiff bases of ethyl glycinate $Ph_2C{=}NCH_2CO_2Et$ are readily alkylated by imines [with ArCH=NAr → $Ph_2C{=}NCH(CHArNHAr)CO_2Et$],[349] and undergo oxidative dimerization [with I_2 → protected threo-3-aminoaspartic acid][350] to lead to members of the aminomethylglycine family. The nickel(II) – benzophenone-L-proline Schiff base (Section 4.1.7) derived from dehydroalanine undergoes Michael addition with amines to give (S)-αβ-diamino acid derivatives (see also Ref. 405).[351]

3-{(4′-Mercaptophenyl)amino}alanine and 4′-mercaptophenylalanine have been prepared from N-trityl-L-serine (Mitsunobu condensation) and L-phenylalanine (p-chlorosulfonylation followed by Sn-HCl reduction), respectively.[352]

4.12 Synthesis of α-Amino Acids Carrying Sulfur-, Selenium-, or Tellurium-containing Side-chains – Several new sulfur-containing amino acids have been prepared (including examples described in Section 6.3). (2S,4S,6S)-4-Hydroxy-5-phenylsulfinylnorvaline has been prepared starting from N-Boc-(S)-allylglycine through bromolactonization, sulfide formation, and routine concluding steps, as a model for a synthesis of the unusual amino acid in ustiloxins A and B.[353]

L- and D-β-(Phenylseleno)alanines have been prepared from β-lactones derived from L- and D-serine, respectively, and used for dehydroalanine synthesis based on oxidative elimination of the phenylseleno group.[354]

4.13 Synthesis of α-Amino Acids Carrying Silicon Functional Groups in Side-chains – Three new silicon-containing DL-amino acids $MeR_2SiCH_2C(NHAc)(CN)CO_2Et$ and corresponding hydantoins have been prepared (see also Ref. 464) through alkylation of ethyl α-(acetamido)cyanoacetate.[355]

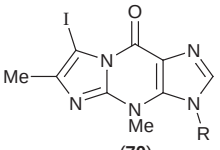
p-Trimethylsilyl-L-phenylalanine enantiomers are available through conventional synthesis and resolution (Ref. 467).

4.14 Synthesis of α-Amino Acids Carrying Phosphorus Functional Groups in Side-chains – Many of the amino acids of this category that are of current interest are phenylalanine derivatives with phosphorus functional groups as arene substituents, and these are prepared from the parent protein amino acid and dealt with in Section 6.3.

The phosphonium salt (S)-$Ph_3P^+CH_2CH(CO_2^-)NHCO_2Me$ serves in efficient

Wittig-type condensations, *e.g.* with (70; R = D-ribofuranosyl moiety), initiating routes for syntheses of ribofuranosylwybutines.[356]

2-Amino-5-phosphonylpentanoic acid [$Ph_2C{=}NCH_2CO_2Et$/BSA/Pd(0)/ethyl (3-acetoxyalk-1-enyl)phosphonate],[357] phosphinothricine and other glutamic acid analogues,[358] and the glutamic acid analogue $HO_2CCH(NH_3^+)CH_2CF_2(PO_3H^-)$[359] have been prepared through standard alkylation protocols applied to synthons derived from glycine and from other simple α-amino acids.

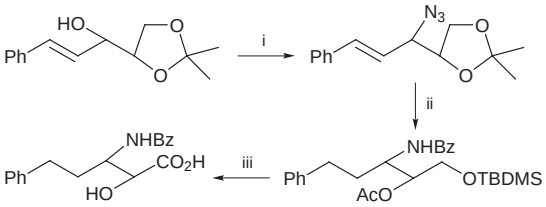


(**70**)

4.15 Synthesis of β-Amino Acids and Higher Homologous Amino Acids – The surge of interest in higher homologues of the α-amino acids has clearly not slowed down. New natural examples of the family, and new syntheses, have been described in the recent literature

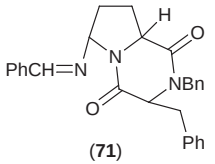
Reviews of addition of homochiral lithium amides to Michael acceptors have appeared,[360,361] and this approach has been used to prepare homochiral N-propargyl- or -allyl-β-amino esters $PhCHMeN(CH_2R)CHR^1CHR^2CO_2R^3$.[362] New examples of Michael acceptors are [S-(E)]- and [R-(E)]-2-[(4-methylphenyl)-sulfinyl]-3-phenylprop-2-enoic acid 1,1-dimethylethyl ester, including SmI_2-mediated reductive elimination of the sulfur function after addition of nitrogen nucleophiles (ammonia, piperazine).[363] Dimethyl 2-phenylselenofumarate readily participates in Michael addition reactions with amines, giving 3-amino-2-phenyl-selenosuccinates in high yields with complete regio- and stereo-selectivity.[364] Ti(IV)-Catalysed Michael addition of O-benzylhydroxylamine to homochiral αβ-unsaturated N-acyl-1,3-oxazolidinones generates disappointing e.e. (up to 42%).[365] Processing of an L-arabinose-derived dioxolane (Scheme 19) gives a vinylogue of phenylisoserine, and the isomeric δ-amino acid $PhCH(NHBz)CH(OH)CH{=}CHCO_2H$ with corresponding stereochemistry was also prepared in this study (from methyl glycidate, generation of the amino alcohol moiety, then chain extension from the ester grouping) through a route employing familiar methodology.[366] Further examples have been described, of the preparation of β-amino acids *via* hydrazines formed between TMS-SAMP and an ω-halogeno-αβ-unsaturated ester (Scheme 20).[367] Opportunities have been established for Ugi condensations in the synthesis of β- and higher amino acids (Refs. 137, 138).

Introduction of two new chiral centres is a feature of Zn-catalysed addition of bis-O-trimethylsilyl ketene acetals $R^1CH{=}C(OSiMe_3)_2$ to N-galactosylimines to produce β-amino acids.[368] This approach based on the Mannich reaction has been carried out with $Me_2C{=}C(OMe)OSiMe_3$ and an imine carrying a novel



Reagents: i, TPP, DEAD, DPPA; ii, iii, standard functional group transformations (7 steps)

Scheme 19



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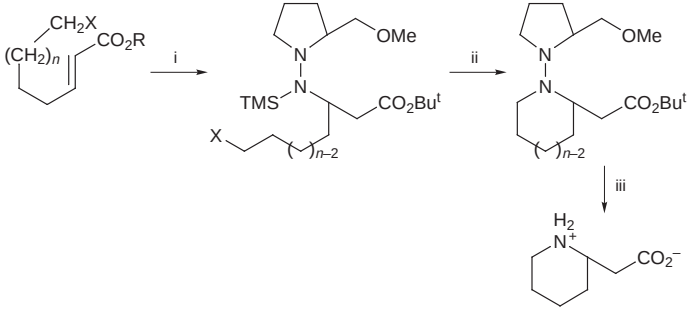
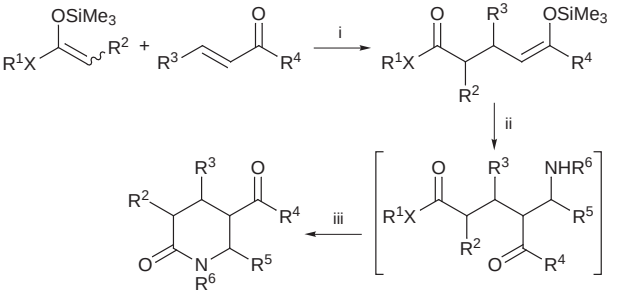


Reagents: i, TMS–SAMP, Bu^{n}Li, THF, −78 °C; ii, SiO_2–EtOAc, then NaI–K_2CO_3/MeOH at reflux; iii, Raney Ni–MeOH, then 6M HCl, followed by Dowex 50WX8–200

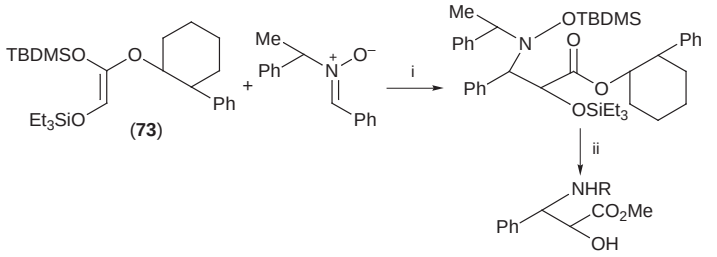
Scheme 20

chiral auxiliary (71), in the presence of $ZnCl_2$, to give (S)-3-amino-3-phenyl-2,2-dimethylpropanoic acid after acid hydrolysis.[369] The usual β-lactam synthesis involving a ketene and an imine ($Bu^tO_2CCH{=}NC_6H_4$-p-OMe + BnOCH=C=O; *cf*. Scheme 17) has been chosen as a route to benzyl N-Boc-isoserinates;[370] the general topic of applications of β-lactams in syntheses of amino acids has been reviewed.[371] Where silyl enolates (72; Michael adducts of ester equivalents with αβ-unsaturated carbonyl compounds) are used in additions to imines, then β-alkoxycarbonyl-δ-lactams are formed (Scheme 21).[372] Homochiral silyl enolates (73) add to homochiral nitrones to give excellent e.e. (98%) in a novel β-phenylisoserine synthesis (Scheme 22).[373] An organoselenium-induced cyclization of an N-acryloyl-L-prolinamide (Scheme 23) may become a valuable general asymmetric synthesis of β-amino acids (as well as to α-amino acids), but formation of unwanted elimination by-products is difficult to control, and routes with fewer steps are not seriously challenged at the moment.[374]



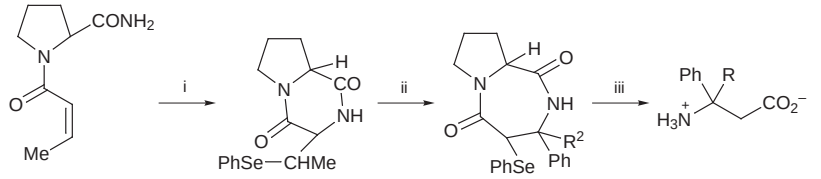
Reagents: i, $SbCl_5$–$Sn(OTf)_2$; ii, $R^5CH{=}NR^6$, $Sc(OTf)_3$, −78 °C; iii, $Hg(OTFA)_2$

Scheme 21



Reagents: i, ZnI_2; ii, functional group development

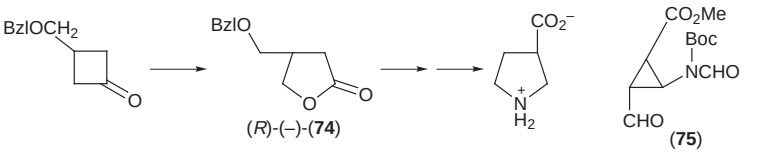
Scheme 22



Reagents: i, PhSeBr, AgOTf, MeCN; ii, $NiCl_2$; iii, $NaBH_4$

Scheme 23

Fluorinated imidoyl chlorides $R_FCCl{=}NR$ condense with lithium enolates of aliphatic esters to give β-enamino esters,[375] and β-amino-αβ-unsaturated alkanoic acid esters are easily prepared by the little-used Blaise reaction (sonicated RCN + $BrCH_2CO_2Et$ in the presence of Zn powder and ZnO → $NH_2CR{=}CHCO_2Et$).[376] These readily undergo α-alkylation since they are substrates for Michael additions to α-methyl-, α-acetoxy-, or α-acetamido-acrylates;[377] condensation with maleic anhydride gives 3-carboxymethyl-4,5-dehydropyrrolid-2-ones.[378] Eschenmoser condensation of N-[(S)-1-phenylethyl)]pyrrolidin-2-thione with ethyl bromoalkanoate/PPh_3 gives the corresponding Z-β-enamino ester that gives the (1R,2R)-α-alkylhomoproline derivative either through catalytic hydrogenation



followed by α-alkylation of the β-unsubstituted derivative,[379] or through catalytic hydrogenation of the α-alkyl α-enamino ester.[380]

A rare example of microbial help in a β-amino acid synthesis is featured in the formation of both β-proline enantiomers *via* (R)-(-)-(74) starting with the racemic cyclobutanone.[381] Amination of cinnamoylamides through epoxidation [polyaniline-supported cobalt(II)-salen complex/O_2] and anilinolysis gives anti-phenylisoserine derivatives in a one-pot procedure,[382] also applied to a synthesis of (-)-bestatin[383] revealing extraordinary control over the stereochemical outcome by the p-substituent of the aromatic ring.[384]

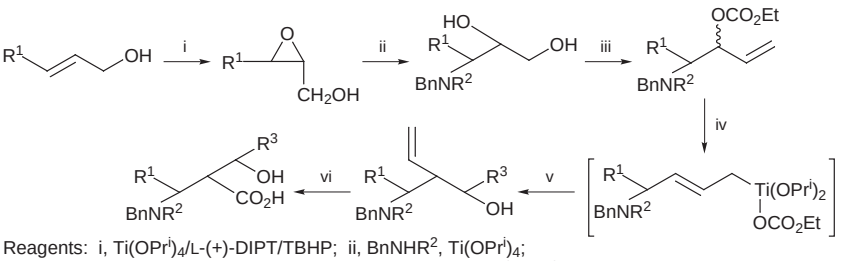
Cyclopropanation of N-Boc-pyrrole by methyl diazoacetate in an improved procedure, followed by ozonolysis and reductive work-up, gives the β-amino acid aldehyde (75) as a single stereoisomer.[385] Homochiral 3-amino-2,2-dimethylcyclobutanecarboxylic acids have been prepared by Curtius rearrangement of the half ester of the corresponding cyclobutanedicarboxylic acid using $(PhO)_2P(O)N_3$,[386] similarly employed for amination of the product of cyclopropanation of tert-butyl cinnamate using the anion of chloromethylphosphonamide to give enantiopure 2-amino-3-phenylcyclopropanephosphonic acid.[387] All four stereoisomers of 2-aminocyclopentanecarboxylic acid have been prepared starting from readily-available enantiomers of 3,4-dimethoxycarbonylcyclopentanone and resolution with (+)- or (-)-ephedrine of amination products at the end of the reaction path.[388] The synthesis of methyl (2S,3S)-3-amino-2-methyl-7-octynoate, a component of onchidin, and the (2R,3R)-diastereoisomer, have been synthesized *via* the β-lactam formed by amination of the corresponding β-hydroxy acid prepared by the Evans route (Section 4.1.3).[389]

Homologation of N-Boc- or N-Z-L-proline by diazomethane-silver benzoate gives excellent yields,[390] and the same applies to the preparation of N-Fmoc β-amino acids[391] including their *in situ* generation during a solid-phase β-peptide synthesis.[392] Homologation of α-amino acids into β-amino acids (see also Ref. 534) has been reviewed.[393] Another classical approach, the easy alkylation of ethyl cyanoacetate followed by reduction of the cyano group, has been used for the synthesis of α-alkyl-β-alanines (see Ref. 166).

cis-4-Hydroxypiperidine-3-carboxylic acid, prepared as the racemate by the reduction of the readily available ketone (a literature account suggesting that baker's yeast reduction was highly enantio- and diastereo-specific could not be confirmed), was used in combinatorial synthesis of bioisosteric carbohydrate mimetics.[394] Cocaine and related tropane alkaloids are not overlooked as sources of cyclic β-amino acids, and N-Fmoc derivatives of tropanes, stripped of certain functional groups, have been described.[395] Elaboration of the Diels-Alder adduct of N-Boc-pyrrole with methyl 3-bromopropiolate has given similarly useful

substrates,[396] and 4-alkylidene homologues have been prepared by the corresponding [4 + 2]-cycloaddition to allenic esters.[397]

3-Amino-2-hydroxyalkanoic acids are easily prepared through aldolization of α-aminoalkanals, and since these are available from α-amino acids and their sensitivity is now well understood, the overall process amounts to the conversion of an α-amino acid into a homologous β-amino acid.[398] The sulfur analogues, β-amino-α-mercaptoalkanoic acids, can be prepared best by p-methoxybenzylsulfenylation of β-amino acid enolates using (2,4-dinitro-C_6H_3)$SSCH_2$(C_6H_4-4-OMe) and replacing the p-methoxybenzyl protecting group as required.[399] The aminohydroxylation procedure introduced by Sharpless continues to offer valuable stereochemical control, shown as applied to methyl (E)-cinnamate (Vol. 29, p. 46; see also Ref. 91),[400] and a route to (2R,3S)-3-(N-toluene-p-sulfonylamino)-4-cyclohexyl-2-hydroxyalkanoic acids from the corresponding αβ-unsaturated esters is another simple example[401] (see Ref. 91 for a synthesis of phenylisoserine using this procedure). The drawback, the need to cleave a sulfonamide to release the amino acid, is less of a problem now that improved methods are available for this step, but the corresponding process with an N-halogenocarbamate sodium salts[402] should prove more convenient. An unusual development of Sharpless epoxides into β-amino acid derivatives *via* 3-amino-1,2-diols involves a chiral allyltitanium intermediate (Scheme 24).[403]

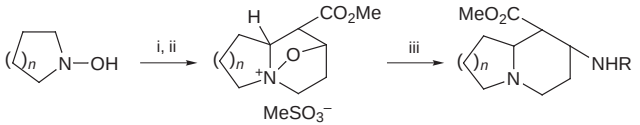


Reagents: i, $Ti(OPr^i)_4$/L-(+)-DIPT/TBHP; ii, $BnNHR^2$, $Ti(OPr^i)_4$;
iii, $NaIO_4$, $H_2C{=}CHMgBr$, $ClCO_2Et$/Py; iv, $Ti(OPr^i)_4$, Pr^iMgCl; v, R^3CHO; vi, O_3

Scheme 24

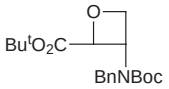
Nucleophilic attack at C-2 of N-toluene-p-sulfinylaziridine 2-carboxylic esters is a relatively rare event (C-3 attack is more usual), and is accomplished, with ring-opening, using $LiAlH_4$. It is highly stereoselective, oxidative manipulation of the products giving α-methyl-β-amino acids.[404] β-Lactams have been found suitable for the preparation of 2,3-diaminoalkanoic acids, in a programme with particular emphasis on the synthesis of analogues of taxol.[405] Other ring-opening processes are essential steps in syntheses of 4-aminopiperidine-3-carboxylic esters (Scheme 25).[406] Approaches to novel α-cyclopentyl (S)-isoserines have been explored, featuring ring-opening of an appropriate epoxide with (R)-α-methylbenzylamine.[407] Poly(L-leucine)-catalysed asymmetric epoxidation of trans-αβ-unsaturated ketones by urea-H_2O_2 in THF with DBU and aminolysis of the homochiral product gives (2R,3S)-β-phenylisoserine.[408] syn-β-Amino-α-hydroxyacids have been prepared through conversion of homochiral α-hydroxy-βγ-

unsaturated esters into allylic carbamates using TosNCO, and their iodocyclization into trans-4,5-disubstituted oxazolidin-2-ones, followed by reductive deiodination.[409] The antibiotic β-amino acid oxetin (76 without protecting groups) has been synthesized as its racemate through Paterno-Buchi cycloaddition of the enecarbamate CH_2=CHN(Bn)CO_2Bu^t to Bu^tO_2COH.[410]



Reagents: i, [O] ⟶ nitrone; ii, [1,3]dipolar cycloaddition to alkadienoate; iii, DABCO, RNH_2

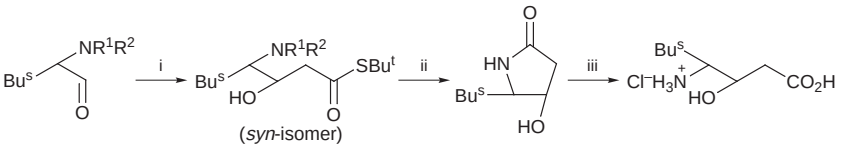
Scheme 25



(**76**)

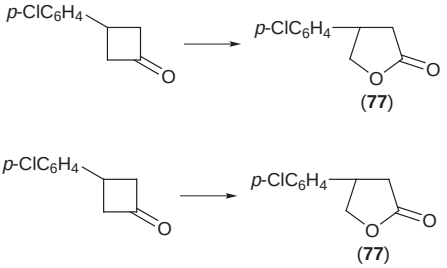
(3R,5R)-3,6-Diamino-5-hydroxyhexanoic acid, the β-amino acid constituent of (+)-negamycin, has been prepared through a route employing mandelonitrile lyase (further details not provided in the abstract source of this information).[411] Further details have been given of the synthesis of N-Boc-Adda from L-serine and (S)-phenyl-lactic acid (see Vol. 29, p. 43),[412] and a new synthesis of N-Boc-(2S,3S,8S,9S)-Adda, employing Pd(0)-catalysed cross-coupling of a syn-homopropargylic ether to a secondary allylic amine bearing an (E)-vinyl iodide, has been reported (see Ref. 768 for a synthesis from an α-amino aldehyde).[413]

γ-Amino acids continue to be almost exclusively synonymous with statines, as seen in a survey of the literature on amino acid synthesis. A route to these from 3-keto-esters,[414] and other pathways from α-aminoalkanals, through aldolization (Scheme 26),[415] through reaction with 3-methylglutaconate to give 2-substituted statines, RCH(NBn_2)CH(CO_2Me)CMe=$CHCO_2$Me,[416] and through syn-selective allyl- or vinyl-magnesium bromide addition [L-leucinal → (-)-N-Boc-statine and (-)-N-Boc-norstatine],[417] feature new developments in synthetic methodology. Other familiar examples of γ-amino acids that continue to attract synthetic interest are (R)-carnitine [see also Vol. 29, p. 48; prepared from (R)-4-trichloromethyloxetan-2-one *via* ethyl (R)-3-hydroxy-4-chlorobutanoate formed through hydrogenolytic ring-opening,[418] from R-(-)-epichlorhydrin by reaction with CH_2=CHMgBr/CuBr and subsequent processing,[419] and from trans-crotonobetaine using *E.coli* 044 K74[420]], racemic baclofen [H_2N-CH_2CH(4-Cl-C_6H_4)CH_2CO_2H, from the [2 + 2]-cycloadduct of dichloroketene with an alkene],[421] R-(-)-baclofen [seven-step route with *Cunninghamella echinulata*-mediated generation of the (3R)-chlorophenyl-lactone (77), by Baeyer-Villiger oxidation of a racemic precursor, as a key step],[422] (-)-detoxinine [prepared in a novel way starting from dichlorodi-isopropylsilane (Scheme 27)],[423] and (S)-vigabatrin after deprotection of CH_2=CHCH(NHBoc)$CH_2CH_2CO_2$Me [from



Reagents: i, $[(menthyl)_2CH_2]_2BCl$ [from (+)-menthone] $\longrightarrow CH_2{=}C(SBu^t)OB[(menthyl)CH_2]_2$; ii, $NH_4{}^+HCO^-/Pd$; iii, $H_3O^+Cl^-$

Scheme 26



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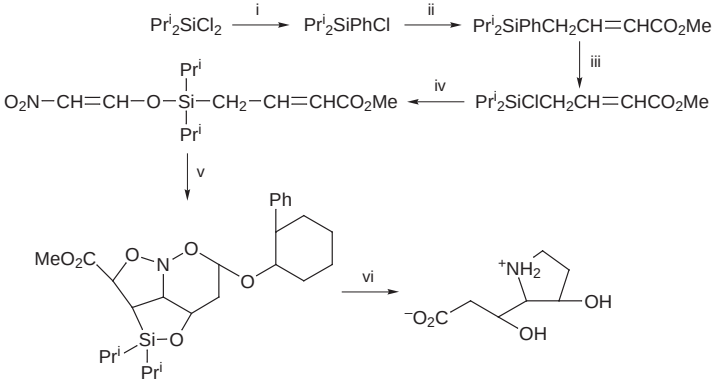
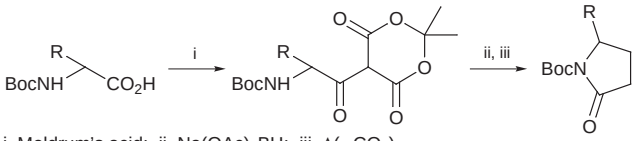


Reagents: i, 1 eq. PhLi; ii, Li, CuCN, $ICH_2CH{=}CHCO_2Me$; iii, HCl, $CHCl_3$; iv, $KOCH{=}CHNO_2$; v, (–)-(2-phenylcyclohexyl) vinyl ether; vi, routine hydrolysis

Scheme 27

(E)-5-phenyl-2-penten-1-ol *via* Sharpless oxidation and oxidative conversion of the phenyl group into CO_2H].[424]

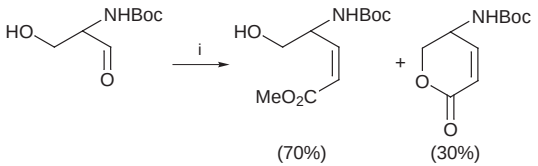
3-Aminocyclopentanecarboxylic acids bearing a 5-[pyrimidin- or purin-1-ylacetyl] grouping have been described (Ref. 347), and δ-amino acid analogues have been synthesized, in each of which the cyclopentane ring is replaced by a glucosamine moiety carrying one of the four nucleobases.[425] Conformationally-restricted β-hydroxy-γ-amino acids have been prepared by Diels-Alder addition to an N-acryloylcamphorsultam [*cf.* Scheme 6, CH_2=CHCO- in place of $(MeS)_2C$=CHCO-; for a synthesis of (3S,2S,1S)-3-amino-2-hydroxycyclohexane-1-carboxylic acid].[426]



Reagents; i, Meldrum's acid; ii, $Na(OAc)_3BH$; iii, $\Delta(-CO_2)$

Scheme 28

Simple homologation of α-amino acid esters (DIBALH then $Ph_3P{=}CHCO_2R$ followed by H_2/Pd-C) to γ-amino acid esters, and introduction of an α-substituent through enolate alkylation, offers an attractive route to γ-amino acids (which may not be applicable if sensitive functional groups are present in the target amino acid).[427] Another standard route in which an N-Boc-L-α-amino acid is condensed with Meldrum's acid leads to γ-lactams (Scheme 28).[428] A frequently-used homologation from the Garner aldehyde (see Section 6.3) involves condensation with the bistrifluoroethyl phosphonate $(CF_3CH_2O)_2P(O)CHMeCO_2Me$ to give γ-amino αβ-unsaturated esters which prove to be amenable to Os-catalysed dihydroxylation.[429] An alternative route to these substrates starts with the addition of lithiated tert-butyl or ethyl propiolates to nitrones $R^1CH{=}N^+R^2O^-$ [→ $R^2N(OH)CHR^1C{\equiv}CCO_2R$ → $R^2NHCHR^1CH_2CH_2CO_2R$ *etc*].[430] Horner-Wadsworth-Emmons alkene synthesis from α-amino-β-hydroxyalkanals involves Z-selectivity when bis(trifluoroethyl)phosphonates are used (Scheme 29).[431]

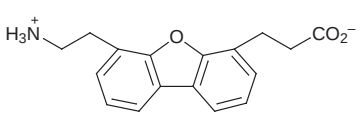


Reagents: i, $(F_3CCH_2O)_2P(O)CH_2CO_2Me$, LiCl, DBU

Scheme 29

Carbon-carbon bond forming syntheses are illustrated by radical cyclization of bromodifluoroacetyl allylamines $BrCF_2CONR^1CH_2CH{=}CHR^2$ to give γ-lactams.[432] γ- and δ-Lactam synthesis is also covered in Ref. 138.

With greater separation of amino and carboxy functions, appropriate synthetic methodology becomes more mundane, and general syntheses do not exist. A synthesis is individually tailored to give access to the particular target, as with 2-[4-(2′-aminoethyl)-6-dibenzofuran-2-yl]propanoic acid (78)[433] and sialyl sugar amino acids (starting from neuraminic acid),[434] a conversion of unprotected D-pentono-1,4-lactones into 5-amino-5-deoxy-D-pentonolactams by manipulating the primary alkanol function (CH_2OH → CH_2Br → CH_2N_3 → CH_2NH_2),[435] and aminomethylthiophen-1-carboxylic acids.[436] The synthesis of conformationally-constrained 3-amino-1-aza-2-oxobicycloalkanecarboxylic acids (*e.g.* 79) through standard methods (such as the Schollkopf synthesis, Section 4.1) has been reviewed.[437] Interesting approaches are still available to be explored, *e.g.*



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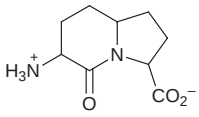
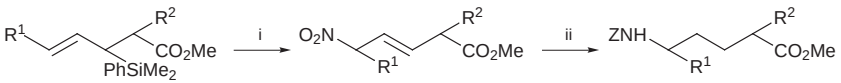


(**79**)

nitration as a means of introducing the δ-amino function into homochiral (E)-crotyl silanes (Scheme 30).[438] 5-(Z-Amino)-2-alkyl-4-hydroxyalkanoic acids have been prepared through condensation of an N-Z-L-α-amino acid with 2-triflyloxy-esters (see also Ref. 414),[439] and δ-amino-βγ-unsaturated alkanoates, *e.g.* $Me_2CHCH(NHMts)CH{=}CHCH(CH_2Ph)CO_2Me$, have been obtained from 4-(aziridin-2-yl)acrylates.[440]



Reagents: i, $NO_2^+BF_4^-$; ii, functional group development

Scheme 30

α-Sulfenylation of 2-(ω-benzenesulfonylaminoalkyl)-1,3-oxazolines gives ω-benzenesulfonylamino-α-(methylthio)alkanoic acids.[441]

An erratum has been published[442] relating to an account of the synthesis of 4-[^{13}C]-, 5-[^{13}C]-, and [^{15}N]-labelled 5-aminolaevulinic acid, currently of interest as a substrate for porphyrin biosynthesis whose photoactivation in tumours is a promising therapeutic approach. A simple condensation of the acid chloride of labelled phthalimidoglycine with the zinc homoenolate of ethyl propionate constitutes this synthesis, and a similar approach giving [2,3-$^{13}C_2$]-5-aminolaevulinic acid uses ethyl [1,2-$^{13}C_2$]bromoacetate.[443] Potassium [^{15}N]phthalimide reacts with tetrahydrofuranyl bromide to give the aminofuran derivative that yields [^{15}N]-labelled 5-aminolaevulinic acid through RuO_4 oxidation.[444]

These δ-amino acids, and the 'δ-nucleo-amino acids' formed by alkylation of protected L-serinols or L-homoserinols with bromoacetates followed by Mitsunobu introduction of thymidine or uracil to give $H_2NCH(CH_2base)CH_2OCHRCO_2H$ and its homologue,[445] or by amination by azidolysis of δ-hydroxy β-(β′-adeninylalkylidene)alkanoic esters and thymidinyl analogues,[446] fall in the category of dipeptide mimetics which are covered in a later Chapter in this Volume.

4.16 Resolution of DL-Amino Acids – Techniques for the separation of enantiomeric amino acids from a racemic mixture fall into several clearly defined categories. Classical laboratory resolution methods are illustrated by separation by fractional crystallization of diastereoisomeric salts formed between N-pivaloyl-DL-tert-leucine with (S)-α-methylbenzylamine for a route to (R)-tert-leucine (Ref. 87), similarly for DL-α-methyl-(4-carboxyphenyl)glycine (Ref. 83), 2-methylamino-3-phenylpropanoic acid (using mandelic acid; Ref. 448), and a synthesis of all stereoisomers of 2-aminocyclopentanecarboxylic acid [(+)- and

(-)-ephedrine, in Ref. 388]. There are cases where a D- or L-amino acid derivative is used for the formation of diastereoisomer mixtures, and of course these operations can be used in reverse for the resolution of DL-amino acids; examples are separations accomplished after the acylation of oxidized Cleland's reagent by N-Boc-L-phenylalanine,[447] combination of (RS)-2-chloro-3-phenylpropanoic acid with ethyl L-phenylalaninate,[448] and of (RS)-2-substituted ω-phenylalkanoic acids with methyl (S)-phenylglycinate,[449] and the corresponding use of an L-β-(N-trimethylammonio)alkanol bromide for efficient resolution of 2,2′-dihydroxy-1,1′-binaphthyl.[450]

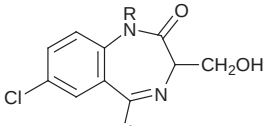
A retroracemization procedure in which a DL-amino acid is condensed with a (S)-2-[(N-alkylprolyl)amino]benzophenone in the presence of nickel(II) nitrate releases the (S)-enantiomer through hydrolysis (*cf.* Ref. 158) in 55-99% e.e.[451] A corresponding deracemization procedure employs an N-phthaloyl-DL-amino acid and (R)-pantolactone (Ref. 105).

The fortuitous preferential crystallization of one enantiomer (initiation of crystallization through seeding with the required enantiomer of the particular target amino acid, or even with an enantiomer of a structurally-related compound) lends itself to large-scale operation. A review has appeared,[452] and recent examples give practical details for DL-methionine hydrochloride,[453] (2RS,3SR)-2-amino-3-chlorobutanoic acid hydrochloride,[454] and DL-α-amino-γ-butyrolactone hydrochloride.[455]

The use of enzymes continues to occupy a prominent place in any list of current resolution options, and is illustrated in the current literature for catalysis of enantioselective hydrolysis: ethyl N-acetyl DL-3-aminobutyrate using lipase from *Candida antartica*,[456] rice bran lipase for a variety of N-acetyl DL-amino acid esters,[457] *Aspergillus niger* lipase for N-protected amino acid esters,[458] long-chain alkyl esters of non-protein amino acids using a microbial protease,[459] also used for syntheses of a Nikkomycin B constituent (Ref. 240) and (S)-4-fluoro-3-nitrophenylalanine (Ref. 326, and resolved using subtilisin, Ref. 141), diethyl acetamidomalonate using α-chymotrypsin to give the (+)-mono-ester (probably the R-enantiomer).[460] α-Chymotrypsin immobilized on Aphron[461] or on porous silica[462] is effective for the continuous-flow resolution of methyl DL-phenylalaninate. The resolution of N-chloroacetyl-DL-7-azatryptophan by carboxypeptidase A,[463] and N-acetyl-DL-(3-trimethylsilyl)alanine using hog kidney acylase (also used for N-acetyl-O-benzylthreonines; Ref. 841) and N-acetyl-DL-(p-trimethylsilyl)phenylalanine using an N-carbamoyl amino acid amidohydrolase[464] (see also Ref. 467) have been described.

A wide variety of sulfur- and selenium-containing N-acetyl-DL-amino acids are substrates for acylase I from *Aspergillus oryzae*[465] (a useful review covers aminoacylase resolution of N-acetyl-DL-amino acids and corresponding use of D-hydantoinases[466]). Acylase resolution (see also Ref. 806) of N-acetyl-β-(2-anthraquinolinyl)alanine has been reported (Ref. 140). N-Carbamoyl-D-amino acid amidohydrolase contained in cell-free extracts of *Blastobacter* sp. A17p-4 catalyses the enantioselective hydrolysis of N-carbamoyl-DL-(p-trimethylsilyl)-phenylalanine.[467] Hydantoins of numerous DL-arylglycines have been shown to be substrates of D-hydantoinase from various bacteria.[468] The peptide amidases

from *Citrus sinensis* and *Stenotrophomonas maltophilia* are effective in the resolution of N-acetyl-DL-amino acid amides[469] (see also Ref. 877 for a use of *Penicillium* amidase, and Ref. 815 for an application of *Pseudomonas putida* amidase). A whole-cell approach exploiting the amidase content of selected bacteria delivers (S)-amino acids with accompanying racemization (in other words, kinetic resolution) when applied to piperidine- and piperazine-2-carboxamides,[470] and the amidase of *Ochrobacterium anthropi* (see also Ref. 270) has been applied to racemic threo-amides to obtain 4-methylthio- and 4-methysulfonyl-(2S,3R)-3-phenylserines.[471] The reverse approach is illustrated in enantioselective N-α-phenylacetylation of amino acids using immobilized penicillin G acylase,[472] and acetylation by p-nitrophenyl acetate of an amino acid anion catalysed by β-cyclodextrin,[473] formation of N-acetyl-D-3-amino-3-phenylpropanoic acid by processing of the racemate by cell-free extracts of *Streptomyces neyagawaensis* SL-387,[474] and O-acetylation of (80) with kinetic resolution, catalysed by immobilized *Mucor miehei* lipase at 60 °C, leading to D- and L-serine after workup.[475]



(**80**)

Antibody-catalysed enantioselective hydrolysis of N-benzyloxycarbonyl-DL-amino acid esters has been reviewed.[476]

A series of papers is appearing dealing with the production of D-amino acids, through the destruction of the L-enantiomer in a racemate by bacterial action (DL-methionine; *e.g. Proteus vulgaris*).[477] In the case of the production of D-lysine, the starting point was the L-enantiomer, with *Comamonas testosteroni* emerging from exploration of numerous species as the favoured agent for digestion of the racemate created by prior chemical racemization.[478] Biocatalytic deracemization (*i.e.* enzyme-mediated dynamic resolution and stereoinversion of easily racemized amino acid derivatives, such as oxazolones and thiazolones) has been reviewed.[479]

Chromatographic and related physical techniques continue to show improving potential when applied to preparative-scale separation of enantiomers.[480] Classical resolution methods associated with classical chiral stationary phases (CSPs), *e.g.* cellulose for the resolution of all the protein amino acids and representative N-dinitrophenyl derivatives,[481] polymer-supported bovine serum albumin (BSA) as stationary phase for the resolution of N-substituted DL-amino acids,[482] and apoenzymes immobilized in a porous polymer membrane[483] for enantioselective transport of amino acid derivatives, and a related ultrafiltration application of BSA for the resolution of DL-tryptophan[484] have been illustrated. Novel CSPs include (R)-phenylglycine-derivatized poly(siloxane)s [efficient separation of N-(3,5-dinitrobenzoyl)-DL-α-amino acid amides],[485] (1→6)-2,5-anhydro-3,4-di-O-

methyl-D-glucitol attached to silica gel for efficient resolution of bulky DL-amino acids (tryptophan and phenylglycine),[486] and CSPs employing the ligand exchange principle, also for use with underivatized amino acids.[487] Uses of CSPs in analytical resolution are covered in parts of Section 7 of this Chapter.

Molecule-imprinted CSPs have been described: cellulose acetate imprinted with Boc-L-glutamic acid shows preferential permeation by L-glutamic acid;[488] methacrylic acid – trimethylolpropane trimethacrylate copolymer imprinted with Boc-L-phenylalanine successfully resolves Boc-DL-phenylalanine;[489] poly(methacrylate)s imprinted with L-phenylalanine anilide have functioned in the capillary electrophoresis mode for resolution of amino acids.[490] Polymers imprinted with N-Boc-L-alanine, -phenylalanine, or -glutamic acid, favour passage of the D-enantiomer of N-Boc-DL-phenylalanine, though with low efficiency, thought to provide evidence of homogeneity of binding sites.[491] The results achieved by chromatography over molecule-imprinted polymers, *e.g.* resolution of DL-amino acids in aqueous buffers,[492] have been reviewed.[493] Such media, prepared by polymerization of an achiral monomer, copper(II) N-(4-vinylbenzyl)iminodiacetic acid, and an amino acid template with ethyleneglycol – dimethylmethacrylate as crosslinking agent, then grafting on to silica gel, give good results with DL-phenylalanine but no resolution was achieved for DL-alanine.[494]

Polymeric membranes bearing the tetrapeptide Asp(OChex)-Ile-Asp(OChex)-Glu(OBzl)O-CH_2- (*i.e.* covalently-bonded through its C-terminal carboxy group), that have been imprinted by Boc-D- or L-tryptophan, allow L-amino acids to permeate preferentially, under electrodialysis conditions.[495] L-Tryptophan has been shown to permeate a (+)-poly{2-[dimethyl(10-pinanyl)silyl]norbornadiene} membrane more readily than its D-enantiomer,[496] and a similar result has been found for membranes made from poly(γ-benzyl-L-glutamate) carrying poly(oxyethylene) side-chains, the polymer adopting a right-handed α-helical conformation.[497] A chiral emulsion liquid membrane [copper(II) N-decyl-L-hydroxyproline in hexanol – decane] offers accelerated transport of D-phenylalanine from the racemate.[498]

Two-phase liquid-liquid extraction is enantioselective when a carbamoylquinine is included in the phase containing an N-protected DL-α-amino acid derivative.[499]

Speculation on mechanisms through which the L-enantiomeric amino acids came to predominate from presumed prebiotic racemic mixtures has featured in this Section over the years, and the mirror-symmetry breaking hypothesis[500] and the broader range of theories[501] will be familiar to regular readers. Surveys across a range of disciplines are published in a Conference Proceedings Volume.[502] Among the ideas with a more recent origin reviewed in the last-mentioned article, the interaction of chiral radiation (*e.g.* circularly-polarized light in the ultraviolet wavelength region) with DL-amino acids at a water-air interface of the prebiotic oceans continues to be developed; electrochemical reduction of phenylglyoxylic acid oxime in magnetic fields has been shown to deliver product with no e.e.;[503] synchrotron grazing incidence X-ray diffraction reveals that amphiphilic DL-amino acid monolayers on water or aqueous glycine separate into islands of opposite chirality.[504] The role of circularly-polarized synchrotron radiation, and

means by which a small initial enantiomer imbalance might have been amplified, are covered in a recent review.[505]

5 Physico-chemical Studies of Amino Acids

5.1 X-Ray Crystal Analysis of Amino Acids and their Derivatives – Amino acids that have received attention this year are: glycine – urea (1:1),[506] L-arginine perchlorate,[507] L-arginine phosphate, deuteriated in guanidino, amino and carboxy groups,[508] L-threonine at 12 K,[509] L-proline hydrate at 100 K,[510] α-methyl-L-proline hydrate,[511] DL-lysine oxalate and its L-form,[512] L-tyrosine perchlorate,[513] L-phenylalanine (S)-mandelate and the D(S)-diastereoisomer of this salt,[514] L-phenylalanine hemisulfate hydrate,[515] O-phospho-L-tyrosine,[516] L-histidine oxalate and the corresponding racemate,[517] (+)-α-methyl-4-carboxyphenylglycine (assigned the S-configuration),[518] [(2′R,4′S)-3′-benzoyl-4′-benzyl-5′-oxo-2′-phenyloxazolidin-4′-yl)]acetic acid and its (2′S,4′R)-analogue carrying 3′-acetyl in place of benzoyl,[519] and 5,7-dichlorokynurenic acid hydrate.[520]

X-ray data collected for amino acid and peptide derivatives have been reviewed.[521] New data for amino acid derivatives cover hexamethyl-L-cystine,[522] L-leucinamide,[523] N-acetyl-L-isoleucinamide and its sarcosine analogue,[524] N-acetyl-L-glutamic acid,[525] N-dodecanoyl-L-serine monohydrate,[526] N-trichloroacetyl-DL- and L-valine and trichloroacetates of these amino acids,[527] N^{α}-pyruvoyl-L-methionine and its copper(II) complex (binding of the metal ion is established to involve the α-keto-amide moiety),[528] the N-Boc-L-phenylalanine – pyridine complex,[529] methyl (R)-α-methylphenylalaninate hydrochloride,[530] 2-alkoxyoxazol-5(4H)-ones,[531] symmetrical anhydrides of N-benzyloxycarbonyl αα-dialkylglycines (of interest through revealing intramolecular interactions between urethane NH and phenyl groups),[532] DL-phenylalanine N-carboxy-anhydride,[533] methyl N-Fmoc homo-β-(S)-leucinate,[534] and (4R,5S)-3-[(2R,3S)-3-hydroxy-2-methyl-7-octynoyl]-4-methyl-5-phenyl-1,3-oxazolidin-2-one.[535]

Several of these studies reach conclusions through incorporating data from spectroscopic techniques (*e.g.*, Raman – IR, Ref. 529).

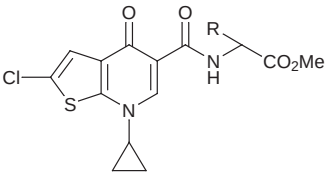
5.2 Nuclear Magnetic Resonance Spectrometry – The usual focus for this section, the frontier science featuring newer instrumental methods and unusual contexts, is illustrated by *in vivo* studies for non-invasive estimations of N-acetyl-L-aspartic acid by ^{1}H-NMR,[536] and for glutamic acid and glutamine (midfield ^{1}H-NMR for quantitative analysis based on areas of α- and β-proton resonances),[537] and the inverse spin-echo difference estimation of the ^{13}C-content of ^{13}C-enriched glutamic acid.[538]

Conventional studies include kinetics of ^{1}H-^{2}H-exchange for the α-protons of protonated methyl glycinate in $^{2}H_2O$.[539] Conformational outcomes from studies employing ^{1}H and ^{13}C-NMR are reported for α-, β- and γ-methylglutamic acids and cyclopentyl and cyclohexyl analogues,[540] kainic acid in water,[541] 2-(carboxycyclopropyl)glycines,[542] sodium N-dodecanoyl-N-methylglycinate (a Z/E-mixture in $^{2}H_2O$),[543] substituted 2,3-diaminopropenoic acid esters,[544] and 1-(N-

acylamino)adamantane-1-carboxylic acid amides.[545] Rotational barriers about C-N bonds for N-α-acylamino acid N′-methylamides have been derived from ^{15}N-NMR spectra,[546] and ^{1}H-^{15}N dipolar coupling studies have been reported for [^{15}N]-tryptophan and π-[^{15}N]-histidine.[547] Solid state studies of amino acids (^{13}C-NMR)[548] and amides of L-amino acids have been described in several recent papers employing ^{1}H-NMR (L-leucinamide,[549] L-alaninamide[550]) and joint NMR-X-ray analysis of L-leucinamide (Ref. 523). ^{13}C-NMR Spectra of amides of N-formyl-alanine, valine, and isoleucine have been reported.[551] As an illustration of a halfway stage between solid-state and solution-phase NMR, 2D J-resolved NMR spectra provide excellent results for N-protected amino acids attached to DMF-swollen Wang resin.[552] ^{19}F-NMR Spectra for N-trifluoroacetyl-amino acids in poly(γ-benzyl-L-glutamate), a chiral liquid crystal medium, show split resonances from which D:L-ratios can be calculated;[553] the equivalent methodology in other branches of NMR [^{2}H-NMR of N-($C^{2}H_3CO$)-derivatives and earlier ^{1}H-NMR applications; see also Ref. 449)] is already well-established.

β-Cyclodextrin-entrapped N-dansyl-L- or -D-leucine,[554] and the enantioselectivity of D-mannitol-based crown ethers towards DL-amino acid esters[555] are examples of structural studies depending on standard NMR instrumentation.

5.3 Optical Rotatory Dispersion and Circular Dichroism – Little new has emerged in this topic area, and what there is [*e.g.* solvent-dependent CD of chromophore-substituted amino acid esters (81)[556]] derives from well-established principles. CD Features are generated within achiral poly(4-carboxyphenylacetylene) when in contact with solutions of homochiral amines and aminoalkanols,[557] and similarly for L- or D-amino acids complexed by achiral gadolinium(III) porphyrins.[558] The last-mentioned study also describes the extraction of the amino acid from aqueous media into a dichloromethane solution of the ligand, and emphasises the secure correlation observed between sign of Cotton effect and absolute configuration of the amino acid.



(**81**)

5.4 Mass Spectrometry – Research activity centred on underivatized amino acids demonstrates state-of-the-art MS methodology. Time-of-flight plasma desorption studies of L-valine and L-leucine revealing the formation of ionic heteroclusters through gas-phase intermolecular reactions,[559] specifically identified to involve pairing in all four chiral combinations when pairs of amino acids are involved (*e.g.* the trimer formed from amino acids A and B → A_2BH^+ → ABH^+ + A_2H^+).[560] Chiral discrimination energies of 0.4 – 4 kJ mol^{-1} derived

from these data can be exploited to allow amino acid enantiomers to be distinguished by mass spectrometry, due to the basicity differences they exhibit when their dimers form part of gas-phase cluster ions. Complexes of α-amino acids with copper(I) ions survive into the gas phase.[561] Related laser desorption MS of tryptophan has provided internal energy data,[562] and has produced evidence of a stable ground state zwitterion structure for arginine through binding energy measurements on gas-phase dimers.[563]

Creation has been established of metastable ions $[MH - CO_2H_2]^+$ from leucine and isoleucine,[564] $[M + H]^+$ ions arising through electrospray MS of strongly basic solutions of amino acids, and $[M - H]^-$ ions from strongly acidic solutions,[565] and of $[M + {}^2H]^+$ ions from amino acids using C^2H_4 and $(C^2H_3)_2CO$ as ionization reagents in CIMS.[566] Consideration of fragment ions in the latter case has demonstrated scrambling of protons (in other words, high proton mobility).

Nickel(II) salt – aliphatic amino acid mixtures behave similarly to corresponding mixtures with copper(II) salts, in yielding $[M + Ni]^+$ ions through FABMS but $[Ni(M-H)M]^+$ ions through electrospray ionization.[567]

Reaction products in the gas phase from cysteine, Me_2Cl^+ and $MeOCH_2^+$ are shown by MS to include S-methylcysteine and N-methylcysteine.[568]

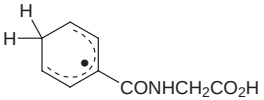
Electrospray ionization mass spectra of PTHs have been fully characterised;[569] three fragmentation pathways have been established for the $[M-H]^-$ ion formed from the PTH of phenylalanine.[570]

5.5 Other Spectroscopic Studies of Amino Acids – Identification of reaction products by routine spectrometric methods is occasionally mentioned elsewhere in this Chapter, but this section is reserved for more unusual studies. These include FTIR study of the effect of glycine and alanine on the water structure of their aqueous solutions,[571] FTIR estimation of self-association of N-Boc-L-α-amino acids in CCl_4[572] and IR-Raman study of amino acids at 18 K over the frequency range 400 – 3800 cm^{-1},[573] non-ionized glycine in a low-temperature argon matrix,[574] and corresponding vibrational spectra for α- and β-alanine,[575] [^{2}H]-doped glycine hydrochloride,[576] N-acetyl-αβ-dehydro-amino acid N′-methylamides,[577] fullerene – amino acid reaction products (see also Ref. 598),[578] and polarized Raman analysis of the vibrational coupling of water of hydration with the amide-I band of N-acetylglycine,[579] UV resonance Raman study of domoic acid,[580] and Raman and ^{1}H-NMR demonstration of the effectiveness of non-polar side-chains in enhancement of water structure by amino acids.[581] Surface-enhanced Raman spectra have been determined for L-phenylalanine,[582] and similarly for GABA,[583] using silver-colloid solutions. An inelastic coherent neutron scattering study augmented by IR-Raman data has been reported for N-[^{2}H]-L-isoleucine.[584]

Further development has been reported of an application of absorption millimetre spectroscopy at 31.42 GHz (see Vol. 29, p.60) in the establishment of a hydrophobicity scale for amino acids.[585]

Electron spin resonance (ESR) spectrometry of UV-irradiated [^{2}H]-, [^{13}C]-, [^{17}O]-labelled tyrosine has allowed the mapping of π-electron spin-density of the neutral phenoxy radical in frozen alkaline solution,[586] and ESR of X-irradiated

single crystals of hippuric acid has demonstrated decarboxylation (formation of carbon centred radicals $PhCONHC^{\cdot}H_2$) and hydrogen radical addition to the phenyl group to give (82).[587] Identification of radicals formed in X-ray-irradiated solid L-alanine has been reported,[588] and ESR properties of this amino acid are also described in support of *in vivo* radiation dosimetry, in a paper[589] that is representative of group of related studies described in a Conference Proceedings volume.



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5.6 Other Physico-chemical Studies of Amino Acids – This Section has settled into a number of sub-sections that are retained in the order in which they have been presented in recent Volumes, but topic areas now deserve to be highlighted through specific sub-headings.

5.6.1 Measurements for Amino Acid Solutions. – Continuation of major studies, into the calorimetry of ternary aqueous systems to establish pairwise cross-interaction coefficients for α-amino acid solutes,[590] enthalpies of interaction of some alkali-metal halides[591] and ammonium methanoate[592] with α-amino acids, appear side-by-side with papers of a less substantial nature (those cited here, are representative of a much larger literature), on solute interactions between NaCl and KCl with glycine, DL-alanine, DL-valine, and DL-leucine in water (Vol. 29, p. 60),[593] with D- and L-serine,[594] of NaCl and $NaNO_3$ with DL-threonine[595] and with glycine and DL-methionine,[596] effects of temperature and pH on the solubilities of common L-α-amino acids,[597] the degree of self-association in water of the remarkably water-soluble fullerene[60] – amino acid adducts (see also Refs. 578, 802, 942, 943),[598] and complex permittivity values for glycine and valine in aqueous organic solvents.[599] Differential scanning calorimetry of aqueous proline over the temperature range -60 °C to 20 °C has been carried out in pursuit of an explanation for the low temperature dependence of the high solubility of this imino acid in water.[600]

Isolated accounts have been published on hydration heat capacities for amino acids in their zwitterionic form,[601] activity coefficients for amino acids through vapour pressure measurements on aqueous solutions,[602] partition coefficients and solubility data for glycine in butan-1-ol – ethanol – water mixtures[603] and in dextran – poly(ethyleneglycol) – water,[604] and distribution of L-tryptophan in aqueous di-(2-ethylhexyl)phosphoric acid – hexane (Vol. 29, p. 2; *cf*. also Ref. 628),[605] de-protonation constants for seleno-DL-cystine and seleno-DL-methionine by potentiometry,[606] dissociation constants for amino acids in dioxan – water at 298 K,[607] apparent molar volumes of ω-amino acids in aqueous guanidine hydrochloride,[608] partial molal volumes of solutions of α-amino acids

in aqueous urea at different temperatures,[609] and partial molal volumes and adiabatic compressibilities of N-acetyl-DL-serinamide and the threonine analogue in aqueous media.[610] Further results for hydrophobicity measurements on amino acids in aqueous media have been obtained using a multichannel taste sensor (Vol. 27, p. 49).[611] Amino acids complexed to metal ions, $M(aa)_n$, where M = Co, Cr, Rh, are well salted-in to water by simple salts.[612]

The explanation for an increase in specific rotation with dielectric constant of the solvent, noted for N-methacryloyl-L-leucine methyl ester,[613] is of continuing interest. Chemiluminescence is generated by adding H_2O_2 and ethidium bromide to aqueous solutions of L-aspartic and L-glutamic acids,[614] and glycine and L-asparagine.[615]

Proline at high concentrations (greater than 4M) prevents the precipitation of lysozyme from aqueous solutions by trichloroacetic acid (the classical protocol for protein isolation from aqueous media).[616] Although this, and the finding that L-histidine cleaves RNA, but not DNA,[617] are isolated observations of a semi-quantitative nature, they deserve to be recorded in the thorough review of amino acid science that this Chapter attempts to offer. L-Seryl-L-histidine cleaves DNA, but its N^{α}-(O,O-di-isopropyl)phosphoryl derivative is less effective in this respect.[618]

5.6.2 Measurements for Solid Amino Acids. – Differential scanning calorimetry of water-containing samples of DL-2-aminobutanoic acid and DL-norleucine have revealed series of similar phase transitions.[619] The optical activity of crystalline L-aspartic acid has been assessed.[620]

5.6.3 Amino Acid Adsorption and Transport Phenomena. – These topics also relate to studies that are described elsewhere in this Chapter, of chromatographic separations (Section 7.5, 7.6) and resolution (Section 4.16) of amino acids. A major topic area is illustrated in a study of the mechanism of adsorption of glutamic acid to a weakly basic ion-exchange resin,[621] of alanine to boehmite,[622] and variation with sodium chloride concentration of the uptake of leucine and phenylalanine by a strong cation exchanger.[623]

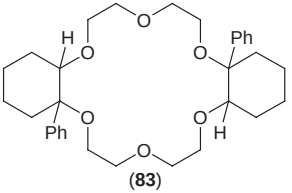
Another topic that is receiving much attention, for its *in vivo* role as well as for its practical applications, is transport of amino acids through membranes: separation of nine different amino acids on the basis of their differing electrostatic attraction for groupings built into a nanofiltration membrane;[624] a similar role for plasticized cellulose triacetate membranes containing large amounts of a quaternary ammonium salt (effectively a supported liquid membrane);[625] transfer of tryptophan and lysine through an impregnated liquid membrane using a strong acid,[626] and of tryptophan hydrochloride through PTFE-supported liquid membranes containing macrocyclic carriers;[627] and extraction of amino acids using PVF-supported liquid membrane with di-(2-ethylhexyl)phosphoric acid as carrier (*cf.* Ref. 605).[628] Transport properties for amino acids through a chitosan membrane have been reviewed,[629] and a review of amino acid transport by crown ethers has appeared.[630]

Size-exclusion chromatographic separation of amino acid mixtures has re-

vealed unexpected behaviour for tryptophan, ascribed to adsorption equilibria involving the stationary phase,[631] and L-thyroxine binding to human serum albumin as column chromatographic medium has been noted.[632] Chelated metal ion affinity chromatographic separation of amino acids and peptides using a Sephadex G10 medium modified by successive reaction with epichlorhydrin and copper(II) iminodiacetate has been described, separation depending on the differing stabilities of the copper(II) complexes of the amino acids.[633] Optimised preparative ion-exchange separation of a mixture of nine amino acids depends on simpler principles.[634]

5.6.4 Host–Guest Studies with Amino Acids – A series of supramolecular complexes between ammonium salts of L-α-amino acids and 2,3,11,12-bis[4-(11-aminoundecanoyl)benzo]-18-crown-6 has been described,[635] and the binding of alkylammonium salts of acids to 18-crown-6 hosts in water – 1,2-dichloroethane mixtures has been correlated with structure.[636] Molecular orbital calculations for relative binding free energies (*i.e.* enantioselectivities) for complexes of ammonium salts of amino acids with synthetic ionophores,[637] and for binding of methyl L-alaninate and L-alanine N′-methylamide to podand ionophore hosts and to C_3-symmetric receptors[638] have been reported.

Preferential binding of the L-enantiomer of an amino acid ester hydrochloride by a chiral crown ether (83) prepared from (R,R)-1-phenyl-1,2-cyclohexanol occurs with low enantioselectivity (14 – 26%),[639] as is also the case for (84), acting as host for aromatic amino acids,[640] and for N-protected amino acids interacting with chiral C_3-symmetric cage-like receptors [(S,S,S)-(+)-85] that carry convergent helically-oriented amide binding sites (Vol. 29, p. 61).[641] Rather better results are achieved with Z-DL-glutamic acid using sapphyrin derivatives [porphyrin-CO-X-CO-porphyrin; X = (1S,2S)-1,2-bisamidocyclohexane][642] and for underivatized amino acids with ammonium 2,3,11,12-bis[4-(10-aminodecylcarbo-



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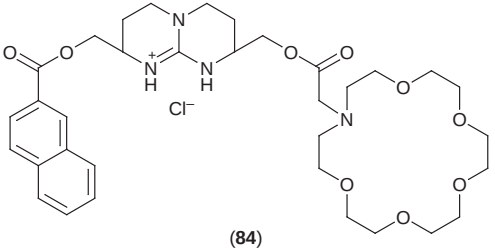

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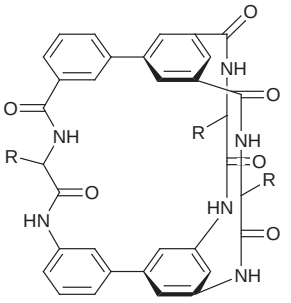
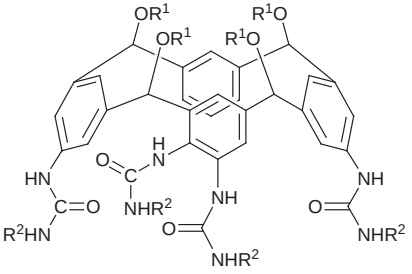


(**85**)

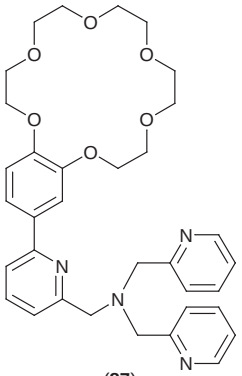
nyl)]benzo-18-crown-6[643] and β-cyclodextrin derivatives (*e.g.* L:D = 33:1 for leucine).[644] The 6-O-monophosphate of β-cyclodextrin shows moderate to good enantioselectivity in favouring complex formation with the D-enantiomer when presented with a racemic amino acid;[645] thermodynamic stability constants of amino acid – cyclodextrin complexation[646] and of complexation of amino acids with mono-[6-(1-pyridinio)-6-deoxy]-α- and γ-cyclodextrins[647] and anilino-β-cyclodextrin analogues[648] have been reported. The association of a copper(II) β-cyclodextrin complex with aromatic amino acids,[649] and the enhanced fluorescence, with intensity in proportion to the concentration of an amino acid in the same solution, of the copper(II) complex of a dansyl-diethylenetriamine-modified β-cyclodextrin,[650] have been described.

Calix[4]arene-based α-aminophosphonates exhibit remarkable selectivity as carriers for membrane transport of the zwitterionic form of aromatic amino acids,[651] and calix[4]arene dimers (86) and amino acid derivatives form homochiral capsules.[652] The binding profile of the novel host (87) for a series of amino acid guests indicates that the two binding sites are 6.5 angstroms apart.[653]



(**86**)

One of these hosts (84), specific for aromatic amino acids, carries a chiral bicyclic guanidine moiety, and a remarkable opposite to this is seen in the arene $[4\text{-}\{(MeO)P(O)_2CH_2C_6H_4\}_2X]^{2-}$ $[Bu_4N]_2$ (X = S, O, *etc.*) that binds to guanidines (especially to N- and C-amide-protected arginines), exactly analogous to the 'arginine fork' postulated for RNA-protein recognition in the AIDS virus.[654]



(87)

Competition between N-Z-amino acids for a novel host (zinc acetate – N-methylmesoporphyrin-II basket with a xylylene-diamide 'handle') favours Z-glycine, but in $CHCl_3$ – water those guests carrying hydrophilic side-chains are preferred.[655]

5.7 Molecular Orbital Calculations for Amino Acids – The unremitting flow of papers describing molecular orbital calculations chosen to simulate the behaviour of an amino acid residue in a polypeptide continues with N-formyl-L-phenylalaninamide,[656] the N-acetyl amino acid N-methylamides of L-alanine,[657] αα-dialkylglycines,[658] valine,[659] asparagine,[660] cis- and trans-2,3-methanomethionines,[661] and phenylalanine analogues with crowded side-chains (Ref. 330), the main differences from previous studies being the gas-phase or solution context of the molecule under scrutiny. Adsorption binding energies of amino acids to ionophores have been calculated (Refs. 637, 638).

Calculated conformational equilibria for N-acetyl alanine-, leucine-, and glutamine-N-methylamides at the water – hexane interface have been presented,[662] and data for N-acetyl-L-prolinamide have been compared with calculations for the free imino acid.[663] The eight most stable conformers of N-methylglycine have been identified, in parallel with similar calculations for the NN-dimethyl analogue.[664] Conformational studies for the glycine zwitterion in aqueous solution[665] and in the gas phase,[666] and corresponding treatment for the L-alanine zwitterion,[667] methyl 2-acetamidoacrylate, and energy calculations for the N-acylimine – enamide tautomers of this compound,[668] kainic acid (Ref. 541), and 2-(carboxycyclopropyl)glycine (Ref. 542) exemplify the thermodynamics context to which MO calculations can contribute (for a review see Ref. 669). This context is also a feature of intramolecular proton transfer within glycine in aqueous solution[670] and the related topic of the proton affinity of protonated glycine,[671] conformational energies of glycine and dithioglycine,[672] free energies of hydration of the coded amino acids in their zwitterionic form,[673] hydration structures of N-acetyl-L-leucinamide and its glutamine analogue,[674] glutamic acid – water interactions,[675] energetics of the conversion of L-arginine into its

N^G-hydroxy derivative,[676] absolute affinities of α-amino acids for copper(I) ions in the gas phase,[677] and Raman vibrational frequencies for L-asparagine[678] and for L-glutamine hydrate.[679] Calculations of standard molal thermodynamic properties of L-α-amino acids at elevated temperatures and pressures underpin a practical purpose, illustrated with effects on lysine and arginine, which exist in aqueous solutions at pH 7 at 25 °C almost entirely as protonated species, but are calculated to be 50% dissociated at 125 °C under pressure.[680] Electron – ion interactional potentials of amino acids have been correlated with physical properties such as hydrophobicity.[681]

Solvent accessibility offered by aromatic side-chains in water-organic solvent mixtures,[682] and similar studies of tryptophan,[683] have been featured in recent papers.

Amino acid radicals receiving attention include glycine in aqueous solutions in relation to their ESR characteristics,[684] tyrosine,[685] and the protonated tryptophyl radical cation $TrpH^{+\cdot}$.[686] The energetics of hydrogen transfer between amino acids in the presence of radicals has been considered.[687]

6 Chemical Studies of Amino Acids

6.1 Racemization – Complete racemization of amino acids liberated during protein hydrolysis occurs using 4M $Ba(OH)_2$ as reagent at 110 °C for 48 h.[688]

Racemization rates for aspartic acid at 60, 80, and 100 °C have been determined, and lead to an activation energy 29.1 kcal mol^{-1} for the process. The stimulus for this work was the derivation of a racemization rate for ambient 12.5 °C, so that age could be determined for a sample of colonial anemone *Gerardia* collected alive at 630 m depth in the sea off the Bahamas.[689] The figure was 250 ± 70 y, on the basis of the D/L-ratio for aspartic acid in samples taken from innermost and outer layers of the trunk, and, not surprisingly, was more readily believed than a radiocarbon date 1800 y.

Such figures derived from amino acid racemization data have become less respected; however, protein at constant temperature, and avoiding turnover through its life cycle, should provide credible data for a living organism. Abstracts of papers given at a Conference 'Perspectives on Amino Acid and Protein Geochemistry', published in Issue 3 of Volume 15 (1998) of the journal *Amino Acids*, include a large number of dating applications. A survey of principles and applications of amino acid racemization dating includes appropriate warnings of sources of inaccuracy,[690] and of course, errors will be introduced where there is uncertainty about the circumstances in which the samples have been placed; for example, stereoinversion at the aspartic acid residue at position 151 of αA-crystallin from human eye lens is a result of UV-B irradiation, but no changes occur in UV-A light.[691] Improved protein recovery from fossils and animal bones, and protocols for preparation of N-alkoxycarbonyl derivatives of methyl and ethyl esters for GLC estimations of aspartic acid obtained from the protein hydrolysates have been described.[692]

6.2 General Reactions of Amino Acids

6.2.1 Thermal Stability of Amino Acids – Isolated observations on this topic have been recorded over the years, but the greater interest in this topic, now that amino acids have been found in samples from extraterrestrial sources and from high temperature vents on the ocean floor, is revealing a lack of disciplined knowledge.

Contradictory claims that simple amino acids have been sublimed unchanged (Ref. 25), and that they self-condense through such treatment (Refs. 784, 786, 787), should be seen with a comment that the undersea vent scenario might make it unreliable to reason from laboratory analogies (Ref. 301). Results from earlier literature under this heading are summarised in Ref. 693.

6.2.2 Reactions at the Amino Group – Replacement of hydrogen at the amino group by simple species (kinetics of N-chlorination by N-chlorosuccinimide[694]) continues a long-running study (Vol. 29, p. 64). A mechanistic study of N-nitrosation of amino acids has been reported.[695]

Replacement of the amino group by halogen with retention of configuration, following the standard protocol, has been applied to D-methionine[696] and δ-phthalimido-L- or D-ornithine[697] for syntheses starting from homochiral amino acids. Conversion of an α-amino acid ester into an α-isocyanoalkanoate avoiding the use of phosgene has been described (DMAP- Boc_2O),[698] and a route to tert-butoxycarbamates from benzamides and acetamides (*e.g.*, AcNHR → AcNBocR → BocNHR) has been described.[699]

N-Acylation by standard procedures has provided N-(benzotriazol-1-ylcarbonyl)amino acids.[700] Lipases catalyse long-chain acylation of L-serine and L-lysine derivatives,[701] and aqueous thioacetic acid, acting as an oxidizing agent, catalyses the acetylation of phenylalanine and leucine, suggesting a primordial role for this compound.[702] Acylation occurs readily in compressed monolayers containing long-chain thioesters of N-acetylglycine and glycine-N′-alkylamides $H_2NCH_2CONH(CH_2)_{15}Me$.[703]

Reaction of an amino acid ester with bis(4-nitrophenyl) carbonate gives hydantoins as well as the expected NN′-carbonyl bis(amino acid ester)s.[704] Removal of the Boc group using BF_3-etherate in CH_2Cl_2 in the presence of molecular sieves requires only room temperature conditions;[705] removal of N-allyloxycarbonyl protection employs $Pd(PPh_3)_4$ – $PhSiH_3$.[706] The preparation of N-Fmoc-amino acids and their use in peptide synthesis has been reviewed.[707] Preparation of N-Fmoc-N-(2-hydroxy-4-methoxybenzyl)amino acids avoiding the formation of N,O-bis(Fmoc) side-products has been described.[708]

Sulfenamides have been prepared with the purpose of generating aminyl radicals through treatment with Bu_3SnH/AIBN.[709] N-Phosphonylation is a clean process when a catalytic amount of 1H-tetrazole accompanies a phosphonyl dichloride in the reagent mixture, since unwanted replacement of both chlorine atoms in sequential reactions with an alcohol and an amino acid is avoided.[710]

N-Silylation of a Boc-amino acid using a silyl triflate in dichloromethane at 0 °C has been described as 'protection of a protecting group', to emphasise the

point that the acidity of the carbamate proton can frustrate synthetic operations elsewhere in the molecule (see also Ref. 724).[711] Unexpected N-acetylation of the N-Fmoc-group of Fmoc-L-seryl and threonyl glycosides has been noted during standard protocols aimed at acetylation of the glucosamine moiety.[712]

N-(α-Picolinoyl)amino acids and peptides prepared from them, are easily deprotected by electrochemical reduction.[713] α-Chloroethyl chloroformate in methanol can be used to debenzylate NN-dialkyl-N-benzylamines.[714]

Schiff base formation is usually routine, and reaction of sn-1-palmitoyl- or stearoyl-2-(9-oxo-nonanoyl)glycerophosphocholine with amino acids is worthy of mention in the context of the unusual reagent.[715]

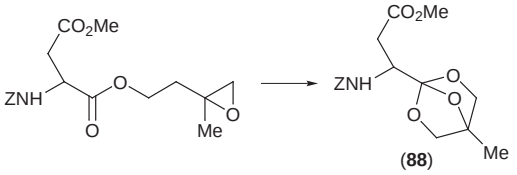
N-Alkylation of amino acids by carbene insertion into an N-H bond (Z_2C: + $RNH_2 \rightarrow RNH\text{-}CHZ_2$) can be accompanied by β-alkylation and ammonium ylide formation; existing knowledge on this topic has been reviewed,[716] and the process applied to an N-protected amino acid amide has been advocated as a new peptide synthesis approach ($R^1NHCHR^2CONH_2$ + $EtO_2CC(=N_2)P(O)(OEt)_2$/ $Rh_2(OAc)_4$/toluene → $R^1NHCHR^2CONHCH[P(O)(OEt)_2]$).[717] Preparation of N-methylamino acids on a solid support uses the Fukuyama method [(2-nitrobenzenesulfonylation, Mitsunobu methylation or the equivalent using MeI/ K_2CO_3, and removal of the sulfonyl protecting group with PhSNa in DMF],[718] also applied to 4-nitrobenzenesulfonamides but in normal solution media.[719] Alkylation procedures have been described that are appropriate for the preparation of N-(ferrocenyl)amino acids,[720] N-[(R)-1-phenylethyl]phenylalanine and N-(3,4,5-trihydroxyphenyl)glycine,[721] N-(homoallylic)-amino acids (from a Schiff base using an allylindium reagent),[722] N-(arylation) using a substituted fluorobenzene,[723] N-(2-hydroxy-4-methoxybenzylation) through reaction with the corresponding aldehyde, followed by $NaBH_4$ reduction, and preparation of their N-Fmoc derivatives after O-protection using trimethylsilyl chloride (see also Ref. 711),[724] N-alkylation (benzophenone Schiff base, HCHO or other alkanal, $NaBH_3CN$),[725] N-vinylidenation (addition of Cl_2C: to a benzophenone Schiff base to generate a dichloroaziridine, then ring opening and dechlorination).[726] Use of the Mitsunobu reaction for the N-monoalkylation of amino acid esters after N-(2,2,5,7,8-pentamethylchroman-6-sulfonyl)ation has been demonstrated.[727] N-Methylation of an N-Boc-amino acid after NaH deprotonation can be accomplished using methyl iodide (Ref. 767).

N^{α}-Alkylation of glycinamide using an alkyl bromide is an essential stage in peptoid synthesis, and repetitive use of an N-alkylation – bromoacetylation sequence has provided peptoid oligomers carrying N-acetylglucosamine residues.[728] N-Benzhydrylation of methyl pyroglutamate using benzhydryl chloride requires prior N-trimethylsilylation and triflic acid catalysis.[729]

A particular form of N-alkylation of amino acids *via* Schiff bases is represented in the Maillard reaction.[730]

N-Oxide formation from an amino acid is a highly stereoselective process; the unusually stable products (see also Vol. 28, p. 59) from N-benzylpipecolic acid and its analogues have been assigned relative configuration through X-ray crystal analysis.[731]

6.2.3 Reactions at the Carboxy Group – Formation of methyl esters has been reported when methanolic plant extracts (*Inga punctata foliar*) containing hydroxypipecolic acids are purified over a cation exchange resin.[732] Improvements to conventional esterification procedures have eased the preparation of tert-butyl esters (only tert-BuOH, anhydrous $MgSO_4$, and H_2SO_4 are needed, though lengthy reaction times in stoppered vessels are involved; the procedure also converts OH groups into tert-butyl ethers).[733] Secondary alkyl esters can be prepared from Z-L-amino acids in organic solvents using a Celite-immobilized proteinase or lipase, but 3 – 4 days reaction times are needed to secure yields around 70%.[734] Amidation of an N-protected amino acid by an alkylamine uses Boc_2O – pyridine (Ref. 698 describes a different outcome with Boc_2O-DMAP) described in a paper that contributes further to the voluminous preceding literature on this process (Vol. 28, p. 62).[735] Preparation of N-Z-amino acid ortho-esters (88) has been accomplished through $Cp_2(Cl)Zr^+$-catalysed rearrangement of 3,4-epoxyalkyl esters.[736]

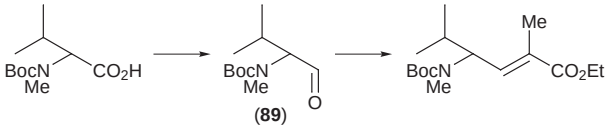


(**88**)

β-(Trimethylsilyl)ethoxymethyl esters can be cleaved using magnesium bromide etherate.[737] Pd/C-Catalysed hydrogenolysis of benzyl esters can be accomplished while preserving 4-methoxybenzyl ethers intact, if conducted in the presence of pyridine.[738]

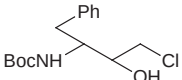
Reactions at the carbonyl group of an amino acid ester may be influenced considerably by the adjacent structural features, as in the reaction of organolithium reagents with methyl N-(phenylfluorenyl) pyroglutamate to give corresponding ketones, ascribed to unusual stabilization of the tetrahedral intermediate [$R^1CO_2Me + R^2Li \rightarrow R^1CR^2(OLi)Me$].[739] Regioselectivity of attack by an aniline on N-protected aspartic and glutamic acid anhydrides is dependent upon the solvent used.[740] Ketones can be formed on solid supports by reaction of the Weinreb amide RCON(Me)OMe with Grignard reagents,[741] also an intermediate in chain extension, *via* the α-aminoaldehyde (89) in a route to γ-amino-αβ-unsaturated esters.[742] An unusual twist is provided by coupling of γ-amino αβ-unsaturated acids to a solid support for use in peptide synthesis, terminated by ozonolysis to release peptide C-terminal aldehydes.[743] Condensation of an α-amino acid ester with $CH_2=C(OLi)OBu^t$ gives a β-ketoester,[744] and formation of α-ketoamides from homologation of the carboxy group proceeds *via* the acyl-cyanophosphorane intermediate $RCOC(=PPh_3)CN$.[745]

α-Amino bromomethyl ketones can be prepared from N-alkoxycarbonylamino acid esters, avoiding the use of diazomethane, through the Kowalski procedure (iodochloromethane and LDA), and have been used in α-aminoalkyl epoxide synthesis on a large scale.[746] α-Aminoalkyl chloromethyl ketones are claimed to



(**89**)

provide the best entry to α-aminoalkyl epoxides through stereoselective reduction,[747] although 3-Boc-aminoalkane-1,2-diols provide the same product through a modified Mitsunobu reaction.[748] Diastereoselective reduction of the chloromethyl ketone from Boc-L-phenylalanine using *Streptomyces nodosus* SC13149 gives the alkanol (90).[749] Amino acid diazoketones, (S)-$R^1R^2NCHRCOCHN_2$, form ketenes on photofragmentation that may be trapped *in situ* with an aldimine to yield an aminoalkyl-β-lactam.[750] Vinyl ketones $BocNHCHR^1COCH{=}CHR^2$ give aminodiol dipeptide isosteres through enantioselective reduction of the carbonyl group followed by aminohydroxylation of the C=C bond[751] and dimerization of methyl ketones $Bn_2NCH(CH_2Ph)COCH_3$ has been accomplished by copper(II) reagents acting on the sodium enolate.[752]



(**90**)

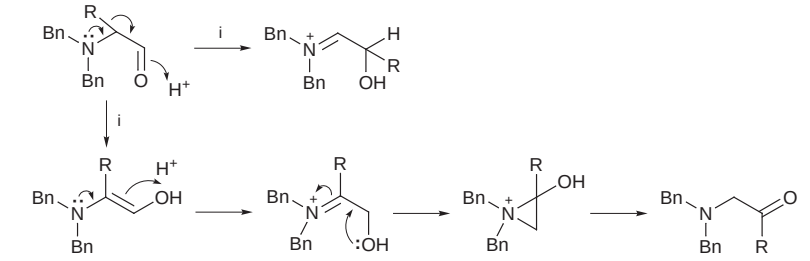
Fmoc-L-phenylalanyl fluoride can be used for O-aminoacylation of oligonucleotides after protection of phosphate groups by β-cyanoethylation.[753]

Tertiary amide enolates derived from amino acids are being used increasingly in general synthesis because of their propensity to electrophilic attack, and the simplest of these processes is re-protonation, illustrated to be enantioselective for an N^{α}-(alkoxycarbonyl)pipecolic acid amide when a chiral amine is used as proton source.[754] Cleavage of secondary amides is effected by N-nitrosation followed by treatment with lithium hydroperoxide and reduction with sodium sulfite.[755] Sodamide – MeCN converts an N,N-dibenzylamino acid ester into the cyanomethyl ketone, a useful source of enaminones $Bn_2NCHR^1CO{-}CH{=}CR^2NH_2$ that are accessed through addition of excess Grignard reagent R^2MgCl.[756]

Reduction of the carboxy group of an amino acid to the aldehyde provides a useful synthesis intermediate, and sets the stage for further functional group elaboration. The DIBAL-H reagent is often used for this conversion, but better results may be achieved in some cases (N-Boc-S,O-isopropylidene-L-serinal from L-serine methyl ester) through lithium aluminium hydride reduction to the primary alcohol, followed by Swern oxidation (methodology already published; Vol. 27, p. 64).[757] The use of DIBAL-H with N-Boc-β-aminonitriles gives N-Boc-β-aminoaldehydes (*e.g.* 89) after the usual work-up.[758] Acetals [*e.g.* (R)-$BocNHCHMeCH(OMe)_2$] derived from these aldehydes can be converted into homoallylic aminoalkanols (R)-$BocNHCHMeCH(OH)CH_2CH{=}CH_2$, syn:anti = 68:32) with tetra-allyltin – TFA,[759] while reductive allylation of amino acids with

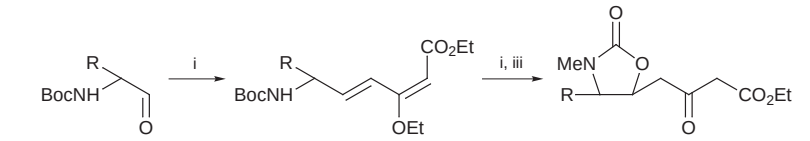
diallylborane gives 1,1-diallyl-2-aminoalkanols (useful as substrates for iodocyclization to 3-allylpyrrolidines).[760] The N-substituent of an N-protected amino aldehyde has a considerable effect on the stereochemical outcome of three-carbon elongation using allylmetal reagents.[761] No such complication arises in the case of one-carbon homologation by Sm(0)/CH_2I_2 [-CHO → -CH(OH)CH_2I].[762]

Boc-α-Aminoaldehydes (*e.g.* 89) have been employed in azapeptide synthesis (condensation with an acylhydrazide and reduction of the C=N bond),[763] and feature in an unusual rearrangement to give ketones, presumed to involve either a 1,2-alkyl shift followed by a 1,2-hydrogen shift, or an equivalent process (Scheme 31).[764] Further examples of synthesis applications for α-aminoaldehydes include



Scheme 31

syn-aldolization to give α-aminoalkyl epoxides,[765] condensation of N-phosphonyl derivatives with a chiral phosphonate $(RO)_2P(O)CH_2CO_2R^*$ [R* = (-)-menthyl] leading to stereoisomer mixtures as a result of easy epimerization at the α-carbon atom.[766] Similar condensation with a 2-ethoxy-(3-ethoxycarbonylallylidene)triphenylphosphorane gives 6-Boc-amino-3-ethoxy-2,4-hexadienoates, used in a synthesis of homochiral N-methyl-oxazolidinones (Scheme 32) through cyclization after N-methylation.[767] Aldolization with (R,E)-crotylsilanes and their S-enantiomers, $MeCH{=}CR^1CH(SiMe_2Ph)CH_2CO_2Me$, leads to 7-(Boc-amino)alk-3-enoates (Ref. 413).[768] Boc-α-Aminoaldehydes react with an allylindium reagent to give allyl alcohols with very modest stereoselectivity.[769]



Scheme 32

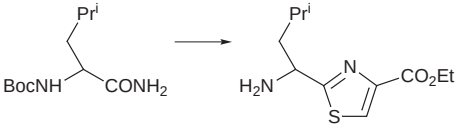
Radical cyclization of N-allyl-α- and -β-aminoaldehydes using Bu_3SnH/AIBN in boiling benzene (*cf.* Ref. 817) leads to hydroxypyrrolidines and hydroxypiperidines.[770]

N-Protected (S)-β-aminoalkanols, prepared from L-amino acids, have nu-

merous uses in their own right, and conversion into corresponding bromoalkanes and attachment through the ether linkage to 3,5-dihydroxybenzoic acid or gallic acid followed by acylation of the amino groups after deprotection, followed by repetition of these steps a number of times, gives highly divergent dendrimers.[771]

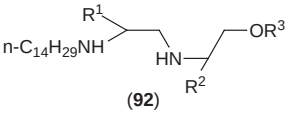
Hypotaurine $H_2NCH_2CH_2SO_2H$ is a special case of an amino acid with an acid group at low oxidation level, and therefore shows the expected tendency to disproportionate into taurine, cysteamine, and cystamine.[772]

Esters of L-proline undergo Curtius rearrangement to give isocyanates,[773] and acylnitroso dienophiles can be generated *in situ* through oxidation by $NaIO_4$, of N-protected amino acid hydroxamates (S)-Boc-NHCHMeCONHOH.[774] Amino acid amides can be developed through the carbonyl group *via* thioamides into thiazolecarboxylates (91)[775] and into analogous oxazoles[776] as dipeptide surrogates, through standard methods.



(**91**)

Kinetics have been determined for copper(I) or zinc-catalysed hydrolysis of alanyl ethyl phosphate, viewed as an analogue of an aminoacyl adenylate.[777] Catalysed enantioselective ester hydrolysis has been a long-running topic in the amino acid field, and this year there are further results from enantioselective hydrolysis of p-nitrophenyl N-dodecanoyl-D- or L-phenylalaninate catalysed by L-histidine-containing surfactants (Z-L-Phe-L-His-L-Leu-OH can generate almost 100% discrimination).[778] A new approach is the involvement of lipophilic homochiral ligands (92) in cationic aggregates [*e.g.*, of cetyltrimethylammonium bromide containing a copper(II) salt], which generates remarkably high enantioselectivity (11 to 35-fold).[779] N-(β-Hydroxyethyl)amino acid amides are unusually easily hydrolysed, due to participation by the hydroxy group of the N-substituent, and therefore they are easily aminolysed and are activated towards peptide bond formation under mild conditions.[780]

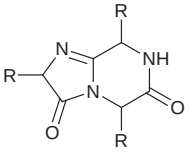


(**92**)

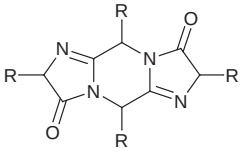
Peroxyl radical attack on [1-^{13}C]leucine has been shown to lead to decarboxylation but also to several products in which the carboxy group is retained, notably 3-methylbutanoic acid.[781]

6.2.4 Reactions at both Amino and Carboxy Groups – Self-condensation of amino acids in concentrated salt solutions (Vol. 28, p. 64) has been established to be a

remarkably simple pathway to peptides, that may have relevance to prebiotic synthesis. Studies of this topic by the pioneers have moved on to specific protocols; *e.g.* alanine and glycine subjected to wetting-drying sequences at 80 °C on alumina, silica, montmorillonite, or hectorite leads to dioxopiperazinediones with the first two minerals but to longer oligopeptides with the second two.[782] Exploration of sequence preferences (those amino acids that self-condense with ease, or condense with other amino acids in mixtures, and those that do not) has also featured in recent studies.[783] Other research groups are studying mechanistic details [kinetics for the copper(II) acetate – glycine – alanine system].[784] Structure-reactivity relationships may emerge for this process from future studies along these lines. Carbonyldi-imidazole accomplishes the oligomerization of L-aspartic acid, L-glutamic acid, and O-phospho-L-serine in aqueous solution in a most efficient manner,[785] as seen for the self-condensation of glycine in an aqueous suspension of zeolite and kaolinite, presumed to occur *via* dioxopiperazine.[786] Sublimation of simple amino acids alanine, valine, leucine, or α-aminoisobutyric acid over silica at 230-250 °C under reduced pressure gives dioxopiperazines and the surprising homologues (93) and (94).[787]



(**93**)

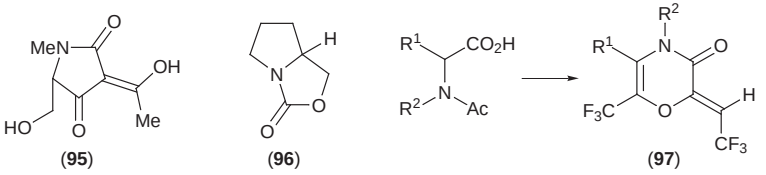


(**94**)

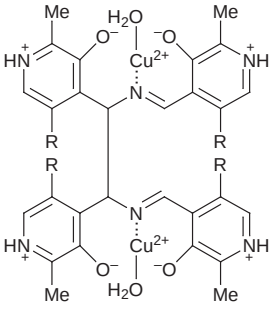
Solid amino acid mixtures bombarded with high energy particles, simulating cosmic ray irradiation, accumulate peptides,[788] a result that is easier to understand than the claimed formation of peptides when the frequency corresponding to the cyclotronic frequencies of amino acids matches that of an irradiating alternating magnetic field combined with electric fields.[789]

Homochiral aziridines obtained from (R)- and (S)-norvaline through successive $LiBH_4$ reduction, O-toluene-p-sulfonylation, and K_2CO_3-mediated cyclization, have been used in an epilachnene synthesis to establish the absolute stereochemistry of the natural azamacrolide from *Epilachna varivestis*.[790] N-Methyl-L-serine has been converted into (95), a tetramic acid analogue of physarobinic acid from *Physarum polycephalum*.[791] Several other uses of amino acids in heterocyclic synthesis are well-established, illustrated this year for syntheses from L-prolinol of the oxazolone (96) and its imines and sulfur analogues,[792] dioxopiperazines from differently-protected aspartic acids,[793] the morpholinone (97) from N-alkyl N-acetylamino acids treated with trifluoroacetic anhydride and pyridine,[794] and oxidized lipid – amino acid reaction products 1-methyl-4-pentyl-1,4-dihydropyridine-3,5-dicarbaldehyde, 1-(5-amino-1-carboxypentyl)pyrrole, and N-Z-1(3)-[1-(formylmethyl)]hexyl-L-histidine.[795] Extraordinary reaction products [98; R = $CH_2OP(O)(OH)O^-$, equal amounts of (R,R)- and (S,S)-isomers], arise from copper(II)-mediated reactions of L-amino acids with pyridoxal 5-phosphate.[796]

A standard synthesis of hydantoins appears in a solid-support version,[797] and



(**95**) (**96**) (**97**)



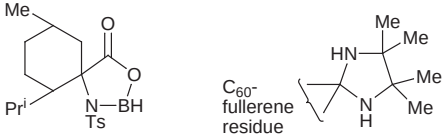
(**98**)

amino acid, acetyl chloride, and trimethylsilyl isothiocyanate reaction mixture gives authentic standards for the revived peptide sequencing method in which the C-terminal residue is converted by cleavage into a thiohydantoin.[798]

Several simple protocols are available that bring about the protection of both amino and carboxy groups of an α-amino acid in one step, though BF_3-etherate condensation must be one of the easiest practical operations of this type. It has been used in an improved procedure for side-chain protection of serine and threonine.[799] The N-toluene-p-sulfonyl derivative of the menthol-derived amino acid reacts with BH_3-THF in nitroethane at 45 °C during 1 h to give the Lewis acid (99; Masumune's catalyst) whose use in asymmetric aldol reactions is illustrated in this work.[800]

An unusual incorporation of an amino acid to form a di-aza-crown ether has been developed, using L-threoninol and di(2-chloroethyl) ether; the route was simple to operate because the secondary alcohol function did not require protection.[801]

[60]Fullerene derivatives of amino acids (Vol. 29, p. 73) have been prepared efficiently, and their properties surveyed; these include one outstanding characteristic, their surprisingly high water-solubility (see also Sections 5.5, 5.6.1).[802] The spiroheterocycle (100) emerges after 20 h from a [60]fullerene – DL-valine –



(**99**) (**100**)

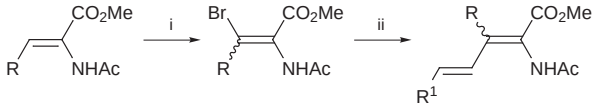
4,4,5,5-tetramethylimidazoline-2-thione mixture in refluxing chlorobenzene under nitrogen.[803] The mechanism proposed seems unlikely, since it requires a carbene intermediate (whose generation in the absence of the fullerene was not tested, but whose presence should be readily demonstrable).

6.2.5 Reactions at the α-Carbon Atom of α- and β-Amino Acids – Nickel peroxide oxidation of N-benzoylamino acids involves N-C^{α} bond cleavage since benzamide is formed in high yield.[804] α-Methylation (MeI/LDA) of ethyl N-benzylidene-β-alaninate and work-up can be achieved sufficiently rapidly to allow production and use of [^{11}C]-β-aminoisobutyric acid within the short time of life of the isotope.[805] α-Proton – ^{2}H-exchange through the classical N-acetylation – cyclization – $^{2}H_2O$ hydrolysis sequence (see also Ref. 539) followed by acylase resolution, gives 2S-[2-^{2}H]kynurenine, also obtained from L-[2-^{2}H]tyrosine, prepared analogously, by ozonolysis.[806]

6.3 Specific Reactions of Amino Acids – Reactions of amino acid side-chains, or reactions that involve amino and carboxy groups as well as side-chains, are covered in this Section. The former category predominates in the literature, reflecting the widespread use of common amino acids as starting materials for the synthesis of other amino acids.

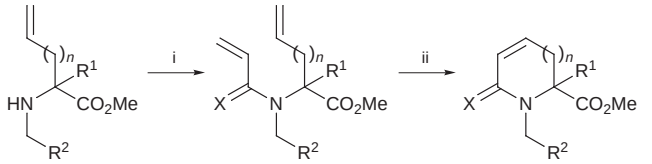
Saturated aliphatic side-chains offer few opportunities for structural modifications, or for the introduction of functional groups, though fenugreek seedlings accomplish the 4-hydroxylation of L-isoleucine, requiring iron(II) salts, ascorbate, 2-oxoglutarate and oxygen (thus implicating a dioxygenase in the process).[807] Industrial-scale conversion of proline into trans-4-hydroxy- and cis-3-hydroxy-L-prolines by *E. coli* proline hydroxylase has been reviewed.[808] Kolbe electrolysis of orthogonally-protected amino acid mixtures gives 2,5-di-amino-adipic acid, 2,6-di-aminopimelic acid, 2,5-di-aminoadipic acid, and 2,7-di-amino-suberic acid.[809]

Unsaturated aliphatic hydrocarbon side-chains offer much scope for functional group elaboration, illustrated for methyl (R,S)-2-(N-diphenylmethylidene)amino-pent-4-ynoate (hydrostannation of give regioisomeric tributylvinylstannane mixtures,[810] nucleophilic addition of N- and C-protected serine, cysteine, and lysine[811]) and coupling of vinylboronic acids to (Z)-β-alkenamide esters *via* a palladium acetate-catalysed Suzuki reaction (Scheme 33) to give α,β-γ,δ-unsaturated amino acids[812] and a simpler version in which indoles are coupled with ethyl α-acetamidoacrylate to give αβ-dehydrotryptophans[813] and cyclopropanation of this substrate with ethyl diazoacetate,[814] ring-closure of N-allyl-α-alkenylglycines through ruthenium-catalysed olefin metathesis to give highly-



Reagents: i, NBS/CH_2Cl_2, then NEt_3; ii, *trans*-$R^1CH{=}CHB(OH)_2$, $Pd(OAc)_2$, Na_2CO_3

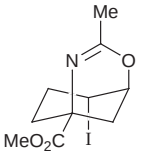
Scheme 33



Reagents: i, $CH_2{=}CHCOCl$ or $CH_2{=}CHCH_2Br$/base; ii, $Ru(Pcy_3)_2Cl_2$, CH_2Cl_2, reflux

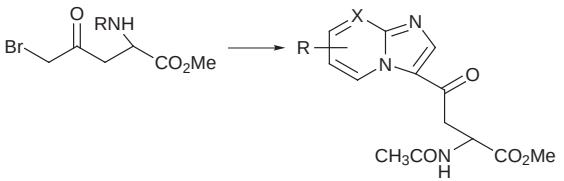
Scheme 34

functionalized 6-and 7-membered ring amino acids (Scheme 34),[815] analogous intermolecular metathesis of homoallylglycine derivatives with aryl- and alkylalkenes using 5 mol% $Cl_2(PCy_3)_2Ru{=}CHPh$ to give modest yields,[816] and diastereoselective cyclization to pyroglutamates of radicals derived from N-bromoacetyl-N-benzylaminoacrylates (esterified with a chiral alkanol) by treatment with AIBN-Bu_3SnH (*cf.* Ref. 770).[817] Catalysed deuteriation of 3,4-dehydroproline and RuO_2 – $NaIO_4$ oxidation gives the 3,4-labelled pyroglutamic acid which by $LiEt_3H$ and Et_3Ge^2H reduction results in (2S,3S,4R,5S)-[3,4,5-$^2H_3$]proline.[818] Pd-Catalysed reaction of dimethyl esters of N-Boc-kainic acid with aryl halides leads to (E)-vinylic substitution products.[819] Manipulation of the alkene moiety of N-acetylaminocyclohex-3-ene carboxylic acid gave (101) from which exo-3-hydroxy-7-azabicyclo[2.2.1]heptane-1-carboxylic acid, a new conformationally-constrained 4-hydroxyproline analogue, was prepared.[820]



(**101**)

α-(ω-Halogenoalkyl)-α-amino acids also provide useful synthesis intermediates for a wide range of applications, the routine use shown in the conversion of β-bromoarylalanines into β-hydroxy-analogues concealing a good deal of mechanistic interest.[821] The organozinc synthon $IZn(NC)CuCH_2CH(CO_2R)NHBoc$, derived from β-iodo-L-alanine, is particularly valuable; for example in Pd-catalysed conversion into (2S,6S)-4-oxo-2,6-diaminopimelic acid or through reaction with functionalized aryl iodides, into L-kynurenine;[822] also its use with (η^3-allyl)tetracarbonyl salts to give, for example, $PhSO_2CH{=}CHCHMeCH_2CH(CO_2R)NHBoc$, epoxidation and Zn-TMSCl-mediated cyclization giving 4,5-disubstituted pipecolic acids.[823] Diastereoselective methylation of 4-oxohomophenylalanine $ZNHCH(CO_2R)CH_2COPh$, formed from the same organozinc synthon, gives the anti-isomer from which all stereoisomers of a Nikkomycin B constituent, 2-amino-3-hydroxy-4-phenylbutanolide, were obtained by appropriate manipulation[824] (see also Ref. 240). 5-Bromo-4-oxonorvaline gives azatryptophans (102; X = N, CH, CR; *cf.* Vol. 28, p. 70) through condensation with pyridinyl- and pyrimidinyl-formamidines,[825] and (S)-2-amino-4-bromobutanoic



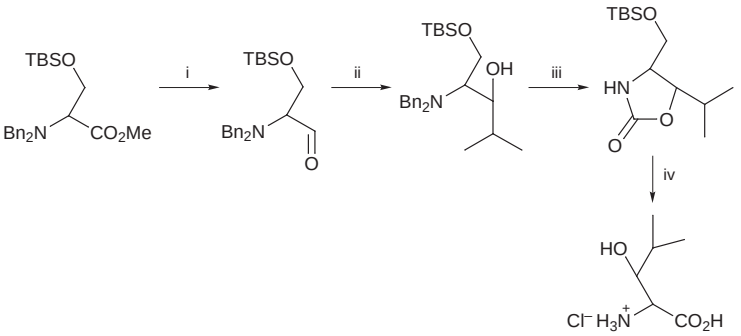
(**102**)

acid (formed from N-Boc-L-homoserine) has been used to construct α-amino acids each carrying one of the four DNA bases in the side-chain.[826]

α-(ω-Hydroxyalkyl)glycines, particularly the prototype β-hydroxyalkylglycine, L-serine, have featured in a rich variety of syntheses in recent years. However, novel pathways are rare; one such pathway is the conversion of nitrate esters of N,C-protected β-hydroxyalkyl-α-amino acids into alkoxy radicals on treatment with tributyltin hydride, from which α-C-centred radicals develop through β-scission.[827] The oxidation pathway from serine to dehydroserine has been established (the dehydration product, dehydroalanine, was the invariable end result of previous attempts) through a careful study of dimethyl sulfoxide – toluene-p-sulfonyl chloride oxidation. The (E)-enol toluene-p-sulfonate was the predominant product.[828] Enol triflates of 3,4-dehydroprolines from 4-hydroxy-prolines are more easily prepared *via* N-(9-phenylfluorenyl)-4-oxoprolines.[829]

The β-lactone of an N-protected L-serine can be opened by soft nucleophiles, as illustrated in a synthesis of S-prenylated L-cysteines.[830] More conventional leaving groups, found in O-mesylates or the β-iodo-alanine derived from N-trityl serine, or homologues derived from threonine or allothreonine, have been employed in intermediates used for syntheses of lanthionines and related cysteine derivatives[831] and L-selenocystine and its [^{77}Se]- and tellurium analogues;[832] in a preparation of γγ′-di-tert-butyl N-Fmoc-γ-carboxy-L-glutamate;[833] and in preparations of β-pyridylalanines using 2-, 3-, and 4-bromopyridines.[834] Mitsunobu processing of a protected serine (*cf.* Ref. 352) is now commonplace. Cycloserine (an isoxazolidinone formed from D-serinamide), undergoes changes in aqueous solutions to form β-(aminoxy)-D-alanine and its cyclic dipeptide (*i.e.* the dioxo-piperazine), as demonstrated by IR studies.[835] Side-chain protection of serine through strong acid-catalysed addition of isobutene has a long history of use, but the surprising demonstration that N-Fmoc- or N-Z-serine pentafluorophenyl esters are appropriate substrates for this reaction will be a welcome short cut, lessening the expense of materials for peptide synthesis.[836] O-Alkylation with a primary alkyl bromide is more difficult but has been accomplished in moderate to good yields over 17 h at room temperature using finely-ground KOH – Aliquat 336.[837] Glycosidation of methyl N-acyl-L-serinates by p-nitrophenyl-D-galactose employing a glycosidase from *Aspergillus oryzae* has been demonstrated,[838] and a representative example of enzymatic hydroxy-group replacement has led to (S)-2,4-diamino[4-^{11}C]butanoic acid after reduction of the intermediate cyanide.[839]

Another example to add to the list of syntheses starting from D-serine, in which the hydroxymethyl side-chain becomes the carboxy group of an amino acid of the L-configurational series, concerns (2S,3S)-β-hydroxyleucine (Scheme 35).[840]

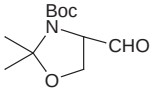


Reagents: i, $LiBH_4$ then Swern oxidation; ii, Pr^iMgCl; iii, $Pd(OH)_2$–H_2 then carbonyl di-imidazole/DMAP; iv, KF, Jones oxidation and conc. HCl

Scheme 35

The oxazolone formed by cyclization of N-acetyl-O-benzyl-L-threonine is easily epimerized and the hydrolysed product mixture can be resolved using hog kidney acylase I, offering an overall conversion into separate samples of N-acetyl-O-benzyl allo-D-threonine and its L-isomer.[841]

The N,O-protected L-serine derivative (103; the Garner aldehyde) continues to be chosen for syntheses of other α-amino acids: N-Boc-D-albizzine (104), *via* the protected (R)-2,3-diaminopropanol;[842] (2R,4R)-2,4-diaminoglutaric acids *via* the



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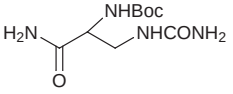
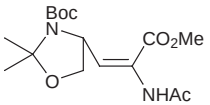


(**104**)

α-aminoacrylate (105);[843] (2S,3S)- and (2S,3R)-m-prenyl-β-hydroxytyrosine present in a novel cyclic heptapeptide from *Aspergillus flavipes*;[844] a maduropeptin moiety;[845] (2S,3S,4R)-phytosphingosine;[846] penaresidin A and related azetidine alkaloids;[847] and the D-O-E segment of vancomycin.[848] For a new synthesis of the Garner aldehyde, see Ref. 902. Conversion of the aldehyde group into ethynyl (-CHO → -C ≡CH) is already well-known and is exploited in a number of these examples, including simple elongation of this carbon chain through Pd-catalysed coupling with aryl and vinyl halides.[849] Unusual control of the stereochemistry of propargylation of the Garner aldehyde with LiC≡C-CH_2OTBS ($SnCl_4$ and HMPA favour syn- and anti-products, respectively) has been noticed.[850] The nitrone (106; *cf*. ref 91, 311) derived from L-serine *via* the



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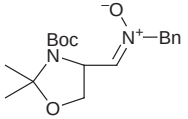

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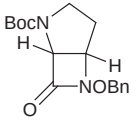


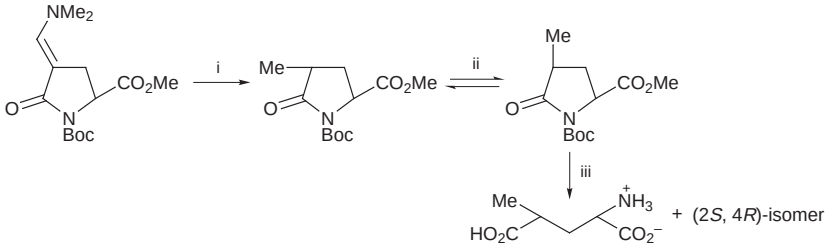
(**107**)

Garner aldehyde has proved useful in 2,3-diaminobutanoic acid synthesis[851] (another use for the Garner aldehyde is described in Ref. 429).

Suitably-protected cis-4-hydroxy-D-proline, elaborated into (2R,4R)-4-amino-pyrrolidine-2,4-dicarboxylic acid through a Bucherer-Bergs synthesis, generates a novel mGluR6 receptor antagonist.[852] trans-3-Hydroxy-L-proline hydroxamate has been cyclized to the β-lactam (107) through the Mitsunobu reaction,[853] as precursor to sulfams and sulfates (SO_3H and OSO_3H respectively, in place of OBn; potential β-lactamase inhibitors).

3-Hydroxyaspartic acid diastereoisomers are intermediates in the route from tartaric acid esters to (2R,3R)- and (2R,3S)-3-hydroxyaspartic acid β-hydroxamates.[854]

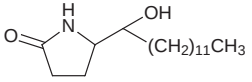
Aspartic and glutamic acids are represented in either acyclic forms or in one of several cyclized forms in synthesis applications: routes to 2-substituted-4-methylene-L-glutamic acids either through alkylation of the 4-methylene-L-pyroglutamate anion or through methylenation after alkylation of ethyl L-pyroglutamate;[855] conversion of tert-butyl 4-dimethylaminoalkylidene-N-Boc-pyroglutamate into chain-extended forms (NMe_2 → H, Me, Et, Ph, C≡CH, $CH{=}CH_2$ with DIBAL-H or a Grignard reagent),[856] conversion of L-pyroglutamic acid into (2S,4S)- and (2S,4R)-4-methylglutamic acid (Scheme 36);[857]



Reagents: i, H_2, Pd–C; ii, KCN, DMF; iii, LiOH, Pr^iOH, then HCl–AcOH and ion-exchange neutralization

Scheme 36

extension of the carboxy group of L-pyroglutamic acid to provide aza-muricatacin (108 and epimer at the side-chain chiral centre), an analogue of the hydroxybutanolide from *Annona muricata*;[858] a synthesis of (-)-bulgecinine (*cf.* Ref. 212) from L-pyroglutaminol[859] and from L-aspartic acid *via* a diazoketone [side-chain = $EtO_2CC(N_2)COCH_2$] (see also Vol. 29, p. 79),[860] and generation of ketones including 4-oxo-L-pipecolic acid from L-aspartic acid (Scheme 37).[861] Introduction of alkyl groups using trialkylaluminium reagents into the 5-position of pyroglutamic acid does not cause ring opening, so hydrogenation (Pt/C) leading to cis-5-alkylprolines can then be accomplished with secure knowledge of



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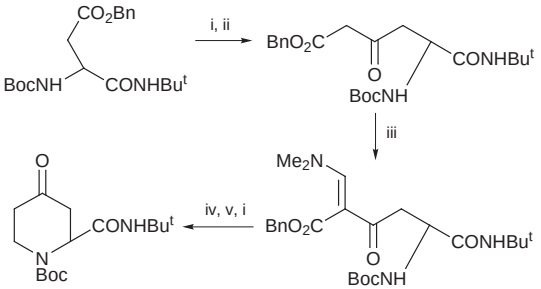
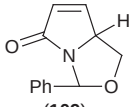


Reagents: i, H_2–Pd/C; ii, carbonyldi-imidazole, $(BnO_2CCH_2CO_2)_2Mg$; iii, DMF–dimethylacetal; iv, HCl; v, Boc_2O/DIPEA

Scheme 37

the stereochemistry of the product.[862] Adapting the carbonyl functions of L-pyroglutamic acid (CO_2H → $CH_2OTBDPS$; >C=O → >CH-CHO] gives a building block for a synthesis of trans-threo-trans-threo-trans-terpyrrolidine, needed for the preparation of potential hosts for molecular recognition studies.[863] Uses for the 3,4-dehydropyroglutaminol synthon (109) include β-amination (though this is a reluctant process in the absence of activating substituents),[864] and conversion into (2S,3R,4S)-epoxyproline *en route* to (2S,3S,4R)-epiminoproline *via* the azido-alcohol.[865] Addition of the imine PhCH=NTs to the enolate derived from the ring carbonyl group of pyroglutamic acid gives the 4-(1′-toluene-p-sulfonylamido)benzyl derivative.[866] A synthesis of spiroacetals from the Tephritid fruit-fly (particularly *Bactrocera*) uses aspartic acid to create the (R)-epoxide (110) through successive brominative deamination with retention of configuration, and borane reduction of the carboxy groups preceding epoxide formation.[867] Further examples include Hofmann rearrangement of N^{α}-protected asparagines to provide β-amino-L-alanine (*alias* 2,3-diaminopropanoic acid);[868] the use of dimethyl (4S,5S)-1-benzyl-2-oxo-3-(9′-phenylfluoren-9′-yl)imidazolone-4,5-dicarboxylate derived from L-aspartic acid for chemoselective ester manipulation to give the γ-hydroxy-α,β-diaminoalkyl functionality required for a component of streptothricin antibiotics;[869] synthesis of (E)- and (Z)-2-amino-4-phenylbut-3-enoic acids (*alias* styrylglycines) from L- and D-aspartic acids respectively.[870]



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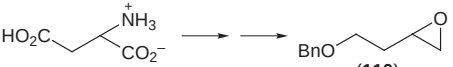
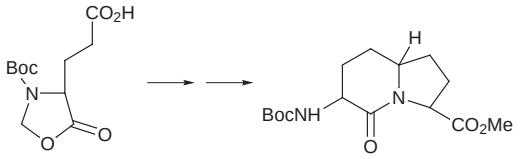


(**110**)

L-β-Aspartic semialdehyde has been used for access to 4-oxo-L-norvaline through homologation with diazomethane.[871] A synthesis of the hydrochloride of the α-semialdehyde has been reported, by ozonolysis of 3-amino-3-vinylpropanoic acid formed from 4-acetoxy-azetidin-2-one[872] and the L-glutamic acid-derived aldehyde, $Boc_2NCH(CO_2Me)CH_2CH_2CHO$, forms the basis for a synth-

esis of C-glycosidic amino acid through aldolization by a glycoside di-anion.[873] An analogous species, formed by ozonolysis of the Birch reduction product of L-phenylalanine, has been condensed with 3-amino-5-oxo-4-phenyl-2,5-dihydro-isoxazole to give β-(isoxazolo[2,3-a]pyrimidin-4-yl)-L-alanine.[874]

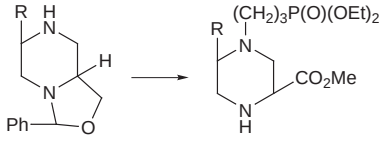
L-Glutamic acid protected at both NH_2 and α-carboxy groups through conversion into the oxazolidinone (111) is transformed into the azabicyclononane derivative (112) through Claisen condensation with the lithium enolate of Z-Glu(OBn)OMe and ensuing tandem cyclization.[875]



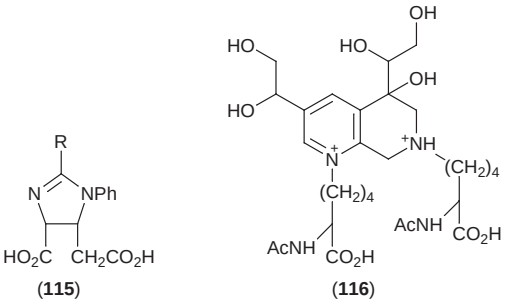
(**111**) (**112**)

Lysine and other α-(ω-aminoalkyl)-α-amino acids in various protected forms have proved to be effective starting materials in syntheses of unusual α-amino acids: L-homoglutamine by oxidation of N^{ε}-Z-L-lysine;[876] (2S,5S)-5-(fluoromethyl)ornithine from (2S,4S)-diaminoadipic acid;[877] N^{γ}-alkylation of the protected 2-(hydroxymethyl)piperazine (113) *en route* to piperazinic acid analogues (114);[878] preparation of homochiral dihydroimidazoles (115) from corresponding β-aminoglutamic acids;[879] and preparation from L-2,4-diaminobutanoic acid, of an amino acid with an adenine-carrying side-chain.[880] The novel N^{ω}-(monomethoxytrityl) group has been advocated for N-protection of an aminoalkyl side-chain, with the advantage of mild cleavage (dichloroacetic and chloroacetic acids) and easy work-up.[881]

A novel protein crosslinking site, threosidine (116), is generated through



(**113**) (**114**)



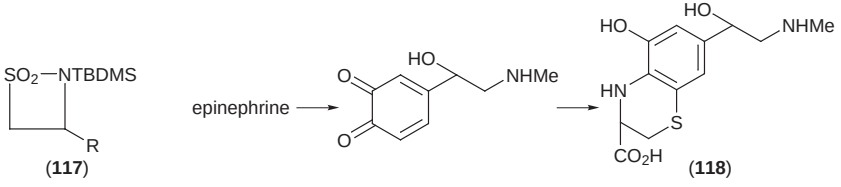
(**115**) (**116**)

condensation of N^{α}-acetyl-L-lysine with D-threose.[882] Condensation of lysine protected at α-NH_2 and carboxy groups, with malondialdehyde and 4-hydroxy-non-2-enal (formed *in vivo* through oxidation of polyunsaturated fatty acids) followed by $NaBH_4$ reduction, provides N^{ε}-propyl derivatives (*cf.* Vol. 29, pp. 81, 82).[883] 1-(N^{α}-Hippuryl-lysyl)-2-hydroxy-2-pentyl-3-(N^{α}-hippuryl-lysylimino)-1,2-dihydropyrrole is the fluorescent product formed from 4-hydroxynon-2-enal and N^{α}-hippuryl-lysine in phosphate buffer at neutral pH, thus identifying the site of attack of this lipid degradation product in proteins as a lysine residue.[884] In a related process, N^{α}-Boc-L-arginine reacts with methylglyoxal to form the novel fluorescent derivative, N^{δ}-(5-hydroxy-4,6-dimethylpyrimidin-2-yl)-L-ornithine, also formed by reactions of arginine derivatives with various sugars and with ascorbic acid, thus providing a clue to damage caused to proteins by this compound.[885] Bis(N-Boc)-protection of the arginine side-chain guanidine becomes more attractive as a result of the effectiveness of $SnCl_4$ as deprotecting reagent.[886]

A review has appeared of applications of L-cysteine as a D-amino acid synthon;[887] these applications are mostly obvious extensions of the well-known uses of L-serine in this respect. One of the many reactions in which L-cysteine behaves differently from serine is its ready formation of an L-thiazolidine-4-carboxylic acid with a carbonyl compound, now shown to be facilitated by microwave radiation.[888] Another growth area of research has developed around sulfur-containing amino acids through the need to identify likely *in vivo* associates for nitric oxide and other nitrogen oxides. Studies of cysteine in this context include determination of the kinetics of nitrosation of L-cysteine, and related reactions,[889] and properties of S-nitrosothiols (for a review see Ref. 890). For S-nitroso-N-acetylcysteine and its penicillamine analogue, the apparently greater thermal stability of the latter is ascribed to steric repression of disulfide formation and therefore, presumably, recapture of nitric oxide.[891] The reversibility of S-nitrosation has been demonstrated through reduction of copper(II) species by S-nitrosothiols.[892]

[^{35}S]-Labelled (S)-homocysteine and L-methionine have been prepared from O-acetyl-(S)-homoserine through (S)-homoserine sulfhydrylase-catalysed thiol exchange with $H_2{}^{35}S$.[893]

Studies of cysteine chemistry leading to sulfur heterocycles cover the formation of the α-sultam (**117**) and its potential in synthesis,[894] uses in trapping sensitive o-quinones (*e.g.* from epinephrine through mild oxidation → 118),[895] photocyclization of methyl N-phthaloylcysteinate to the benzazepin-1,5-dione and other species,[896] and oxidative consequences of attack by the superoxide radical on N-acetylcysteine.[897]



(**117**) (**118**)

Cysteine selenotrisulfide has been prepared through the reaction of L-cysteine with sodium selenite in acid solutions.[898] Cobalt-assisted cleavage of disulfide bonds, *e.g.* conversion of N-acetyl-L-cystine to S-alkyl-, -aryl- or -acyl-mercapturic acids, has been established using Zn together with a trace of $CoCl_2$, and an organic halide.[899] One-electron oxidation potentials of α-carbon-centred radicals of cysteine methyl ester, cystine, and related amino acids have been determined to throw light on their redox chemistry.[900] Spontaneous oxidation of methionine in samples prepared for analysis has not been sufficiently recognized as a source of underestimation of this amino acid; the effects on the generation of methionine sulfoxide, of co-solutes and other factors present in physiological samples have been intelligently established through an HPLC study of OPA-mercaptoethanol condensation products.[901] The L-methionine side-chain provides the aldehyde function of the D-Garner aldehyde in a synthesis from the N-Boc-amino acid, through oxidative elimination after reduction of the carboxy function and cyclization with $Me_2C(OMe)_2/BF_3$.[902]

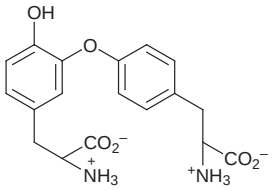
Phenylalanine side-chain modifications (see also Section 4.10) originating in the electrophilic substitution chemistry of benzene are illustrated in preparations of p-(N-thioaroylamino)-L-phenylalanines,[903] of o- and p-phosphinophenyl derivatives of glycine and alanine through nucleophilic phosphination of 2- and 4-fluorophenylglycine and -alanine with PhP(R)K (R = Me, Ph).[904] p-(Chlorosulfonyl)ation is described in Ref. 352. L-4-(Phosphonofluoromethyl)phenylalanine has been obtained similarly, through substitution of L-p-iodophenylalanine.[905] Pd-Catalysed Stille cross-coupling of methyl N-Boc-4-(trimethylstannyl)-L-phenylalaninate with aryl and vinyl iodides and triflates gives 4-aryl and 4-vinyl homologues.[906]

Tyrosine side-chain protection through (2-adamantyloxycarbonyl)ation of its copper complex has been advocated.[907] (2,4-Dimethylpent-3-yloxycarbonyl)ation of Boc-L-tyrosine gives a suitably protected intermediate for peptide synthesis since the group is stable towards piperidine but completely cleaved by HF,[908] and O-[bis(2-cyanoethyl)thiophosphonyl]ation has been effected using a phosphoramidite, catalysed by 1H-tetrazole.[909]

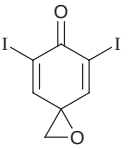
Tyrosine is the source of 4-iodo-L-phenylalanine, through reaction with NaI and Chloramine-T in aqueous media at pH 7, a procedure applied to give the [^{131}I] and [^{123}I]isotopomers,[910] and the iodo-compound lies along a pathway to 4-ethynyl-L-phenylalanine through application of the Heck reaction.[911] De-amination and de-iodination accompanying the formation of various radicals is the fate of this amino acid through irradiation in aqueous media.[912] [^{18}F]Acetyl hypofluorite is the key reactant in routes to 6-[^{18}F]fluoro-L-m-tyrosine and its 2-[^{18}F]-isomer, also the 6-[^{18}F]fluoro-β-fluoromethylene homologue.[913] Reimer-Tiemann formylation of N-Boc-L-tyrosine and application of the Dakin reaction leads to m-hydroxy-O-benzyl-L-tyrosine.[914] 3-Nitrotyrosine formation from the amino acid, together with nitrous and nitric acids, with peroxonitrous acid has been shown through ^{15}N-CIDNP NMR to involve radical intermediates.[915]

(S,S)-Isodityrosine (119) has been prepared from methyl Boc-L-tyrosinate, after conversion into the m-iodo analogue followed by Pd-catalysed coupling with N-Boc-β-iodo-L-alanine.[916] Horseradish peroxidase mediates the C-O-

coupling of dibromo- and dichloro-tyrosines to give substituted isodityrosines.[917] Biomimetic oxidative coupling of ethyl N-acetyl-3,5-di-iodo-L-tyrosinate *via* the usual aryloxydienone intermediate gives N-acetyl-L-thyroxine,[918] also obtained from the unprotected di-iodotyrosine through reaction with epoxide (120) formed from the corresponding benzyl alcohol by sodium bismuthate oxidation.[919]

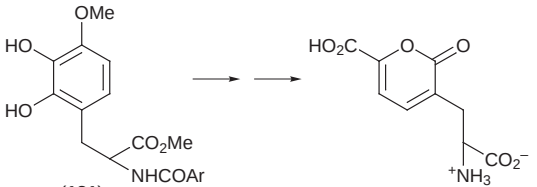


(**119**)



(**120**)

Oxidative cleavage of the catechol moiety of the DOPA analogue (121) prepared by Erlenmeyer and Schollkopf syntheses gave the muconate, followed by recyclization to give stizolobinic acid in a biomimetic route.[920]



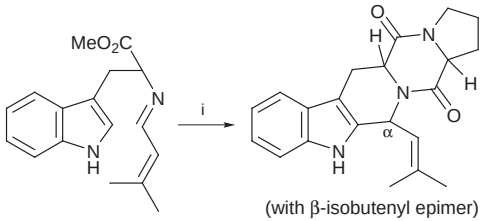
(**121**)

N-Acyl-α-arylglycines owe their presence in diagnostic kits to their role as chemiluminescence substrates, peroxidase-catalysed oxidation by hydrogen peroxide leading to 3-acylaminobenzo[b,d]furan-2(3H)-ones.[921]

Histidine side-chain protection that restrains interference by the imidazole moiety in the various operations involved in peptide synthesis is accomplished by N^{π}-(2-adamantyloxymethyl)ation[922] or N^{π}- and N^{τ}-allylation,[923] these groups being increasingly used in other contexts, and in the latter case offering the convenience of Pd-mediated cleavage. Full protection of L-histidine is mandatory for the introduction of an α-alkyl group using species generated by silver(I)-catalysed radical decarboxylative oxidation of an alkanoic acid in the presence of ammonium persulfate in 10% sulfuric acid.[924] During esterification of N^{α}-acetyl-L-histidine with diazomethane, some 1′-methylation is observed,[925] but a careful study of N^{τ}-(4-nitrophenyl)ation and H_2/Pd-C cleavage does not substantiate the claim that N^{τ}-N^{π}-migration occurs.[926] N^{α}-Acetylhistidine undergoes nucleophilic addition to catecholamines through its side-chain nitrogen atoms, to give C-2 and C-6 adducts,[927] an observation that will have significance in *in vivo* processes.

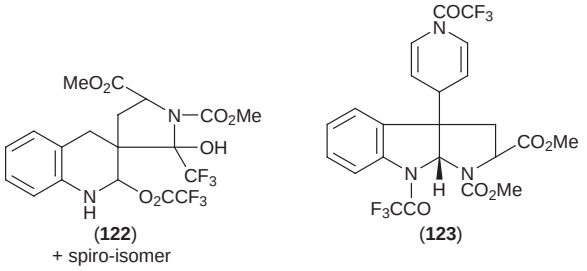
Tryptophan side-chain chemistry provides a rich mixture of indole substitution processes, including intramolecular cyclization to the aliphatic moiety. Photochemical versions of these processes are covered in the next Section, while Pictet-

Spengler condensations continue to be predominant in the solution chemistry of tryptophan, leading to indole alkaloids[928] and oxindole alkaloids[929] (proceeding *via* different tetracyclic ketone intermediates), and leading to the pentacyclic fumitremorgin cell cycle inhibitors through a different pathway (Scheme 38).[930] Adding pyridine to the previously-studied N-methoxycarbonyl-L-tryptophanate – trifluoroacetic anhydride reaction mixture leads to further new products (122 and its stereoisomer) and (123) as well as a trifluoroacetylated indole derivative.[931] TFA Dimerization of tryptophan derivatives generates δ_1, δ_1'-trans-indolines.[932] Oxidation of tryptophan by H_2O_2 gives the known products oxindolylalanine > 3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carboxylic acid, > N-formylkynurenine > dioxindolylalanine > kynurenine > 5-hydroxytryptophan (there are some surprises in the order of yields of reaction products).[933] γ-Irradiation gives the same major product, with N-formylkynurenine and 4-, 5-, 6-, and 7-hydroxylated tryptophans.[934] Oxidation by the dibromine radical anion or by peroxidase-catalysed processes has been studied with particular reference to the involvement of oxygen and superoxide.[935]



Reagent: i, Fmoc-L-Pro-Cl/py, then piperidine–CH_2Cl_2

Scheme 38



6.4 Effects of Electromagnetic Radiation on Amino Acids – Much of the current chemistry of tyrosine and tryptophan falls into this category, but photochemical and related consequences for an increasing range of common amino acids are being studied.

Photodecomposition of aliphatic amino acids in water at 248 nm[936] and 266 nm[937] is a result of two-photon excitation of water so as to generate radicals that are the effective reagents in the process. Four dipeptides formed from valine and

methionine undergo efficient peptide bond cleavage under irradiation at 193 nm.[938]

Radicals are formed by irradiation of L-alanine with a 3.4 MeV/amu ^{59}Co ion beam,[939] and the radical anion $MeCH{\cdot}CO_2^-$ formed in this way can be detected by its extraordinary UV absorption characteristics (λ_{max} 350 nm, (1100 $M^{-1}cm^{-1}$).[940] UV-Irradiated aqueous amino acids in contact with TiO_2 fragment into ammonia, nitrates, and CO_2, somewhat whimsically called photocatalysed mineralization,[941] while photolysis of [C_{60}]fullerenes with ethyl L-alaninate and acetaldehyde or iminodiacetic esters gives fulleropyrrolidines (*cf.* Vol. 29, p.73);[942] methyl esters of morpholino- and piperidino-acetic acids lead to analogous products while the free acids undergo decarboxylation to give dialkylaminomethylfullerenes.[943] Selective destruction of L-alanine and L-aspartic acid by ionizing radiation, reported previously, is unexpectedly prevented by glycine, thought to be due to the reaction of the glycine α-radical with alanine to regenerate the more stable alanine α-radical.[944]

A review of the extensive use of 5-aminolaevulinic acid in photodynamic therapy of cancer has been published,[945] focussing on underlying mechanisms (the wider literature from the medical perspective cannot be accommodated here).

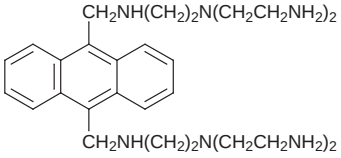
Aromatic and heteroaromatic acids provide substrates for all the various branches of photochemistry, represented by pyridinoline and deoxypyridinoline (effects of UV, visible, X-ray and γ-irradiation),[946] β-homotyrosine (fluorescence characteristics),[947] histidine [fluorescence quenching through binding to the dizinc(II) complex of the fluorophore (124)],[948] and phosphorescence of tryptophan, tyrosine, phenylalanine, proline, and histidine (the latter three amino acids generate only about one-hundredth of the emission of tryptophan).[949]

Tryptophan studies cover generation of its neutral radical through 355 nm laser flash photolysis in the presence of N-hydroxypyridin-2-thione;[950] photosensitized oxidation in Triton X-100 micelles[951] and by singlet oxygen or electron transfer pathways;[952] fluorescence characteristics of L-tryptophan and N^{α}-acetyl-L-tryptophanamide in micelles;[953] and photoexcited triplet states studied by spin-lattice relaxation with respect to the influence of solvent and salts.[954] N-Nitroso-L-tryptophan, S-nitrosothiols, and other nitric oxide derivatives can be analysed by chemiluminescence spectroscopy after photolysis.[955]

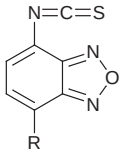
7 Analytical Methods

7.1 Introduction – General reviews cover amino acid analysis[956] and peptide and protein hydrolysis.[957] Occasionally, there are papers that cover all standard analytical methods from the point of view of one particular amino acid, and that has arisen in the current literature for homocysteine[958,959] and tryptophan.[960] In one of these broad studies,[956] data on the variation in performance of different laboratories on homocysteine analysis have been considered.

7.2 Gas-liquid Chromatography – Results have appeared from conventional protocols followed for mixtures: of N(O,S)-isobutyloxycarbonyl derivatives of



(**124**)



(**125**)

amino acid methyl esters[961] (including the use of N- and P-selective detectors[962]), of N(O) pentafluorobenzil derivatives of amino acid pentafluorobenzyl esters,[963] and of N-ethoxycarbonylamino acid ethyl esters from hydrolysates of samples of oil paintings.[964]

The mass spectrometer as detector is an essential feature of analyses of ^{13}C-enriched leucine, isoleucine, and valine mixtures, rendered suitable for GLC through derivatization with o-phenylenediamine after conversion into α-keto-acids using L-leucine dehydrogenase.[965] The GC-MS combination has also been applied to estimating the [^{15}N]amino acid content of samples derivatized as their N-trifluoroacetyl isopropyl esters,[966] for N-heptafluorobutyroyl pentafluoro-benzyl esters of N(O)-TMS-amino acids using [^{13}C],[^{2}H]-labelled amino acid standards,[967] for dansyl- and Z-[^{13}C, ^{2}H]-labelled amino acids,[221] for N-perfluoro-acyl alkyl esters using CH_4-CIMS, shown to generate [M+H], [M+C_2H_5], and [M+H−C_3H_6] ions,[968] and for amino acid mixtures in the form of their N-ethoxycarbonyl trifluoroethyl esters.[969] Considerable care has been taken to develop a one-step derivatization protocol [N(O,S)]-ethoxycarbonylation and ethyl esterification using ethyl chloroformate, applied for stable isotope analysis of amino acids.[970]

Particular amino acids targeted in GC-MS analysis include S-carboxymethyl-L-cysteine,[971] 3-chlorotyrosine aiming at attomole sensitivity for human tissue samples,[972] eight known pipecolic acids in plants,[973] and non-protein amino acids in cycad seeds (including the previously-reported species-specific cycasindene, see also Ref. 35).[974]

Estimation of D:L-ratios for amino acids has been achieved through GLC over a CSP similar to Chirasil-Val, though more sensitive, of samples derivatized as N-pivaloyl methyl esters,[975] and for trifluoroethyl esters of N- and N,O-isobutoxy-carbonyl derivatives for separations over Chirasil-D-Val for serine and threonine have been reported.[976]

7.3 Ion-exchange Chromatography – Most amino acids analysers are now based on HPLC instrumentation (Section 7.5), and the ninhydrin reagent protocol is still favoured, *e.g.* for homocysteine;[977] a new ion-exchange analyzer based on traditional lines has been described.[978] Exploration of continuous rotating annular chromatography technique for cation-exchange separation of amino acid mixtures has been reported;[979] another novel technique for contin-uous monitoring of eluate containing underivatized amino acids is based on evaporative light-scattering.[980]

7.4 Thin-layer Chromatography – A review[981] covers estimation of D:L-ratios for derivatized amino acids using TLC plates impregnated, for example,[982] with (1R,3R,5R)-2-azabicyclo[3.3.0]octane-3-carboxylic acid.

7.5 High-performance Liquid Chromatography – Studies under this heading, dealing with mixtures of amino acids, are grouped together, first in the category of conventional approaches to the analysis of free amino acids and derivatized samples, then into various categories of modified stationary phases.

Amino acids such as iodo- and di-iodotyrosine, tri-iodothyronine, and thyroxine[983] are well-suited to an HPLC analysis protocol that employs a UV or fluorescence detector, and so are histidine and its 1- and 3-methyl derivatives in muscle (analysed after OPA derivatization),[984] dityrosine in spinal fluid (λ_{exc} 285 nm, λ_{em} 410 nm)[985] and in plasma proteins and haemoglobin,[986] pyridinoline and its deoxy-homologue,[987] and hydroxylysylpyridinoline and its lysyl analogue.[988] The Beckman Cross-Links kit for HPLC analyis of pyridinoline and its deoxy-homologue is superior to others and HPLC is an attractive alternative to immunoassays.[989] The preceding group of analyses of protein crosslinking amino acids is joined by desmosine (from elastin),[990] and histidinohydroxylysinonorleucine (after Fmoc derivatization), a more recently discovered trifunctional collagen crosslink.[991] These are markers for osteoporosis and other afflictions, and another amino acid of this type is N-phenylpropionylglycine, whose presence in urine indicates the level of medium-chain acyl coenzyme A dehydrogenase deficiency.[992] Quantitation at 412 nm has been achieved for cysteine and N-acetylcysteine after post-column reaction with 5,5α-dithiobis(2-nitrobenzoic acid), a classical derivatization reagent for thiols.[993]

A lengthy procedure for hydroxyproline estimation employs nitrous acid deamination of primary amino acids, extraction of N-nitrosoimino acids into ethyl ethanoate, de-nitrosation with HBr, then derivatization with dabsyl chloride and HPLC analysis.[994] A sensitive procedure for the HPLC analysis of L-DOPA and its 3-O-methyl derivative in blood platelets employs electrochemical detection.[995]

Unusual analytes or techniques are illustrated in estimation of S-adenosyl-L-methionine in blood,[996] microwave-induced plasma MS as detector system for HPLC of S-(2-aminoethyl)-cysteine, cystathionine, and lanthionine,[997] and seleno-amino acids[998] (including ICP-MS for analysis of selenocysteine, selenomethionine, and methylselenocysteine in yeast[999]) and N^G-dimethyl-L-arginine in blood plasma.[1000]

Derivatization intended to raise the sensitivity of amino acid analysis, at the same time calling for simplified HPLC instrumentation, remains the standard approach. N-Fmoc amino acids[1001] (see also Ref. 988) are well established fluorescent derivatives, whose use has been extended to diastereoisomer formation through treatment of enantiomer mixtures of amino acids with N-Fmoc-L-amino acid N-carboxyanhydrides prior to HPLC analysis for determination of their D:L-ratios.[1002] Fluoresceamine derivatives[1003] are not completely out of fashion, and benzoxadiazolyl derivatives are definitely in favour (derivatization of homocysteine using 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate; other exam-

ples are scattered throughout Section 7).[1004] PTC-Amino acids give reliable results (Ref. 11).

New proposals for sensitive fluorescent derivatives include N-(3-indolylacetyl)-amino acids,[1005] N-(acridin-9-yl)amino acids,[1006] 2-(4-hydrazinocarbonylphenyl)-4,5-diphenylimidazole-derivatized carnitines,[1007] and Schiff bases formed with 3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde.[1008]

OPA Derivatives formed between amino acids and an o-phthaldialdehyde – thiol reagent cocktail (3-mercaptopropionic acid has been advocated[1009]) have been chosen for analyses of glutamine,[1010] the NO synthase-related basic amino acids (N^{ω}-hydroxy-L-arginine, L-arginine, and its mono- and di-methyl derivatives),[1011] N-methylated lysines,[1012] argininosuccinic acid,[1013] neurotransmitter amino acids,[1014] and amino acid mixtures in physiological samples.[1015] The 5-aminolaevulinic acid – OPA derivative has been determined at trace levels through electrochemical detection.[1016] A noticeable increase in interest in 6-aminoquinolylamino acids (AQC-amino acids formed by the AccQ-Tag procedure[1017]) follows from their high sensitivity (from 12 fmol for threonine to 1.8 pmol for tryptophan[1018]) based on measurements at the fluorescence maximum 395 nm (λ_{exc} 250 nm) for derivatives separated over C_{18} silica using the ion-pair elution technique. The derivatization reagent, 6-aminoquinolyl N-hydroxysuccinimidylcarbamate, causes no complications in the interpretation of a chromatogram, when a quaternary eluent system is used,[1019] and a measure of its efficiency is shown in improved separation of 24 derivatized amino acids in 45 minutes.[1020]

An amino acid is released at each cycle of an Edman sequence determination of a polypeptide as a 2-anilinothiazol-5(4H)-one, and instead of conversion into a phenylthiohydantoin (PTH), its reaction with 4-aminofluorescein at one cycle, and with aminotetramethylrhodamine at the next, generates considerably simplified interpretations of HPLC traces.[1021] Non-fluorescent PTHs and dansylamino acids have been detected by indirect time-resolved fluorescence generated by the inclusion of europium(III) chelates to the eluent.[1022] Determination of D:L-ratios for PTHs has been accomplished through HPLC using mobile phases containing various chiral selectors,[1023] while the normal PTH analysis protocol in which no discrimination of enantiomers is intended, is employed in an improved system,[1024] and in a sensitive estimation of 1-aminocyclopropanecarboxylic acid in plant tissues.[1025] This employs MS detection, as does an even more sensitive protocol based on 4-(3-pyridinylmethylaminocarboxypropyl)PTHs.[1026] Diastereoisomer-forming derivatization has been explored using a series of p-substituted heteroaryl isothiocyanates (125),[1027] and using 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (the advanced Marfey reagent)[1028] and HPLC separation. In the former case, the purpose was to find a derivatizing isothiocyanate that did not introduce racemization, and an electron-donating p-substituent was found to be effective. The Marfey reagent study showed that the LL-diastereoisomer is not always eluted ahead of the LD-isomer. A chiral isothiocyanate is more effective for the estimation of D:L-ratios and the fluorescent reagent (-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2-oxabenzo-1,3-diazole has been advocated.[1029]

Commercial chiral stationary phases (CSPs) of the Pirkle type have been used

to estimate D-amino acid trace contaminants in L-amino acid samples derivatized by 4-fluoro-7-nitro-2,1,3-benzoxadiazole.[1030] Experimental CSPs include porous graphite coated with an N-substituted L-phenylalanine,[1031] and human serum albumin for HPLC of dansylamino acids.[1032] C_{18}-Silica coated with N,S-dioctyl-D-penicillamine as a chiral ligand-exchange phase for complexing with copper(II) ions and providing excellent resolution of amino acid enantiomers,[1033] and graphite coated with an N-substituted L-proline as chiral selector has been compared with other CSPs for ligand-exchange separation of enantiomers of amino acids.[1034] A quinine carbamate-based chiral anion exchanger has been explored for the resolution of DNP-amino acids.[1035] Mercaptopropylsilica gel derivatized with a benzamide, $-CH_2CH_2O\text{-}p\text{-}C_6H_4\text{-}CONR^1R^2$, in which NR^1R^2 is a homochiral 3,4-diaminopyrrolidinamide,[1036] has been used as a novel CSP for the HPLC resolution of DL-β-amino acid esters.[1037] Imprinted polymers for HPLC or CZE resolution of aromatic DL-amino acids have been reviewed,[1038] and the broader field of enantiomer HPLC separation over homochiral polymers has been surveyed.[1039]

Dansylamino acids can be resolved using hydroxypropyl-β-cyclodextrin as chiral selector.[1040] Systems based on cyclodextrins, for the estimation of enantiomer ratios using HPLC with electrochemical detection have been reviewed,[1041] and a brief but broader review[1042] has also appeared.

7.6 Capillary Zone Electrophoresis (CZE) and Related Analytical Methods – Textbook support for CZE and related techniques reflects their value in separation of mixtures for analysis.[1043] The subject has been reviewed.[1044]

Dramatic illustrations continue to appear: the separation and identification of a mixture of 12 amino acids and 9 carbohydrates, employing amperometric detection after separation,[1045] and estimation of the glutamic acid content of a single cell by quantifying the laser-induced fluorescence of NADH generated by passage, after CZE separation, through a column carrying glutamate dehydrogenase and glutamate-pyruvate transaminase.[1046]

In appropriate cases, UV quantitation is used for CZE of underivatized amino acids: oxidation products of tyrosine and DOPA,[1047] S-adenosyl-L-homocysteine,[1048] and indirect means in which a salicylate or benzoate is used as a UV-absorbing buffer additive.[1049] But the usual approach is the same as for HPLC analysis, *i.e.* derivatization prior to separation, and the same general emphasis on a few derivatization protocols applies to both techniques. Thus, recent studies have involved 6-aminoquinolinylamino acids,[1050] PTHs (CZE),[1051] PTHs of 3- and 4-hydroxyproline after clearing primary amines using OPA (MEKC),[1052] fluorescein TH of glutamic acid,[1053] OPA-chiral thiol derivatives (MEKC),[1054] naphthalene-2,3-dicarbaldehyde derivatives of aspartic and glutamic acids,[1055] 1-methoxycarbonylindolizine-3,5-dicarbaldehyde derivatized amino acids,[1056] 4-aminosulfonyl-7-fluoro-2,1,3-benzoxadiazole as derivatization reagent for homocysteine and other thiols (worse results were obtained using ammonium 4-aminosulfonyl-7-fluoro-2,1,3-benzoxadiazole-4-sulfonate),[1057] and DNP derivatives (magnesium, cadmium and zinc salt additives offer better separations).[1058] The inclusion of a cyclodextrin in the buffer enhances the fluorescence of NDA-

derivatized glutamic and aspartic acids and therefore increases the sensitivity of CZE analysis of these amino acids.[1059]

Fluorescein THs have been detected at 10 zmol levels (*i.e.* 10^{-20} mol) using CZE.[1060]

The resolution of DL-amino acids on the analytical scale by CZE techniques has been reviewed.[1061] Chiral stationary phases are applicable to CZE and the related MEKC and MECC techniques, with AQC-DL-amino acids,[1062] dansyl-DL-amino acids,[1063] and 7-nitro-2-oxabenzo-1,3-diazolyl-DL-amino acids,[1064] the last-mentioned being resolved using heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin in the buffer. Chiral separations (MEKC) employing hydroxypropyl-β-cyclodextrins as buffer additives have been reviewed.[1065] The alternative derivatization approach, generation of diastereoisomers, has been illustrated with the use of the OPA – chiral thiol reagent system for aspartic and glutamic acids (tetra-O-acetyl-1-thio-β-D-glucopyranose was the best co-reagent).[1066] Creation of molecule-imprinted polymers for CZE resolution of enantiomers has been explored, using L-phenylalanine anilide as print molecule, and methacrylic acid and 2-vinylpyridine as monomers.[1067]

7.7 Assays for Specific Amino Acids – Colorimetric procedures based on established principles allow estimation of hydroxyproline at trace levels,[1068] and the use of spectrophotometric enzymic assays is also standard practice, *e.g.* for GABA in plant samples[1069] and glutamic acid in tissue through deamination using glutamic dehydrogenase, followed by derivatization by formazan formation.[1070] An unusual approach, using TLC with a stationary phase carrying *Agrobacterium tumefaciens* harbouring lac2 fused to a gene that is regulated by autoinduction, has been applied to the characterization of N-acyl-L-homoserine lactones in biological samples.[1071]

The trend shown in the literature, on which this Section is based, continues towards the development of sensors designed for the quantification of individual amino acids in physiological samples. A chemiluminescence-generating system centred on an immobilized D-amino acid oxidase has been described for estimating the D-enantiomer content of samples of coded amino acids.[1072] New technology for amino acid oxidase electrodes based on iridium-dispersed carbon paste media allows estimations of amino acids in solutions down to 10^{-5} M,[1073] and immobilization on an oxygen electrode of the L-amino acid oxidase present in *Vipera ammodyter* venom extracts provides a novel amperometric sensor for L-amino acids.[1074] Immobilized glutamate oxidase using a hydrogen peroxide electrode permits quantitation of L-glutamic acid, L-glutamine, and GABA,[1075] and more sophistication is introduced into this system when a calixarene ammonium ionophore is incorporated.[1076] Analogous estimation of L-glutamine requires a mixed L-glutamate oxidase – glutaminase electrode.[1077]

L-Phenylalanine generates a response from an immobilized L-phenylalanine dehydrogenase sensor in a flow injection system,[1078] also the basis of corresponding macroelectrodes for L-alanine, L-serine, L-aspartic acid, and L-arginine[1079] and miniaturized versions of these.[1080] A carbon paste electrode carrying tyrosinase, salicylate hydroxylase, and L-phenylalanine dehydrogenase serves for

the assay of L-phenylalanine based on the quantitation of NADH produced.[1081] An amperometric assay for L-glutamic acid is based on use of an immobilized thermophilic L-glutamate dehydrogenase electrode.[1082]

The benefits of flow-injection amperometry have been exploited in a biosensor carrying tryptophan 2-mono-oxygenase for analysis of L-tryptophan or L-phenylalanine.[1083] The first on-line sensor for GABA employs glutamate oxidase and catalase immobilized on a glassy carbon electrode together with bovine serum albumin and horseradish peroxidase.[1084]

8 References

1. Enantioselective Synthesis of β-Amino acids, ed. E. Juaristi, Wiley-VCH, New York, 1997.
2. Protein Structure Analysis: Preparation, Characterization and Microsequencing, eds. R.M. Kamp, T. Choli-Papadopoulou, and B. Wittmann-Liebold, Springer Verlag, Berlin, 1997.
3. Ref. 2; *e.g.* Rapid HPLC, F. Godt and R.M. Kamp, p. 49; HPLC analysis of D- and L-amino acids, D.Vollenbroich and K.Krause, p. 249; HPLC of PITC and DABITC derivatives, T. Choli-Papadopoulou, Y. Skendros, and K. Katsani, p.137.
4. Amino Acids and Peptides, by G.C.Barrett and D.T.Elmore, Cambridge University Press, Cambridge, 1998.
5. S.D.Heys, J.Broom, and O.Eremin, in L-Arginine, Biological Aspects and Clinical Applications, ed. O.Eremin, Landes, Austin, Texas, 1997, p.1.
6. E.Juaristi, *An.Quim.Int.Ed.*, 1997, **93**, 135.
7. K.Imai, M.Kato, Y.Huang, H.Ichihara, T.Fukushima, T.Santa, and H.Homma, *Yakugaku Zaishi*, 1997, **117**, 637 (*Chem.Abs.*, 1998, **128**, 44963).
8. G.Kreil, *Ann.Rev.Biochem.*, 1997, **66**, 337.
9. M.H.Park, Y.B.Lee, and Y.A. Joe, *Biol.Signals*, 1997, **6**, 115 (*Chem.Abs.*, 1998, **127**, 216447).
10. R.A.Azevedo, P.Arruda, W.L.Turner, and P.J.Lea, *Phytochemistry*, 1997, **46**, 395.
11. T.Okayasu, M.Ikeda, K.Akimoto, and K.Sorimachi, *Amino Acids*, 1997, **13**, 379.
12. S.W.Taylor and J.H.Waite, in Prolyl Hydroxylase, Protein Disulfide Isomerase, and Other Structurally-Related Proteins, ed. N.A.Guzman, Dekker, New York, 1998, p.97.
13. S.Kim, B.M.Merrill, R.Rajpurohit, A.Kumar, K.L.Stone, V.V.Papov, J.M.Schneiders, W.Szer, S.H.Wilson, W.K.Paik, and K.R.Williams, *Biochemistry*, 1997, **36**, 5185.
14. M.H.Baslow, *J.Neurochem.*, 1997, **68**, 1335.
15. M.H.Baslow and S.Yamada, *Exp.Eye Res.*, 1997, **64**, 283.
16. H.T.Alborn, T.C.J.Turlings, T.H.Jones, G.Stenhagen, J.H.Loughrin, and J.H.Tomlinson, *Science*, 1997, **276**, 945.
17. Y.Guo, M.Gavagnin, E.Mollo, G.Cimino, N.A.Hamdy, I.Fakhr, and M.Pansini, *Nat.Prod.Lett.*, 1997, **9**, 281.
18. M.V.D'Auria, C.Giannini, L.Minale, A.Zampella, C.Debitus, and M.Frostin, *J.Nat.Prod.*, 1997, **60**, 814.
19. N.N.Gessler, L.I.Kharchenko, T.E.Parlovskaya, and V.Y.Bykhovskii, *Prikl.Biokhim.Mikrobiol.*, 1996, **32**, 666.
20. D.Xi, Y.-G.Peng, and J.S.Ramsdell, *Nat.Toxins*, 1997, **5**, 74.

21. I.Apostal, J.Levine, J.Lippincott, J.Leach, E.Hess, C.B.Glascock, M.J.Weickert, and R.Blackmore, *J.Biol.Chem.*, 1997, **272**, 28980.
22. F.Morishita, Y.Nakanishi, S.Kaku, Y.Furukawa, S.Ohta, T.Hirata, M.Ohtani, Y.Fujisawa, Y.Muneoka, and O.Matsushima, *Biochem.Biophys.Res.Commun.*, 1997, **240**, 354.
23. J.R.Cronin and S.Pizzarello, *Science*, 1997, **275**, 951.
24. J.L.Bada, *Science*, 1997, **275**, 942.
25. M.H.Engel and S.A.Macko, *Proc.SPIE – Int.Soc.Opt.Eng.*,Vol. 3111 (Instruments, Methods, and Missions for the Investigation of Extraterrestrial Microorganisms), Ed. R.B.Hoover, SPIE, Bellingham, Washington, 1997, p. 82 (*Chem.Abs.*, 1998, **127**, 216734).
26. S.Yabushita and A.Allen, *Astron.Geophys.*, 1997, **38**, 15.
27. R.Sakai, H.Kamiya, M.Murata, and K.Shimamoto, *J.Am.Chem.Soc.*, 1997, **119**, 4112.
28. J.J.Wu Won, B.E.Chalker, and J.A.Rideout, *Tetrahedron Lett.*, 1997, **38**, 2525.
29. T.T.Teai, P.Raharivelomanana, J.-P.Bianchini, R.Faure, P.M.V.Martin, and A.Cambon, *Tetrahedron Lett.*, 1997, **38**, 5799.
30. D.Karentz, W.C.Dunlap, and I.Bosch, *Mar.Biol.*, 1997, **129**, 343.
31. P.S.Paremeswaran, S.Y.Kamat, and V.R.Hegde, *J.Nat.Prod.*, 1997, **60**, 802.
32. I.-J.Ryoo, K.-S.Song, J.-P.Kim, W.-G.Kim, H.Koshino, and I.-D.Yoo, *J.Antibiot.*, 1997, **50**, 256.
33. K.Shin-ya, J.-S.Kim, K.Furihata, Y.Hayakawa, and H.Seto, *Tetrahedron Lett.*, 1997, **38**, 7079.
34. H.R.N.Marona, G.G.Ortega, E.P.Schenkel, and J.Huet, *Acta Farm.Bonaerense*, 1996, **15**, 159 (*Chem.Abs.*, 1998, **127**, 106612).
35. M.Pan, T.J.Mabry, J.M.Beale, and B.M.Mamiya, *Phytochemistry*, 1997, **45**, 517.
36. Y.Aoyagi, S.Takasaki, S.Fujihala, A.Kasuga, and R.Sugaharai, *Phytochemistry*, 1997, **46**, 1095.
37. K.Pari, P.J.Rao, C.Derakumar, and J.N.Rastogi, *J.Nat.Prod.*, 1998, **61**, 102.
38. E.J.Dumdei, J.S.Simpson, M.J.Garson, K.A.Byriel, and C.H.L.Kennard, *Aust.J.-Chem.*, 1997, **50**, 139.
39. X.Fu, L.-M.Zeng, J.-Y.Su, and M.Pais, *J. Nat.Prod.*., 1997, **60**, 695.
40. K.Kanoh, S.Kohno, T.Asari, T.Harada, J.Katada, M.Muramatsu, H.Kawashima, H.Sekiya, and I.Uno, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 2847.
41. A.J.L.Cooper, *Chemtracts*, 1997, **10**, 440.
42. T.Sano and K.Kaya, *Phytochemistry*, 1997, **44**, 1503.
43. U.Papke, E.M.Gross, and W. Francke, *Tetrahedron Lett.*, 1997, **38**, 379.
44. N.M.Kelly, A.Sutherland, and C.L.Willis, *Nat.Prod.Rep.*, 1997, **14**, 205.
45. K.Jahnisch, *Liebigs Ann./Recl.*, 1997, 757.
46. P.Trapencieris, I.Kalvins, L.Kaulina, and V.Kauss, *Org.Process Res.Dev.*, 1997, **1**, 259.
47. I.Koshishii, H.Takayama, N.Aimi, K.Yamaguchi, H.Toyoda, and T.Imanari, *Chem.Pharm.Bull.*, 1997, **45**, 344.
48. E.Aller, R.T.Buck, N.J.Drysdale, L.Ferris, D.Haigh, C.J.Moody, N.D.Pearson, and J.B.Sanghera, *J.Chem.Soc., Perkin Trans.I*, 1996, 2879.
49. D.Albanese, F.Corcella, D.Landini, A.Maia, and M.Penso, *J.Chem.Soc., Perkin Trans.I*, 1997, 247.
50. C.Agami, F.Couty, L.Hamon, and O.Venier, *J.Org.Chem.*, 1997, **62**, 2106.
51. J.C.Shattuck and J.Meinwald, *Tetrahedron Lett.*, 1997, **38**, 8461.
52. D.Ma and Z.Ma, *Tetrahedron Lett.*, 1997, **38**, 7599.

53. J.Cacciola, R.S.Alexander, J.M.Fevig, and P.F.W.Stouten, *Tetrahedron Lett*., 1997, **38**, 5741.
54. M.E.Jung and Y.H.Jung, *Synlett*, 1995 (Special Issue), 563.
55. H.Kotsuki, T.Ohishi, and T.Araki, *Tetrahedron Lett*., 1997, **38**, 2129.
56. P.Bravo, G.Cavicchio, M.Crucianelli, A.Poggiali, and M.Zanda, *Tetrahedron: Asymmetry*, 1997, **8**, 2811.
57. M.A.Loreto, P.A.Tardella, L.Tedeschi, and T.Tofani, *Tetrahedron Lett*., 1997, **38**, 5717.
58. M.S.Gulzar, M.Akhtar, and D.Gani, *J.Chem.Soc., Perkin Trans.I*, 1997, 649.
59. D.A.Evans and S.G.Nelson, *J.Am.Chem.Soc*., 1997, **119**, 6452.
60. C.Greck and J.P.Genet, *Synlett*., 1997, 741.
61. J.F.Bower, R.Jumnah, A.C.Williams, and J.M.J.Williams, *J.Chem.Soc., Perkin Trans.I*, 1997, 1411.
62. A.S.Demir, *Pure Appl.Chem*., 1997, **69**, 105.
63. J.A.Marco, M.Carda, J.Murga, F.Gonzalez, and E.Falomir, *Tetrahedron Lett*., 1997, **38**, 1841.
64. C.J.Moody, A.P.Lightfoot, and P.T.Gallagher, *Synlett*., 1997, 659.
65. V.A.Soloshonok and V.P.Kukhar, *Tetrahedron*, 1997, **53**, 8307.
66. O.Tamura, N.Mita, N.Kusaka, H.Suzuki, and M.Sakamoto, *Tetrahedron Lett*., 1997, **38**, 429.
67. M.G.Le Pironnec, J.L.Guinamant, A.Robert, and M.Baudy-Floc'h, *Synthesis*, 1997, 229.
68. C.P.Decicco and P.Grover, *Synlett*., 1997, 529.
69. N.Zheng, J.D.Armstrong, J.C.McWilliams, and R.P.Volante, *Tetrahedron Lett*., 1997, **38**, 2817.
70. Q.Dai and R.Chen, *Org.Prep.Proced.Int*., 1997, **29**, 580.
71. Q.Dai and R.Chen, *Synth.Commun*., 1997, **27**, 17.
72. Q.Dai and R.Chen, *Synth.Commun*., 1997, **27**, 3341.
73. Q.Ding and R.Chen, *Synth.Commun*., 1997, **27**, 1653.
74. T.Ibuka, N.Mimura, H.Ohno, K.Nakai, M.Akaji, H.Habashita, H.Tamamura, Y.Miwa, T.Taga, N.Fujii, and Y.Yamamoto, *J.Org.Chem*., 1997, **62**, 2982.
75. L.Antolini, M.Bucciarelli, E.Caselli, P.Davoli, A.Forni, I.Moretti, F.Prati, and G.Torre, *J.Org.Chem*., 1997, **62**, 8784.
76. K.Jahnisch, *Tetrahedron Lett*., 1997, **38**, 227.
77. C.Strassler, A.Linden, and H.Heimgartner, *Helv.Chim.Acta*, 1997, **80**, 1528.
78. M.Pasto, A.Moyano, M.A.Pericas, and A.Riera, *J.Org.Chem*., 1997, **62**, 8425.
79. G.Roussel, E.G.Zamora, A.-C.Carbonnelle, and R.Beugelmans, *Tetrahedron Lett*., 1997, **38**, 4401.
80. J.R.Hanrahan, P.C.Taylor, and W.Errington, *J.Chem.Soc., Perkin Trans.I*, 1997, 493.
81. C.Dominguez, J.Ezquerra, L.Prieto, M.Espada, and C.Pedregal, *Tetrahedron: Asymmetry*, 1997, **8**, 571.
82. T.S.Yokum, M.G.Bursavich, S.A.Piha-Paul, D.A.Hall, and M.L.McLaughlin, *Tetrahedron Lett*., 1997, **38**, 4013.
83. E.Nazie, P.Cardinael, A.-R.Schoofs, and G.Coquerel, *Tetrahedron: Asymmetry*, 1997, **8**, 2913.
84. M.Horikawa, T.Nakajima, and Y.Ohfune, *Synlett*., 1997, 253.
85. N.Moss, J.-M.Ferland, S.Goulet, I.Guse, E.Malenfant, L.Plamondon, R.Plante, and R.Deziel, *Synthesis*, 1997, 32.
86. H.Kuang and M.D.Distefano, *J.Am.Chem.Soc*., 1998, **120**, 1072.

87. B.M.Adger, U.C.Dyer, I.C.Lennon, P.D.Tiffin, and S.E.Ward, *Tetrahedron Lett*., 1997, **38**, 2153.
88. N.A.Petasis and I.A.Zavialov, *J.Am.Chem.Soc*., 1997, **119**, 445.
89. N.A.Petasis, A.Goodman, and I.A.Zavialov, *Tetrahedron*, 1997, **53**, 16463.
90. G.Dyker, *Angew.Chem.Int.Ed*., 1997, **36**, 1700.
91. M.Bruncko, G.Schlingloff, and K.B.Sharpless, *Angew.Chem.Int.Ed*., 1997, **36**, 1483; see also K.B.Sharpless and G.Li, *PCT Int.Appl.WO*, 97 44,312 (*Chem.Abs*., 1998, **128**, 23137).
92. G.Casiraghi, G.Rassu, and F.Zanardi, *Chemtracts*, 1997, **10**, 318.
93. E.Medina, A.Vidal-Ferron, A.Moyano, M.A.Pericas, and A.Riera, *Tetrahedron: Asymmetry*, 1997, **8**, 1581.
94. P.Merino, E.Castillo, F.L.Merchan, and T.Tejero, *Tetrahedron: Asymmetry*, 1997, **8**, 1725.
95. A.Dondini, F.Junquera, F.L.Merchan, P.Merino, M.-C.Scherrmann, and T.Tejero, *J.Org.Chem*., 1997, **62**, 5484.
96. A.Dondini and D.Perrone, *Aldrichim. Acta*, 1997, **30**, 35.
97. K.Kobayashi, H.Akamatsu, S.Irisawa, M.Takahashi, O.Morikawa, and H.Konishi, *Chem.Lett*., 1997, 503.
98. N.Voyer, J.Roby, S.Chenard, and C.Barberis, *Tetrahedron Lett*., 1997, **38**, 6505.
99. D.Hoppe and T.Henre, *Angew.Chem.Int.Ed*., 1997, **36**, 2282.
100. J.Zhu, C.Deur, and L.S.Hegedus, *J.Org.Chem*., 1997, **62**, 7704.
101. M.Braun and K.Opdenbusch, *Liebigs Ann./Recl*., 1997, 141.
102. D.Enders and U.Reinhold, *Tetrahedron: Asymmetry*, 1997, **8**, 1895.
103. A.V.Belyankin, A.R.Khomutov, Y.N.Zhukov, O.N.Kartasheva, and R.M.Khomutov, *Russ.Chem.Bull*., 1997, **46**, 133.
104. I.M.Lefebvre and S.A.Evans, *J.Org.Chem*., 1997, **62**, 7532.
105. M.Calmes, J.Daunis, and N.Mai, *Tetrahedron: Asymmetry*, 1997, **8**, 1641; *Tetrahedron*, 1997, **53**, 13719.
106. C.Palomo, I.Ganboa, B.Odriozola, and A.Linden, *Tetrahedron Lett*., 1997, **38**, 3093.
107. D.J.Ager, I.Prakash, and D.R.Schaad, *Aldrichim. Acta*, 1997, **30**, 3.
108. W.Yuan and V.J.Hruby, *Tetrahedron Lett*., 1997, **38**, 3853.
109. S.Liao, M.D.Shenderovich, J.Liu, and V.J.Hruby, *Tetrahedron*, 1997, **53**, 16645.
110. J.Liu, S.Liao, Y.Han, W.Qiu, and V.J.Hruby, *Tetrahedron: Asymmetry*, 1997, **8**, 3213.
111. Y.Han, S.Liao, W.Qiu, C.Cai, and V.J.Hruby, *Tetrahedron Lett*., 1997, **38**, 5135.
112. J.S.Sabol, G.A.Flynn, D.Friedrich, and E.W.Huber, *Tetrahedron Lett*., 1997, **38**, 3687.
113. F.Jeanjean, N.Perol, J.Gore, and G.Fournet, *Tetrahedron Lett*., 1997, **38**, 7547.
114. A.V.Purandare and S.Natarajan, *Tetrahedron Lett*., 1997, **38**, 8777.
115. M.H.Kress, C.Yang, N.Yasuda, and E.J.J.Grabowski, *Tetrahedron Lett*., 1997, **38**, 2633.
116. E.Juaristi, D.Madrigal, and C.Huerta, *Soc.Quim.Mex*., 1997, **41**, 200.
117. A.Mazon and C.Najera, *Tetrahedron: Asymmetry*, 1997, **8**, 1855.
118. K.N.Kriel, N.D.Emslie, and G.H.P.Roos, *Tetrahedron Lett*., 1997, **38**, 109.
119. G.Cardillo, L.Gentilucci, A.Tolomelli, and C.Tomasini, *Tetrahedron Lett*., 1997, **38**, 6953.
120. J.Malmquist, J.Carlsson, K.E.Markides, P.Pettersson, P.Olsson, K.Sunnerheim-Sjoeberg, and S. Sjoeberg, in Cancer: Neutron Capture Therapy, ed. Y.Mishima, Plenum, New York, 1996, p. 131.
121. W.Q.Liu, B.P.Roques, and C.Garbay, *Tetrahedron Lett*., 1997, **38**, 1389.

122. F.A.Bennett, D.J.Barlow, A.N.O.Dodoo, R.C.Hider, A.B.Lansley, J.Lawrence, C.Marriott, and S.S.Bansai, *Tetrahedron Lett*., 1997, **38**, 7449.
123. H.Miyabe, C.Ushiro, and T.Naito, *Chem.Commun*., 1997, 1789.
124. A.G.Myers, B.H.Yang, H.Chen, L.McKinstry, D.J.Kopecky, and J.L.Gleason, *J.Am.Chem.Soc*., 1997, **119**, 6496.
125. A.B.Smith, A.B.Benowitz, D.A.Favor, P.A.Sprengeler, and R.Hirschmann, *Tetrahedron Lett*., 1997, **38**, 3809.
126. M.J.O'Donnell and Z.Fang, *Hecheng Huaxue*, 1996, **4**, 303 (*Chem.Abs*., 1998, **127**, 95538).
127. S.Hatakeyama, H.Matsumoto, K.Fukuyama, Y.Mukugi, and H.Irie, *J.Org.Chem*., 1997, **62**, 2275.
128. S.Hatakeyama, M.Yoshida, T.Esumi, Y.Iwabuchi, H.Irie, T.Kawamoto, H.Yamada, and M.Nishizawa, *Tetrahedron Lett*., 1997, **38**, 7887.
129. N.Chida, J.Takeoka, K.Ando, N.Tsutsumi, and S.Ogawa, *Tetrahedron*, 1997, **53**, 16287.
130. R.Badorrey, C.Cativiela, M.D.Diaz de Villegas, J.A.Galvez, and Y.Lapena, *Tetrahedron: Asymmetry*, 1997, **8**, 311.
131. L.Lay, M.Meldal, F.Nicotra, L.Panza, and G.Russo, *Chem.Commun*., 1997, 1469.
132. J.F.Knifton, in Applications of Homogeneous Catalysis by Organometallic Compounds, Vol. 1, eds. B.Cornils and W.A.Herrmann, VCH, Weinheim 1996, p.159.
133. J.-J.Lin and J.F.Knifton, *Jiemian Kexue Huizhi*, 1996, **19**, 121 (*Chem.Abs*., 1997, **126**, 144521).
134. J.-J.Lin and J.F.Knifton, *Catal.Lett*., 1997, **45**, 139.
135. M.Beller, M.Eckert, F.Vollmuller, S.Bogdanovic, and H.Geissler, *Angew.Chem.-Int.Ed*., 1997, **36**, 1494.
136. K.M.Short, B.W.Ching, and A.M.M.Mjalli, *Tetrahedron*, 1997, **53**, 6653; K.M.Short and A.M.M.Mjalli, *Tetrahedron Lett.*, 1997, **38**, 359.
137. R.Bossio, C.F.Marcos, S.Marcaccini, and R.Pepino, *Tetrahedron Lett.*, 1997, **38**, 2519.
138. G.C.B.Harriman, *Tetrahedron Lett.*, 1997, **38**, 5591.
139. T.A.Keating and R.W.Armstrong, *J.Org.Chem*., 1998, **63**, 867.
140. T.Matsubara, J.Shinohara, and M.Hisido, *Macromolecules*, 1997, **30**, 2651.
141. C.Vergne, M.Bois-Choussy, J.Ouazzani, R.Beugelmans, and J.Zhu, *Tetrahedron: Asymmetry*, 1997, **8**, 391.
142. T.Kitagawa and N.Akiyama, *Chem.Pharm.Bull*., 1997, **45**, 1865.
143. J.-P.Mazaleyrat, A.Gaucher, J.Savrda, and M.Wakselman, *Tetrahedron: Asymmetry*, 1997, **8**, 619.
144. J.-P.Mazaleyrat, A.Gaucher, Y.Goubard, J.Savrda, and M.Wakselman, *Tetrahedron Lett.*, 1997, **38**, 2091.
145. C.Alvarez-Ibarra, A.G.Csaky, M.L.Quiroga, and D.Ramirez, *Tetrahedron*, 1997, **53**, 2189.
146. C.Alvarez-Ibarra, A.G.Csaky, E.Martinez-Santos, and M.L.Quiroga, *Tetrahedron*, 1997, **53**, 3679.
147. A.Mazon, C.Najera, J.Ezquerra, and C.Pedregal, *Tetrahedron Lett.*, 1997, **38**, 2167.
148. E.Ohler and S.Kanzler, *Liebigs Ann./Recl*., 1997, 1437.
149. S.Kroger and G.Haufe, *Amino Acids*, 1997, **12**, 363.
150. E.A.Mash, L.J.Williams, and S.S.Pfeiffer, *Tetrahedron Lett.*, 1997, **38**, 6977.
151. B.Lygo and P.G.Wainwright, *Tetrahedron Lett.*, 1997, **38**, 8595.
152. P.Sun and Y.Zhang, *Synth.Commun*., 1997, **27**, 4173.
153. W.L.Scott, C.Zhou, Z.Fang, and M.J.O'Donnell, *Tetrahedron Lett.*, 1997, **38**, 3695;

D.L.Griffith, M.J.O'Donnell, R.S.Pottorf, W.L.Scott, and J.A.Porco, *Tetrahedron Lett.*, 1997, **38**, 8821.
154. M.J.O'Donnell, C.W.Lugar, R.S.Pottorf, C.Zhou, W.L.Scott, and C.L.Cwi, *Tetrahedron Lett.*, 1997, **38**, 7163.
155. R.Badorrey, C.Cativiela, M.D.Diaz de Villegas, and J.A.Galvez, *Tetrahedron*, 1997, **53**, 1411.
156. R.Badorrey, C.Cativiela, M.D.Diaz de Villegas, and J.A.Galvez, *Synthesis*, 1997, 747.
157. L.Battistini, F.Zanardi, G.Rassu, P.Spanu, G.Pelosi, G.G.Fava, M.B.Ferrari, and G.Casiraghi, *Tetrahedron: Asymmetry*, 1997, **8**, 2975.
158. V.I.Tararov, T.F.Saveleva, N.Y.Kuznetsov, N.S.Ikonnikov, S.A.Orlova, Y.N.Belokon, and M.North, *Tetrahedron: Asymmetry*, 1997, **8**, 79.
159. Y.N.Belokon, K.A.Kochetkov, T.D.Chirkina, N.S.Ikonnikov, S.A.Orlova, V.V.Smirnov, and A.A.Chesnokov, *Mendeleev Commun.*, 1997, 137.
160. V.A.Soloshonok, D.V.Avilov, V.P.Kukhar, L.V.Meervelt, and N.Mischenko, *Tetrahedron Lett.*, 1997, **38**, 4903.
161. V.A.Soloshonok, D.V.Avilov, V.P.Kukhar, L.V.Meervelt, and N.Mischenko, *Tetrahedron Lett.*, 1997, **38**, 4671.
162. I.K.Mosevich, O.F.Kuznetsova, O.S.Fedorova, and M.V.Korsakov, *Radiokhim.*, 1996, **38**, 511.
163. M.Jacob, M.L.Roumestant, P.Viallefont, and J.Martinez, *Synlett.*, 1997, 691.
164. T.-L.Yeh, C.-C.Liao, and B.-J.Uang, *Tetrahedron*, 1997, **53**, 11141.
165. J.M.Lerestif, L.Toupet, S.Sinbandhit, F.Tonnard, J.P.Bazureau, and J.Hamelin, *Tetrahedron*, 1997, **53**, 6351.
166. M.A.Stark and C.J.Richards, *Tetrahedron Lett.*, 1997, **38**, 5881.
167. V.A.Soloshonok, A.Vadim, A.D.Kacharov, D.V.Avilov, K.Ishikawa, N.Nagashima, and T.Hayashi, *J.Org.Chem.*, 1997, **62**, 3470.
168. S.Kotha, E.Brahmachary, A.Kuki, K.Lang, D.Anglos, B.Singaram, and W.Chrisman, *Tetrahedron Lett.*, 1997, **38**, 9031.
169. S.Kotha and E.Brahmachary, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 2719.
170. M.Kirihata, T.Morimoto, I.Ichimoto, and M.Takagaki, in Cancer: Neutron Capture Therapy, ed. Y.Mishima, Plenum, New York, 1996, p.151.
171. B.Kayser, K.Polborn, W.Steglich, and W.Beck, *Chem.Ber./Recl.*, 1997, **130**, 171.
172. M.J.O'Donnell, N.Chen, C.Zhou, A.Murray, C.P.Kubiak, F.Yang, and G.G.Stanley, *J.Org.Chem.*, 1997, **62**, 3962.
173. F.Lamaty, R.Lazaro, and J.Martinez, *Tetrahedron Lett.*, 1997, **38**, 3385.
174. S.Achamlal, A.Elachqar, A.El Hallaoui, S.El Hajji, M.L.Roumestant, and P.Viallefont, *Amino Acids*, 1997, **12**, 257.
175. D.A.Alonso, A.Costa, and C.Najera, *Tetrahedron Lett.*, 1997, **38**, 7943.
176. S.D.Debenham, J.S.Debenham, M.J.Burk, and E.J.Toone, *J.Am.Chem.Soc.*, 1997, **119**, 9897.
177. M.J.O'Donnell, I.A.Esikova, A.Mi, D.F.Shullenberger, and S.Wu, *ACS Symp.Ser.*, 1997, **659** (Phase Transfer Catalysis), 124.
178. C.Zhou, *Hecheng Huaxue*, 1996, **4**, 325 (*Chem.Abs.*, 1998, **127**, 95539).
179. R.Grandel and U.Kazmaier, *Tetrahedron Lett.*, 1997, **38**, 8009.
180. D.Gonzalez, V.Schapiro, G.Seoane, T.Hudlicky, and K.Abboud, *J.Org.Chem.*, 1997, **62**, 1194.
181. S.Bouifraden, J.-P.Lavergne, J.Martinez, P.Viallefont, and C.Riche, *Tetrahedron: Asymmetry*, 1997, **8**, 949.
182. M.J.Koen, J.Morgan, J.T.Pinhey, and C.J.Sherry, *J.Chem.Soc., Perkin Trans.I*, 1997, 487.

183. J.Morgan, J.T.Pinhey, and C.J.Sherry, *J.Chem.Soc., Perkin Trans.I*, 1997, 613.
184. H.Matsunaga, T.Ishizuka, and T.Kunieda, *Tetrahedron*, 1997, **53**, 1275.
185. N.Katagiri, M.Okada, Y.Morishita, and C.Kaneko, *Tetrahedron*, 1997, **53**, 5725.
186. P.Kremminger and K.Undheim, *Tetrahedron*, 1997, **53**, 3687.
187. J.Efskind, T.Benneche, and K.Undheim, *Acta Chem.Scand.*, 1997, **51**, 942.
188. S.Rodbotten, T.Benneche, and K.Undheim, *Acta Chem.Scand.*, 1997, **51**, 873.
189. K.Hammer, T.Benneche, H.Hope, and K.Undheim, *Acta Chem.Scand.*, 1997, **51**, 392.
190. M.J.I.Andrews and A.B.Tabor, *Tetrahedron Lett.*, 1997, **38**, 3063.
191. S.Neset, H.Hope, and K.Undheim, *Tetrahedron*, 1997, **53**, 10459.
192. A.Pecunioso, D.Papini, B.Tamburini, and F.Tinazzi, *Org.Prep.Proced.Int.*, 1997, **29**, 218.
193. K.Hammer and K.Undheim, *Tetrahedron*, 1997, **53**, 2309.
194. K.Hammer and K.Undheim, *Tetrahedron*, 1997, **53**, 5925.
195. K.Hammer and K.Undheim, *Tetrahedron*, 1997, **53**, 10603.
196. T.Gan and J.M.Cook, *Tetrahedron Lett.*, 1997, **38**, 1301.
197. R.Liu, P.Zhang, T.Gan and J.M.Cook, *J.Org.Chem.*, 1997, **62**, 7447.
198. T.Gan, R.Liu, P.Yu, S.Zhao, and J.M.Cook, *J.Org.Chem.*, 1997, **62**, 9298.
199. V.Ojea, S.Conde, M.Ruiz, M.C.Fernandez, and J.M.Quintela, *Tetrahedron Lett.*, 1997, **38**, 4311.
200. R.Chinchilla, L.R.Falvello, N.Galindo, and C.Najera, *Angew.Chem.Int.Ed.*, 1997, **36**, 995.
201. M.G.B.Drew, M.Fengler-Veith, L.M.Harwood, and A.W.Jahans, *Tetrahedron Lett.*, 1997, **38**, 4521; R.Angell, M.Fengler-Veith, H.Finch, L.M.Harwood, and T.T.Tucker, *ibid.*, 4517; R.Angell, M.G.B.Drew, M.Fengler-Veith, H.Finch, L.M.Harwood, A.W.Jahans, and T.T.Tucker, *ibid.*, 3107.
202. L.M.Harwood, G.Hamblett, A.I.Jimenez-Diaz, and D.J.Watkin, *Synlett.*, 1997, 935.
203. D.M.Bender and R.M.Williams, *J.Org.Chem.*, 1997, **62**, 6690.
204. P.Remuzon, M.Soumeillant, C.Dussy, and D.Bouzard, *Tetrahedron*, 1997, **53**, 17711.
205. K.Hoffmann and D.Seebach, *Chimia*, 1997, **51**, 90.
206. A.Kless, A.Boerner, D.Heller, and R.Selke, *Organometallics*, 1997, **16**, 2096.
207. M.Kitamura, M.Yoshimura, M.Tsukamoto, and R.Noyori, *Enantiomer*, 1996, **1**, 281.
208. A.Tungler and K.Fodor, *Catal.Today*, 1997, **37**, 191 (*Chem.Abs.*, 1998, **127**, 234560); R.Kuwano and Y.Ito, *Kagaku*, 1997, **52**, 38 (*Chem.Abs.*, 1998, **127**, 331702).
209. P.J.Pye, K.Rossen, R.A.Reamer, N.N.Tsou, R.P.Volante, and P.J.Reider, *J.Am.Chem.Soc.*, 1997, **119**, 6207.
210. B.Basu, S.K.Chattopadhyay, A.Ritzen, and T.Frejd, *Tetrahedron: Asymmetry*, 1997, **8**, 1841.
211. B.Adger, U.Berens, M.J.Griffiths, M.J.Kelly, R.McCague, J.A.Miller, C.F.Palmer, S.M.Roberts, R.Selke, U.Vitinius, and G.Ward, *Chem.Commun.*, 1997, 1713.
212. M.J.Burk, J.G.Allen, and W.F.Kiesman, *J.Am.Chem.Soc.*, 1998, **120**, 657.
213. A.Javidan, K.Schafer, and S.G.Pyne, *Synlett.*, 1997, 100.
214. S.G.Pyne, K.Schafer, B.W.Skelton, and A.H.White, *Chem.Commun.*, 1997, 2267.
215. Q.-S.Hu and C.-M.Hu, *Chin.J.Chem.*, 1997, **15**, 286 (*Chem.Abs.*, 1998, **127**, 234573).
216. O.H.Ryu, J.Y.Ju, and C.S.Shin, *Process Biochem.*, 1997, **32**, 201.
217. Y.Zhang, M.Wang, and W.Wu, *Zhongguo Yaoke Daxue Xuebao*, 1996, **27**, 500 (*Chem.Abs.*, 1997, **126**, 154642).
218. D.Wuester-Botz, R.Kelle, M.Frantzen, and C.Wandrey, *Biotechnol.Prog.*, 1997, **13**, 387.

219. M.Maeda, K.Takata, A.Toyoda, T.Niitsu, M.Iwakura, and K.Shibata, *J.Ferment.-Bioeng*., 1997, **83**, 113 (*Chem.Abs*., 1997, **126**, 209222).
220. K.Okamoto, K.Kino, and M.Ikeda, *Biosci., Biotechnol., Biochem*., 1997, **61**, 1877.
221. D.A.Skladnev, O.V.Mosin, T.A.Egorova, C.V.Eremin, and V.I.Shvets, *Biotekhnologiya*, 1996, 25.
222. K.Dallmann, L.Orosz, and B.Szajani, *Biotechnol.Lett*., 1997, **19**, 123.
223. J.-S.Zhao and S.-K.Yang, *Huaxue Xuebao*, 1997, **55**, 196 (*Chem.Abs*., 1997, **126**, 237439).
224. H.-Y.Cho and K.Soda, *Biosci., Biotechnol., Biochem*., 1997, **61**, 1216.
225. J.Park, J.Tai, C.A.Roessner, and A.I.Scott, *Bioorg.Med.Chem.Lett*., 1996, **4**, 2179.
226. A.Galkin, L.Kulakova, H.Yamamoto, K.Tanizawa, H.Tanaka, N.Esaki, and K.Soda, *J.Ferment.Bioeng*., 1997, **83**, 299.
227. G.Krix, A.S.Bommarius, K.Drauz, M.Kottenhahn, M.Schwarm, and M.-R.Kula, *J.Biotechnol*., 1997, **53**, 29.
228. A.Sutherland and C.L.Willis, *Tetrahedron Lett*., 1997, **38**, 1837.
229. J.P.G.Malthouse, T.B.Fitzpatrick, J.J.Milne, L.Grehn, and U.Ragnarsson, *J.Pept.Sci*., 1997, **3**, 361.
230. T.Kimura, V.P.Vassilev, G.-J.Shen, and C.-H.Wong, *J.Am.Chem.Soc*., 1997, **119**, 11734.
231. D.P.Henderson, M.C.Shelton, I.C.Cotterill, and E.J.Toone, *J.Org.Chem*., 1997, **62**, 7910.
232. D.J.Ager, I.G.Fotheringham, S.A.Laneman, D.P.Pantaleone, and P.P.Taylor, *Chim. Oggi*, 1997, **15**, 11.
233. H.Motoyama and H.Anazawa, *Kagaku to Seibutsu*, 1997, **35**, 123.
234. T.Shibatani, *Kikan Kagaku Sosetsu*, 1997, **33**, 35 (*Chem.Abs*., 1998, **127**, 346622).
235. A.Ozaki, *Bio.Ind*., 1997, **14**, 31.
236. N.J.Turner, *Curr.Org.Chem*., 1997, **1**, 21.
237. E.Zymanczyk-Duda and B.Lejczak, *Wiad.Chem*., 1997, 51, 293.
238. N.Esaki, S.Nakamori, T.Kurihara, S.Furuyoshi, and K.Soda, in *Biotechnology*, Second Edition, Vol. 6, 1996, eds. H.-J.Rehm and G.Reed, p. 503.
239. A.Bongini, M.Panunzio, E.Bandini, G.Martelli, and G.Spunta, *J.Org.Chem*., 1997, **62**, 8911.
240. H.Kapeller, W.G.Jary, W.Hayden, and H.Griengl, *Tetrahedron: Asymmetry*, 1997, **8**, 245.
241. A.Tuch, M.Saniere, Y.Le Merrer, and J.-C.Depezay, *Tetrahedron: Asymmetry*, 1997, **8**, 1649.
242. S.Sasaki, Y.Hamada, and T.Shioiri, *Tetrahedron Lett*., 1997, **38**, 3013.
243. H.Noguchi, T.Aoyama, and T.Shioiri, *Tetrahedron Lett*., 1997, **38**, 2883.
244. C.Mazzini, L.Sambri, H.Regeling, B.Zwanenburg, and G.J.F.Chittenden, *J.Chem.Soc., Perkin Trans.I*, 1997, 3351.
245. J.E.Baldwin, S.J.Bamford, A.M.Fryer, M.P.W.Rudolph, and M.E.Wood, *Tetrahedron*, 1997, **53**, 5233.
246. J.E.Baldwin, S.J.Bamford, A.M.Fryer, M.P.W.Rudolph, and M.E.Wood, *Tetrahedron*, 1997, **53**, 5255; J.E.Baldwin, A.M.Fryer, M.R.Spyvee, R.C.Whitehead, and M.E.Wood, *Tetrahedron*, 1997, **53**, 5273.
247. M.D.Bachi and A.Melman, *J.Org.Chem*., 1997, **62**, 1896.
248. O.Miyata, Y.Ozawa, I.Ninomiya, and T.Naito, *Synlett*., 1997, 275.
249. H.Maeda and G.A.Kraus, *J.Org.Chem*., 1997, **62**, 2314.
250. M.Kawamura and K.Ogasawara, *Heterocycles*, 1997, **44**, 129.
251. Y.Nakada, T.Sugahara, and K.Ogasawara, *Tetrahedron Lett*., 1997, **38**, 857.

252. R.C.F.Jones, K.J.Howard, and J.S.Snaith, *Tetrahedron Lett*., 1997, **38**, 1647.
253. D.J.Hyett, J.B.Sweeney, A.Tavassoli, and J.F.Hayes, *Tetrahedron Lett*., 1997, **38**, 8283.
254. D.S.Matteson and J.-J.Yang, *Tetrahedron: Asymmetry*, 1997, **8**, 3855.
255. D.A.Wacker, F.E.Lovering, R.J.Bridges, C.Willis, R.Bartlett, and A.R.Chamberlin, *Synlett.*, 1997, 503.
256. H.Baumgartner, A.C.O'Sullivan, and J.Schneider, *Heterocycles*, 1997, **45**, 1537.
257. M.Maeda, F.Okazaki, M.Murayama, Y.Tachibana, Y.Aoyagi, and A.Ohta, *Chem.-Pharm.Bull*., 1997, **45**, 962.
258. T.Wirth, *Angew.Chem.Int.Ed*., 1997, **36**, 225.
259. C.L.Wysong, T.S.Yokum, M.L.McLaughlin, and R.P.Hammer, *Chem.Tech*., 1997, **27**, 26.
260. P.Damhaut, C.Lemaire, A.Plenevaux, C.Brinhaye, L.Christiaens, and D.Comar, *Tetrahedron*, 1997, **53**, 5785.
261. K.Tomiyoshi, K.Amed, S.Muhammad, T.Higuchi, T.Inoue, K.Endo, and D.Yang, *Nucl.Med.Commun*., 1997, **18**, 169.
262. B.Trost and X.Ariza, *Angew.Chem.Int.Ed*., 1997, **36**, 2635.
263. C.Cativiela, M.D.Diaz de Villegas, J.A.Galvez, and Y.Lapena, *Tetrahedron*, 1997, **53**, 5891.
264. M.R.Mish, F.M.Guerra, and E.M.Carreira, *J.Am.Chem.Soc*., 1997, **119**, 8379.
265. T.Kawabata, T.Wirth, K.Yahiro, H.Suzuki, and K.Fuji, *ICR Annual Rep*., 1997, **3**, 36 (*Chem.Abs*., 1998, **127**, 121981).
266. D.Ma and H.Tian, *J.Chem.Soc., Perkin Trans.I*, 1997, 3493.
267. I.V.Ekhato and Y.Huang, *J.Labelled Compd.Radiopharm*., 1997, **39**, 1019.
268. H.M.L.Davies and N.Kong, *Tetrahedron Lett.*, 1997, **38**, 4203.
269. S.N.Osipov, A.S.Golubev, N.Sewald, and K.Burger, *Tetrahedron Lett.*, 1997, **38**, 5965.
270. J.van Betsbrugge, D.Tourwe, B.Kaptein, H.Kierkels, and R.Broxterman, *Tetrahedron*, 1997, **53**, 9233.
271. D.Ma, Z.Ma, J.Jiang, Z.Yang, and C.Zheng, *Tetrahedron: Asymmetry*, 1997, **8**, 889.
272. P.L.Ornstein, T.J.Bleisch, M.B.Arnold, R.A.Wright, B.G.Johnson, and D.D.Schoepp, *J.Med.Chem*., 1998, **41**, 346.
273. E.C.Taylor and B.Hu, *Heterocycles*, 1997, **45**, 241.
274. S.Hanessian, U.Reinhold, and G.Gentile, *Angew.Chem.Int.Ed*., 1997, **36**, 1881.
275. V.V.Tverezovsky, M.S.Baird, and I.G.Bolesov, *Tetrahedron*, 1997, **53**, 14773.
276. M.J.Sloan and K.L.Kirk, *Tetrahedron Lett*., 1997, **38**, 1677.
277. R.R.Srivastava, R.R.Singhaus, and G.W.Kabalka, *J.Org.Chem*., 1997, **62**, 4476.
278. Y.Ohfune, K.Nanba, I.Takada, T.Kan, M.Horikawa, and T.Nakajima, *Chirality*, 1997, **9**, 459; Y.Ohfune and M.Horikawa, *Yuki Gosei Kagaku Kyokaishi*, 1997, **55**, 982 (*Chem.Abs*., 1998, **128**, 3856).
279. A.Avenoza, J.H.Busto, M.Paris, J.M.Peregrina, and C.Cativiela, *J.Heterocycl.-Chem*., 1997, **34**, 1099.
280. A.Avenoza, C.Cativiela, M.Paris, J.M.Peregrina, and B.Saenz-Torre, *Tetrahedron: Asymmetry*, 1997, **8**, 1123.
281. F.A.Davis, C.-H.Liang, and H.Liu, *J.Org.Chem*., 1997, **62**, 3796.
282. F.J.Lakner and L.P.Hager, *Tetrahedron: Asymmetry*, 1997, **8**, 3547.
283. N.A.Sasaki, M.Dockner, A.Chiaroni, C.Riche, and P.Potier, *J.Org.Chem*., 1997, **62**, 765; erratum *ibid*., 9388.
284. P.Karoyan and G.Chassaing, *Tetrahedron Lett*., 1997, **38**, 85; *Tetrahedron: Asymmetry*, 1997, **8**, 2025.

285. E.Lorthiois, I.Marek, and J.-F.Normant, *Tetrahedron Lett*., 1997, **38**, 89.
286. M.J.S.Carpes, P.C.M.L.Miranda, and C.R.D.Correia, *Tetrahedron Lett*., 1997, **38**, 1869.
287. C.Wittland, U.Floerke, and N.Risch, *Synthesis*, 1997, 1291.
288. V.Collot, M.Schmitt, A.K.Marwah, B.Norberg, and J.-J.Bourguignon, *Tetrahedron Lett*., 1997, **38**, 8033.
289. G.C.Bolton, J.C.Hodges, and J.R.Rubin, *Tetrahedron*, 1997, 53, 6611.
290. R.Badorrey, C.Cativiela, M.D.Diaz de Villegas, and J.A.Galvez, *Tetrahedron Lett*., 1997, **38**, 2547.
291. C.Agami, D.Bihan, R.Morgentin, and C.Puchot-Kadouri, *Synlett*, 1997, 799.
292. G.M.Makara and G.R.Marshall, *Tetrahedron Lett*., 1997, **38**, 5069.
293. C.Palomo, I.Ganboa, C.Cuevas, C.Boschetti, and A.Linden, *Tetrahedron Lett*., 1997, **38**, 4643.
294. P.Laszlo, *Recherche*, 1997, 26.
295. J.D.Sutherland and J.N.Whitfield, *Tetrahedron*, 1997, **53**, 11493.
296. L.Joshi, *Acta Cienc.Indica, Chem*., 1996, **22**, 85 (*Chem.Abs*., 1998, **127**, 91803).
297. K.Kobayashi, M.Kohara, M.Dokiya, and H.Yanagawa, *Viva Origino*, 1997, **25**, 167.
298. M.Kohara, T.Gamo, H.Yanagawa, and K.Kobayashi, *Chem.Lett*., 1997, 1053.
299. S.Miyakawa, H.Tamura, K.Kobayashi, and A.Sawaoka, *Jpn.J.Appl.Phys., Part 1*, 1997, **36**, 4481.
300. K.Kobayashi, T.Kaneko, C.Ponnamperuma, T.Oshima, H.Yanagawa, and T.Saito, *Nippon Kagaku Kaishi*, 1997, 823 (*Chem.Abs*., 1998, **128**, 112129).
301. M.R.Amin, D.B.Harper, J.M.Moloney, C.D.Murphy, J.A.K.Howard, and D.O'Hagan, *Chem.Commun*., 1997, 1471 (for an erratum, see *Ibid*., p.1815).
302. C.Zhang, C.Ludin, M.K.Eberle, H.Stoeckli-Evans, and R.Keese, *Helv.Chim.Acta*, 1998, **81**, 174.
303. M.K.Eberle, R.Keese, and H.Stoeckli-Evans, *Helv.Chim.Acta*, 1998, **81**, 182.
304. S.Kroger and G.Haufe, *Liebigs Ann.Chem./Recl*., 1997, 1201.
305. O.Varela, *Pure Appl.Chem*., 1997, **69**, 621.
306. G.Arsequell and G.Valencia, *Tetrahedron: Asymmetry*, 1997, **8**, 2839.
307. B.V.Rao, U.M.Krishna, and M.K.Gurjar, *Synth.Commun*., 1997, **27**, 1335.
308. P.Merino, S.Franco, F.L.Merchan, and T.Tejero, *Tetrahedron: Asymmetry*, 1997, **8**, 3489.
309. G.delle Monache, M.C.Di Giovanni, D.Misiti, and G.Zappia, *Tetrahedron: Asymmetry*, 1997, **8**, 231.
310. C.J.Easton and M.C.Mervett, *Tetrahedron*, 1997, **53**, 1151.
311. K.Wisniewski, *Wiad. Chem*., 1997, **51**, 63.
312. G.Bitan, D.Muller, R.Hasher, E.V.Gluhov, and C.Gilon, *J.Chem.Soc., Perkin Trans.I*, 1997, 1501.
313. D.Xu, K.Prasad, O.Repic, and T.J.Blacklock, *Tetrahedron: Asymmetry*, 1997, **8**, 1445.
314. C.Cativiela, M.D.Diaz De Villegas, and J.A.Galvez, *Tetrahedron: Asymmetry*, 1997, **8**, 1605.
315. H.Buttner, F.Gerum, and T.Severin, *Carbohydr.Res*., 1997, **300**, 265.
316. Reviews: K.L.Dueholm and P.E.Nielsen, *New J.Chem*., 1997, **21**, 19; P.E.Nielsen and G.Haaima, *Chem.Soc.Rev*., 1997, **26**, 73; D.R.Corey, *Trends Biotechnol*., 1997, **15**, 224; Review of PNA – nucleic acid complexes: M.Eriksson and P.E.Nielsen, *Quart.Rev.Biophys*., 1996, **29**, 369; see also, E.Uhlman, D.W.Will, G.Breipohl, D.Languer, and A.Ryte, *Angew.Chem.Int.Ed*., 1996, **35**, 2632; A.C.van der Laan, R.Brill, R.G.Kuimelis, E.Kuyl-Yeheskiely, J.H.van boom, A.Andrus, and R.Vinayak, *Tetrahedron Lett*., 1997, **38**, 2249.

317. G.Breipohl, D.W.Will, A Peyman, and E.Uhlmann, *Tetrahedron*, 1997, **53**, 14671.
318. S.L.Miller, *Nat.Struct.Biol.*, 1997, **4**, 167.
319. G.Lowe and T.Vilaivan, *J.Chem.Soc., Perkin Trans.I*, 1997, 539, 547, 555.
320. C.Alvarez-Ibarra, A.G.Csaky, M.Ortega, M.J.de la Morena, and M.L.Quiroga, *Tetrahedron Lett.*, 1997, **38**, 4501.
321. B.M.Trost and G.R.Dake, *J.Am.Chem.Soc.*, 1997, **119**, 7595.
322. D.T.Davies, K.Goodall, N.Kapur, M.O'Brien, and A.F.Parsons, *Synth.Commun.*, 1997, **27**, 3815.
323. M.A.Loreto, F.Pompei, P.A.Tardella, and D.Tofani, *Tetrahedron*, 1997, **53**, 15853.
324. U.Kazmaier, *Liebigs Ann./Recl.*, 1997, 285.
325. M.Aidene, F.Barbot, and L.Miginiac, *J.Organomet.Chem.*, 1997, **534**, 117.
326. R.Beugelmans, E.G.Zamora, and G.Roussi, *Tetrahedron Lett.*, 1997, **38**, 8189.
327. Y.Satoh, C.Gude, K.Chan, and F.Firooznia, *Tetrahedron Lett.*, 1997, **38**, 7645.
328. Z.S.Arnold, *J.Pept.Sci.*, 1997, **3**, 354.
329. Y.S.Park and P.Beak, *J.Org.Chem.*, 1997, **62**, 1574.
330. S.N.Rao, M.F.Chan, S.S.Projeta, and V.N.Bahji, *J.Pept.Res.*, 1997, **49**, 145.
331. S.E.Gibson, N.Guillo, R.J.Middleton, A.Thuillez, and M.J.Tozer, *J.Chem.Soc., Perkin Trans.1*, 1997, 447.
332. S.E.Gibson, N.Guillo, and M.J.Tozer, *Chem.Commun.*, 1997, 637.
333. D.Ma, H.Tian, H.Sun, A.P.Kozikowski, S.Pshenichkin, and J.T.Wroblewski, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 1195.
334. E.C.Taylor, L.D.Jennings, Z.Mao, B.Hu, J.-G.Jun, and P.Zhou, *J.Org.Chem.*, 1997, **62**, 5392.
335. S.Kotha and E.Brahmachary, *Tetrahedron Lett.*, 1997, **38**, 3561.
336. B.Ye, Z.-J.Yao, and T.R.Burke, *J.Org.Chem.*, 1997, **62**, 5428.
337. J.Meiwes, M.Schudok, and G.Kretzschmar, *Tetrahedron: Asymmetry*, 1997, **8**, 527.
338. T.S.Yokum, P.K.Tungaturthi, and M.L.McLaughlin, *Tetrahedron Lett.*, 1997, **38**, 5111.
339. M.W.Majchrzak, J.N.Zobel, and D.J.Obradovich, *Synth.Commun.*, 1997, **27**, 3201.
340. B.P.Clark and J.R.Harris, *Synth.Commun.*, 1997, **27**, 4223.
341. A.Alami, A.El Hallaoui, A.Elachqar, M.L.Roumestant, and P.Viallefont, *Bull.-Chem.Soc.Belg.*, 1996, **105**, 767.
342. A.N.Bowler, A.Dinsmore, P.M.Doyle, and D.W.Young, *J.Chem.Soc., Perkin Trans.1*, 1997, 1297.
343. P.Kolar, A.Petric, and M.Tisler, *J.Heterocycl.Chem.*, 1997, **34**, 1067.
344. F.A.Sloek, B.Ebert, Y.Lang, P.Krogsgaard-Larsen, S.M.Lenz, and U.Madsen, *Eur.-J.Med.Chem.*, 1997, **32**, 329.
345. R.Waelchli, C.Beerli, H.Meigel, and L.Revesz, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 2831.
346. P.Ciapetti, F.Soccolini, and M.Taddei, *Tetrahedron*, 1997, **53**, 1167.
347. S.Jordan, C.Schwemler, W.Kosch, A.Kretschmer, E.Schwenner, U.Stropp, and B.Mielke, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 681; S.Jordan, C.Schwemler, W.Kosch, A.Kretschmer, U.Stropp, E.Schwenner, and B.Mielke, *ibid.*, p. 687.
348. R.M.Adlington, J.E.Baldwin, D.Catterick, and G.J.Pritchard, *Chem.Commun.*, 1997, 1757.
349. V.Dryanska, I.Pashkuleva, and D.Tasheva, *Synth.Commun.*, 1997, **27**, 1849.
350. C.Alvarez-Ibarra, A.G.Csakye, B.Colmenero, and M.L.Quiroga, *J.Org.Chem.*, 1997, **62**, 2478.
351. A.S.Sagiyan, A.E.Avetisyan, S.M.Djamgaryan, L.R.Djibaryan, E.A.Gyulumyan,

S.K.Grigoryan, N.A.Kuzmina, S.A.Orlova, N.S.Ikonnikov, V.S.Larichev, V.I.Tararov, and Y.N.Belokon, *Izv.Akad.Nauk, Ser.Khim.*, 1997, **46**, 483.
352. H.S.M.Lu, M.Volk, Y.Kholodenko, E.Gooding, R.M.Hochstrasser, and W.F.DeGrado, *J.Am.Chem.Soc.*, 1997, **119**, 7173.
353. C.A.Hutton and J.M.White, *Tetrahedron Lett.*, 1997, **38**, 1643.
354. M.Sakai, K.Hashimoto, and H.Shirahama, *Heterocycles*, 1997, **44**, 319.
355. R.J.Smith, S.Bratovanov, and S.Bienz, *Tetrahedron*, 1997, **53**, 13695.
356. T.Itaya, T.Kanai, and T.Iida, *Tetrahedron Lett.*, 1997, **38**, 1979.
357. M.Attolini, M.Maffei, B.Principato, and G.Pfeiffer, *Synlett.*, 1997, 384.
358. N.R.Kurdyumova, V.V.Ragulina, and E.N.Tsvetkov, *Mendeleev Commun.*, 1997, 69.
359. A.M.Kawamoto and M.M.Campbell, *J.Fluorine Chem.*, 1997, **81**, 181.
360. S.G.Davies and O.Ichihara, *Yuki Gosei Kagaku Kyokaishi*, 1997, **55**, 42; S.G.Davies and D.R.Fenwick, *Tetrahedron: Asymmetry*, 1997, **8**, 3387.
361. M.E.C.Polywka, *Spec.Chem.*, 1996, **16**, 5S, 8S.
362. Y.Takayama, S.Okamoto, and F.Sato, *Tetrahedron Lett.*, 1997, **38**, 8351.
363. H.Matsuyama, N.Itoh, M.Yoshida, N.Kamigata, S.Sasaki, and M.Iiyoda, *Chem.-Lett.*, 1997, 375; H.Matsuyama, N.Itoh, M.Yoshida, and M.Iiyoda, *Phosphorus, Sulfur, Silicon Relat.Elem.*, 1997, **120 and 121**, 475.
364. M.Bella, F.D'Onofrio, R.Margarita, L.Parlanti, G.Piancatelli, and A.Mangoni, *Tetrahedron Lett.*, 1997, **38**, 7917.
365. L.Falborg and K.A.Jorgenson, *J.Chem.Soc., Perkin Trans.1*, 1997, 2823.
366. J.S.Yadav, S.Chandrasekar, and P.K.Sasmal, *Tetrahedron Lett.*, 1997, **38**, 8765.
367. D.Enders and J.Wiedemann, *Liebigs Ann./Recl.*, 1997, 699.
368. H.Kunz, A.Burgard, and D.Schanzenbach, *Angew.Chem.Int.Ed.*, 1997, **36**, 386.
369. F.Guenoun, T.Zair, F.Lamaty, M.Pierrot, R.Lazaro, and P.Viallefont, *Tetrahedron Lett.*, 1997, **38**, 1563.
370. A.Abouabdellah, J.-P.Begue, D.Bonnet-Delpon, and T.T.T.Nga, *J.Org.Chem.*, 1997, **62**, 8826.
371. I.Ojima and F.Delaloge, *Chem.Soc.Rev.*, 1997, **26**, 377.
372. S.Kobayashi, R.Akiyama, and M.Moriwaki, *Tetrahedron Lett.*, 1997, **38**, 4819.
373. S.Jost, Y.Gimbert, A.E.Greene, and F.Fotiadu, *J.Org.Chem.*, 1997, **62**, 6672.
374. S.-K.Chung, T.-H.Jeong, and D.-H.Kang, *Tetrahedron: Asymmetry*, 1997, **8**, 5.
375. S.Fustero, B.Pina, and A.Simon-Fuentes, *Tetrahedron Lett.*, 1997, **38**, 6771.
376. A.S.-Y.Lee, R.-Y.Cheng, and O.-G.Pan, *Tetrahedron Lett.*, 1997, **38**, 443.
377. C.Cave, Y.Le Porhiel-Castillon, V.Daley, C.Riche, A.Chiaroni, and J.D'Angelo, *Tetrahedron Lett.*, 1997, **38**, 8703.
378. C.Cave, A.Gassama, J.Mahuteau, J.D'Angelo, and C.Riche, *Tetrahedron Lett.*, 1997, **38**, 4773.
379. A.Bardou, J.P.Celerier, and G.Lhommet, *Tetrahedron Lett.*, 1997, **38**, 8507.
380. J.Blot, A.Bardou, C.Bellec, M.-C.Fargeau-Bellassoued, J.P.Celerier, G.Lhommet, D.Gardette, and J.-C.Gramain, *Tetrahedron Lett.*, 1997, **38**, 8511.
381. C.Mazzini, J.Lebreton, V.Alphand, and R.Furstoss, *J.Org.Chem.*, 1997, **62**, 5215.
382. B.C.Das and J.Iqbal, *Tetrahedron Lett.*, 1997, **38**, 2903.
383. A.De, P.Basak, and J.Iqbal, *Tetrahedron Lett.*, 1997, **38**, 8383.
384. A.De, S.Ghosh, and J.Iqbal, *Tetrahedron Lett.*, 1997, **38**, 8379.
385. C.Bubert and O.Reiser, *Tetrahedron Lett.*, 1997, **38**, 4985.
386. K.Burgess, S.Li, and J.Rebenspies, *Tetrahedron Lett.*, 1997, **38**, 1681.
387. S.Hanessian, L.-D.Cantin, S.Roy, D.Andreotti, and A.Gomtsyan, *Tetrahedron Lett.*, 1997, **38**, 1103.

388. D.Noteberg, J.Branalt, I.Kvarnstrom, B.Classon, B.Samuelsson, U.Nillroth, U.H.Danielson, A.Karlen, and A.Hallberg, *Tetrahedron*, 1997, **53**, 7975.
389. M.Fernandez-Suarez, L.Munoz, R.Fernandez, and R.Riguera, *Tetrahedron: Asymmetry*, 1997, **8**, 1847.
390. I.A.O'Neil, C.L.Murray, A.J.Potter, and S.B.Kalindjian, *Tetrahedron Lett*., 1997, **38**, 3609.
391. A.Leggio, A.Liguori, A.Procopio, and G.Sindona, *J.Chem.Soc., Perkin Trans.I*, 1997, 1969; E.P.Ellmerer-Mueller, D.Broessner, N.Maslouh, and A.Tako, *Helv.Chim. Acta*, 1998, **81**, 59.
392. R.E.Marti, K.H.Bleicher, and K.W.Bair, *Tetrahedron Lett*., 1997, **38**, 6145.
393. D.Seebach and J.L. Matthews, *Chem.Commun*., 1997, 2015.
394. E.Byrgesen, J.Nielsen, M.Willert, and M.Bols, *Tetrahedron Lett*., 1997, **38**, 5697.
395. P.E.Thompson and M.T.W.Hearn, *Tetrahedron Lett*., 1997, **38**, 2907.
396. S.Singh and G.P.Basmadian, *Tetrahedron Lett*., 1997, **38**, 6829.
397. N.P.Pavri and M.L.Trudell, *Tetrahedron Lett*., 1997, **38**, 7993.
398. M.Xiao, X.Wu, B.Liang, Y.Muramatsu, S.Matsumoto, Y.Hasegawa, and Z.Li, *Huaxue Shiji*, 1996, **18**, 324.
399. L.Bischoff, C.David, L.Martin, H.Meudal, B.-P.Roques, and M.-C.Fournie-Zaluski, *J.Org.Chem*., 1997, **62**, 4848.
400. J.Rudolph, P.C.Sennhenn, C.P.Vlaar, and K.B.Sharpless, *Angew.Chem.Int.Ed*., 1996, **35**, 2810.
401. T.T.Upadhya and A.Sudalai, *Tetrahedron: Asymmetry*, 1997, **8**, 3685.
402. G.Li, H.H.Argent, and K.B.Sharpless, *Angew.Chem.Int.Ed*., 1996, **35**, 2813.
403. X.Teng, A.Kasatkin, Y.Kawanaka, S.Okamoto, and F.Sato, *Tetrahedron Lett*., 1997, **38**, 8977.
404. F.A.Davis, G.V.Reddy, and C.-H.Liang, *Tetrahedron Lett*., 1997, **38**, 5139.
405. G.Moyna, H.J.Williams, and A.I.Scott, *Synth.Commun*., 1997, **27**, 1561.
406. F.M.Cordero, F.Machetti, F.De Sarlo, and A.Brandi, *Gazz.Chim.Ital*., 1997, **127**, 25.
407. M.Diaz and R.M.Ortuno, *Tetrahedron: Asymmetry*, 1996, **7**, 3465.
408. B.M.Adger, J.V.Barkley, S.Bergeron, M.W.Cappi, B.E.Flowerdew, M.P.Jackson, R.McCague, T.C.Nugent, and S.M.Roberts, *J.Chem.Soc., Perkin Trans.I*, 1997, 3501.
409. H.Sugimura, M.Miura, and N.Yamada, *Tetrahedron: Asymmetry*, 1997, **8**, 4089.
410. T.Bach and J.Schroder, *Liebigs Ann./Recl*., 1997, 2265; for stereochemical aspects see S.A.Fleming and J.J.Gao, *Tetrahedron Lett*., 1997, **38**, 5407.
411. J.Jang, Y.Lee, and Y.Ahn, *Bull.Korean Chem.Soc*., 1997, **18**, 254 (*Chem.Abs*., 1997, **126**, 305759).
412. F.D'Aniello, A.Mann, A.Schoenfelder, and M.Taddei, *Tetrahedron*, 1997, **53**, 1447.
413. J.S.Panek and T.Hu, *J.Org.Chem*., 1997, **62**, 4914.
414. R.V.Hoffman and J.Tao, *J.Org.Chem*., 1997, **62**, 2292.
415. C.Gennari, D.Moresca, A.Vulpetti, and G.Pain, *Tetrahedron*, 1997, **53**, 5593.
416. N.Piveteau, P.Audin, and J.Paris, *Synlett*., 1997, 1269.
417. G.Veeresha and A.Datta, *Tetrahedron Lett*., 1997, **38**, 5223.
418. C.E.Song, J.K.Lee, I.O.Kim, and J.H.Choi, *Synth.Commun*., 1997, **27**, 1009.
419. M.M.Kabat, A.R.Daniewski, and W.Burger, *Tetrahedron: Asymmetry*, 1997, **8**, 2663.
420. J.M.Obon, U.R.Maiquez, M.Canovas, H.-P.Kleber, and J.L.Ibarra, *Enzyme Microb. Technol*., 1997, **21**, 531.
421. F.Coelho, M.B.M.De Azevedo, R.Boschiero, and P.Resende, *Synth.Commun*., 1997, **27**, 2455.

422. C.Mazzini, J.Lebreton, V.Alphand, and R.Furstoss, *Tetrahedron Lett*., 1997, **38**, 1195.
423. S.E.Denmark, A.R.Hurd, and H.J.Sacha, *J.Org.Chem*., 1997, **62**, 1668.
424. M.Alcon, M.Poch, A.Moyano, M.A.Pericas, and A.Riera, *Tetrahedron: Asymmetry*, 1997, **8**, 2967.
425. R.A.Goodnow, A.-R.Richou, and S.Tam, *Tetrahedron Lett*., 1997, **38**, 3195.
426. D.Xiao, P.J.Carroll, S.C.Mayer, A.J.Pfizenmayer, and M.M.Joullie, *Tetrahedron: Asymmetry*, 1997, **8**, 3043.
427. S.Hanessian and R.Schaum, *Tetrahedron Lett*., 1997, **38**, 163.
428. M.Smrcina, P.Majer, E.Majerova, T.A.Guerassina, and M.A.Eissenstadt, *Tetrahedron*, 1997, **53**, 12867.
429. P.M.Kauppinen and A.M.P.Koskinen, *Tetrahedron Lett*., 1997, **38**, 3103.
430. J.-N.Denis, S.Tchertchian, A.Tomassini, and Y.Vallee, *Tetrahedron Lett*., 1997, **38**, 5503.
431. F.Ruebsam, A.M.Evers, C.Michel, and A.Giannis, *Tetrahedron*, 1997, **53**, 1707.
432. K.Uneyama, T.Yanagiguchi, and H.Asai, *Tetrahedron Lett*., 1997, **38**, 7763.
433. H.Bekele, C.L.Nesloney, K.W.McWilliams, N.M.Zacharias, P.Chitnumsub, and J.W.Kelly, *J.Org.Chem*., 1997, **62**, 2259.
434. J.Gervay, T.M.Flaherty, and C.Nguyen, *Tetrahedron Lett*., 1997, **38**, 1493.
435. V.Bouchez, I.Stasik, D.Beaupere, and R.Uzan, *Tetrahedron Lett*., 1997, **38**, 7733.
436. B.Peschke, K.Madsen, B.S.Hansen, and N.L.Johansen, *Bioorg.Med.Chem.Lett*., 1997, **7**, 1969.
437. S.Hanessian, G.McNaughton-Smith, H.-G.Lombart, and W.D.Lubell, *Tetrahedron*, 1997, **53**, 12789.
438. C.E.Masse, B.S.Knight, P.Stavropoulos, and J.S.Panek, *J.Am.Chem.Soc*., 1997, **119**, 6040.
439. R.V.Hoffman and J.Tao, *Tetrahedron*, 1997, **53**, 7119; J.Tao and R.V.Hoffman, *J.Org.Chem*., 1997, **62**, 6240.
440. H.Tamamura, *Chem.Commun.*, 1997, 2327.
441. A.Rottmann and J.Liebscher, *Tetrahedron: Asymmetry*, 1997, **8**, 2433.
442. J.Wang and A.I.Scott, *Tetrahedron Lett*., 1997, **38**, 739; erratum, *ibid*., p. 2587.
443. R.A.Bunce, C.L.Schilling, and M.Rivera, *J.Labelled Compd.Radiopharm*., 1997, **39**, 669.
444. K.Iida, Y.Takao, T.Ogai, and M.Kajiwara, *J.Labelled Compd.Radiopharm*., 1997, **39**, 797.
445. K.-H.Altmann, C.S.Chiesi, and C.Garcia-Echeverria, *Bioorg.Med.Chem.Lett*., 1997, **7**, 1119; C.Garcia-Echeverria, D.Husken, C.S.Chiesi, and K.-H.Altmann, *ibid*., p.1123; see also M.Fujii, K.Yamamoto, J.Hidaka, and T.Ohtsu, *Tetrahedron Lett*., 1997, **38**, 417.
446. M.Cantin, R.Schutz, and C.J.Leumann, *Tetrahedron Lett*., 1997, **38**, 4211.
447. C.A.Evans, L.Bernier, J.Dugas, and T.S.Mansour, *Tetrahedron Lett*., 1997, **38**, 7657.
448. T.Shiraiwa, A.Ohta, H.Miyazaki, Y.Gogun, and H.Kurokawa, *Chirality*, 1997, **9**, 386.
449. T.Yabuchi, T.Ooi, and T.Kusumi, *Chirality*, 1997, **9**, 550.
450. F.Toda and K.Tanaka, *Chem.Commun*., 1997, 1087.
451. B.B.De and N.R.Thomas, *Tetrahedron: Asymmetry*, 1997, **8**, 2687.
452. T.Shiraiwa, *Bunri Gijutsu*, 1996, **26**, 346.
453. T.Shiraiwa, H.Miyazaki, T.Watanabe, and H.Kurokawa, *Chirality*, 1997, **9**, 48.
454. T.Shiraiwa, H.Miyazaki, A.Ohta, K.Motonaka, E.Kobayashi, M.Kubo, and H.Kurokawa, *Chirality*, 1997, **9**, 656.

455. T.Shiraiwa, H.Miyazaki, A.Ohta, Y.Waki, M.Yasuda, T.Morishita, and H.Kurokawa, *Chem.Pharm.Bull.*, 1996, **44**, 2322.
456. V.M.Sanchez, F.Rebelledo, and V.Gotor, *Tetrahedron: Asymmetry*, 1997, **8**, 37.
457. N.W.Fadnavis and V.Jadhav, *Tetrahedron: Asymmetry*, 1997, **8**, 2361.
458. L.E.Janes and R.J.Kazlauskas, *Tetrahedron: Asymmetry*, 1997, **8**, 3719.
459. T.Miyazawa, H.Minowa, T.Miyamoto, K.Imagawa, R.Yanagihara, and T.Yamada, *Tetrahedron: Asymmetry*, 1997, **8**, 367.
460. V.I.Tararov, Y.N.Belokon, A.Singh, and V.S.Parmar, *Tetrahedron: Asymmetry*, 1997, **8**, 33.
461. G.J.Lye, *Biotechnol.Tech.*, 1997, **11**, 611.
462. L.J.Liu, P.Yang, and R.X.Zhuo, *Chin.Chem.Lett.*, 1997, **8**, 413.
463. J.D.Brennan, C.W.V.Hogue, B.Rajendran, K.J.Willis, and A.G.Szabo, *Anal.Biochem.*, 1997, **252**, 260.
464. A.Tanaka, H.Yamanaka, and T.Kawamoto, *Ann.N.Y.Acad.Sci.*, 1996, **799**, 762.
465. A.S.Bommarius, K.Drauz, K.Gunther, G.Knaup, and M.Schwarm, *Tetrahedron: Asymmetry*, 1997, **8**, 3197.
466. K.Drauz, *Chimia*, 1997, **51**, 310.
467. Y.Tsuji, H.Yamanaka, T.Fukui, T.Kawamoto, and A.Tanaka, *Appl.Microbiol.Biotechnol.*, 1997, **47**, 114; H.Yamanaka, T.Kawamoto, and A.Tanaka, *J.Ferment.-Bioeng.*, 1997, **84**, 181.
468. M.J.Garcia and R.Azerad, *Tetrahedron: Asymmetry*, 1997, **8**, 85.
469. U.Stelkes-Ritter, G.Beckers, A.S.Bommarius, K.Drauz, K.Gunther, M.Schwarm, and M.-R.Kula, *Biocatal.Transform.*, 1997, **15**, 205.
470. E.Eichhorn, J.P.Roduit, N.Shaw, K.Heinzmann, and A.Kiener, *Tetrahedron: Asymmetry*, 1997, **8**, 2533.
471. B.Kaptein, T.J.G.M.Van Dooren, W.H.J.Boesten, T.Sonke, A.L.L.Duchateau, Q.B.Broxterman, and J.Kamphuis, *Org.Process.Res.Dev.*, 1998, **2**, 10.
472. M.Fite, M.Capellas, M.D.Benaiges, G.Caminal, P.Clapes, and G.Alvaro, *Biocatal.-Biotransform.*, 1997, 14, 317.
473. O.S.Tee, T.A.Gadosy, and J.B.Giorgi, *Can.J.Chem.*, 1997, **75**, 83.
474. M.-C.Chung, H.-J.Lee, C.-H.Lee, H.-K.Chun, and Y.-H.Kho, *J.Microbiol.Biotechnol*, 1997, **7**, 329.
475. A.Avdagic and V.Sunjic, *Helv.Chim.Acta*, 1998, **81**, 85.
476. F.Tanaka, K.Kinoshita, R.Tanimura, and J.Fujii, *Chemtracts*, 1997, **10**, 1039.
477. E.Takahashi, M.Furui, H.Seko, and T.Shibatani, *Appl.Microbiol.Biotechnol.*, 1997, **47**, 173.
478. E.Takahashi, M.Furui, and T.Shibatani, *Biotechnol.Lett.*, 1997, **19**, 245; E. Takahashi, M.Furui, H.Seko, and T.Shibatani, *Appl.Microbiol.Biotechnol.*, 1997, **47**, 347.
479. H.Stecher and K.Faber, *Synthesis*, 1997, 1.
480. E.R.Francotte, in Chiral Separations, Ed. S.Ahuja, American Chemical Society, Washington, 1997, p. 271; homochiral chromatographic media: A.Peter and G.Toth, *Anal.Chim.Acta*, 1997, **352**, 335; chiral membranes: S.Tone, *Kagaku Kogaku*, 1998, **62**, 93 (*Chem.Abs.*, 1998, **128**, 115205).
481. S.Yuasa, T.Fukuhara, M.Isoyama, M.Tanaka, and A.Shimada, *Biomed.Chromatogr.*, 1997, **11**, 276.
482. H.Hofstetter, O.Hofstetter, and V.Schurig, *J.Chromatogr.A*, 1997, **764**, 35.
483. B.B.Lakshmi and C.R.Martin, *Nature*, 1997, **388**, 758.
484. S.Poncet, J.Randon, and J.L.Rocca, *Sep.Sci.Technol.*, 1997, **32**, 2029.
485. M.H.Hyun and C.S.Min, *Tetrahedron Lett.*, 1997, **38**, 1943.

486. T.Kakuchi, T.Satoh, H.Kanai, S.Umeda, T.Hatakeyama, and K.Yokota, *Enantiomer*, 1997, **2**, 273.
487. B.Polak and W.Golkiewicz, *Chem.Anal.*, 1996, **41**, 931.
488. J.Izumi, M.Yoshikawa, and T.Kitao, *Maku*, 1997, **22**, 149 (*Chem.Abs.*, 1997, **127**, 66125).
489. R.J.Ansell and K.Mosbach, *J.Chromatogr., A*, 1997, **787**, 55.
490. J.M.Lin, T.Nakagama, X.Z.Wu, K.Uchiyama, and T.Hobo, *Fresenius' J.Anal. Chem.*, 1997, **357**, 130.
491. C.J.Allender, K.R.Brain, and C.M.Heard, *Chirality*, 1997, **9**, 233.
492. C.Yu, O.Ramstrom, and K.Mosbach, *Anal.Lett.*, 1997, **30**, 2123.
493. L.I.Andersson, I.A.Nicholls, and K.Mosbach, *Adv.Mol.Cell.Biol.*, 1996, **15B**, 651; I.A.Nicholls, *Ibid.*, 671.
494. S.Vidyasankar, M.Ru, and F.H.Arnold, *J.Chromatogr.A*, 1997, **775**, 51.
495. M.Yoshikawa, J.-I.Izumi, and T.Kitao, *Polym.J.*, 1997, **29**, 205; M.Yoshikawa, J.-I.Izumi, T.Kitao, and S.Sakamoto, *Macromol.Rapid Commun.*, 1997, **18**, 761.
496. T.Aoki, M.Onshima, K.Shinohara, T.Kaneko, and E.Oikawa, *Polymer*, 1997, **38**, 235.
497. K.Inoue, A.Miyahara, and T.Itaya, *J.Am.Chem.Soc.*, 1997, **119**, 6191.
498. P.J.Pickering and J.B.Chaudhuri, *J.Membr.Sci.*, 1997, **127**, 115.
499. K.-H.Kellner, A.Blasch, H.Chmeil, M.Lammerhofer, and W.Lindner, *Chirality*, 1997, **9**, 268.
500. V.A.Avetisov and V.I.Goldanskii, *Usp.Fiz.Nauk*, 1996, **168**, 873; *Ross.Khim.Zh.*, 1996, **40**, 131.
501. R.Popa, *J.Mol.Evol.*, 1997, **44**, 121; W.Wang, *Beijing Daxue Xuebao, Ziran Kexueban*, 1997, **33**, 265 (*Chem.Abs.*, 1998, **127**, 25896).
502. Astronomical and Biochemical Origins: Search for Life in the Universe, eds. C.B.Cosmovici, S.Bowyer, and D.Wertheimer, Editrice Compositori, Bologna, Italy, 1997 (*e.g.*, *Chem.Abs.*, 1998, **127**, 274412, 316014).
503. M.Hiroi and M.Sato, *Kobe Shosen Daigaku Kiyo, Dai-z-rui: Shoken, Rikogakuhen*, 1997, **45**, 51 (*Chem.Abs.*, 1998, **128**, 23114).
504. I.Weissbuch, M.Berfeld, W.Bouwman, K.Kjaer, J.Als-Nielsen, M.Lahav, and L.Lieserowitz, *J.Am.Chem.Soc.*, 1997, **119**, 933.
505. S.Miki, *Kagaku*, 1997, **52**, 70.
506. G.Smith, K.E.Baldry, C.H.L.Kennard, and K.A.Byriel, *Aust.J.Chem.*, 1997, **50**, 737.
507. N.Srinivasan and R.K.Rajaram, *Z.Kristallogr.*, 1997, **212**, 311.
508. Z.Cheng, Y.Cheng, L.Guo, and D.Xu, *Z.Kristallogr.*, 1997, **212**, 221.
509. J.Janczak, D.Zobel, and P.Luger, *Acta Crystallogr., Sect.C: Cryst.Struct.Commun.*, 1997, **C53**, 1901.
510. J.Janczak and P.Luger, *Acta Crystallogr., Sect.C: Cryst.Struct.Commun.*, 1997, **C53**, 1954.
511. R.A.Toscano, R.G.Enriquez, W.F.Reynolds, G.A.Magos, and D.Guecco, *Acta Crystallogr., Sect.C: Cryst.Struct.Commun.*, 1997, **C53**, 1690.
512. J.Venkatraman, M.M.Prabu, and M.Vijayan, *J.Pept.Res.*, 1997, **50**, 77.
513. W.Yang, T.Jin, and S.Zhang, *Beijing Daxue Xuebao, Ziran Kexueban*, 1996, **32**, 413 (*Chem.Abs.*, 1997, **126**, 212400).
514. K.Okamura, K.-I.Aoe, H.Hiramatsu, N.Nishimura, T.Sato, and K.Hashimoto, *Anal.Sci.*, 1997, **13**, 355.
515. T.Kawakita, *ACS Symp.Ser.*, **667**(Separation and Purification by Crystallization), p.111.
516. T.Suga, C.Inubashi, and N.Okabe, *Acta Crystallogr., Sect.C: Cryst.Struct.Commun.*, 1998, **C54**, 83.

517. M.M.Prabu, H.G.Nagendra, S.Suresh, and M.Vijayan, *J.Biomol.Struct.Dyn.*, 1996, **14**, 387.
518. C.Wilson, J.A.K.Howard, D.E.Jane, D.C.Sunter, and J.C.Watkins, *Acta Crystallogr., Sect.C: Cryst.Struct.Commun.*, 1997, **C53**, 909.
519. A.D.Abell, R.A.Edwards, and M.D.Oldham, *J.Chem.Soc., Perkin Trans. I*, 1997, 1655.
520. M.Kubicki, H.A.R.Bassyouni, and P.W.Codding, *Acta Crystallogr., Sect.C: Cryst.-Struct.Commun.*, 1997, **C53**, 739.
521. C.Toniolo, M.Crisma, and F.Formaggio, *Biopolymers*, 1997, **40**, 627.
522. A.Privett, T.Barclay, and W.Cordes, *J.Chem.Crystallogr.*, 1997, **27**, 45.
523. Y.Wang, P.S.Belton, H.Tang, N.Wellner, S.C.Davies, and D.L.Hughes, *J.Chem. Soc., Perkin Trans.II*, 1997, 899.
524. R.Puluti, C.De Sena, and C.Giancola, *J.Therm.Anal.*, 1997, **48**, 1249.
525. A.Dobson and R.E.Gerkin, *Acta Crystallogr., Sect.C: Cryst.Struct.Commun.*, 1997, **C53**, 73.
526. B.Schade, J.-H.Fuhrhop, V.Hubert, M.Weber, and P.Luger, *Acta Crystallogr., Sect.C: Cryst.Struct.Commun.*, 1997, **C53**, 1070.
527. A.R.Ofial, S.Q.Dou, V.G.Krishnan, H.Paulus, H.Fuess, and A.Weiss, *Z.Naturforsch., A: Phys.Sci.*, 1997, **52**, 249.
528. T.Y.Sliva, A.M.Duda, V.M.Amirkhanov, I.Fritski, T.Glowiak, and H.Kozlowski, *J.Inorg.Biochem.*, 1997, **65**, 67.
529. L.Van Meervelt, C.Bruyneel, H.Morisse, and T.Zeegers-Huyskens, *J.Phys.Org.-Chem.*, 1997, **10**, 825.
530. M.Crisma, G.Valle, A.Polese, F.Formaggio, C.Toniolo, and J.Kamphuis, *Z.Kristallogr., New Cryst.Struct.*, 1997, **212**, 113.
531. M.Crisma, G.Valle, F.Formaggio, C.Toniolo, and A.Bagno, *J.Am.Chem.Soc.*, 1997, **119**, 4136.
532. M.Crisma, F.Formaggio, G.Valle, C.Toniolo, M.Saviano, R.Iacovino, L.Zaccaro, and E.Benedetti, *Biopolymers*, 1997, **42**, 1.
533. H.Kanazawa, H.Uekusa, and Y.Ohashi, *Acta Crystallogr., Sect.C: Cryst.Struct.-Commun.*, 1997, **C53**, 1154.
534. E.Benedetti, R.Iacovino, C.Pedone, F.Rossi, M.Saviano, C.Isernia, D.Montesarchio, L.De Napoli, and G.Piaccalli, *Lett.Pept.Sci.*, 1997, **4**, 129.
535. R.Carballo, M.Fernandez-Suarez, L.Munoz, R.Rignera, and C.Maichle-Mossmer, *Acta Crystallogr., Sect.C: Cryst.Struct.Commun.*, 1997, **C53**, 1312.
536. A.H.Wilman and P.S.Allen, *J.Magn.Reson., Ser.B*, 1996, **113**, 203.
537. R.W.Prost, L.Mark, M.Mewissen, and S.-J.Li, *Magn.Reson.Med.*, 1997, **37**, 615.
538. V.F.Wendisch, A.A.de Graaf, and H.Salim, *Anal.Biochem.*, 1997, **245**, 196.
539. A.Rios and J.P.Richard, *J.Am.Chem.Soc.*, 1997, **119**, 8375.
540. N.Todeschi, J.Gharbi-Benarous, V.Arulmozhi, F.Acher, R.Azerad, and J.-P. Girault, *J.Chem.Inf.Comput.Sci.*, 1997, **37**, 372.
541. N.Todeschi and J.Gharbi-Benarous, *Bioorg.Med.Chem.*, 1997, **5**, 1943.
542. N.Evrard-Todeschi, J.Gharbi-Benarous, A.Cosse-Barbi, G.Thirot, and J.-P.Girault, *J.Chem.Soc., Perkin Trans.II*, 1997, 2677.
543. G.Cerichelli, L.Luchetti, and G.Mancini, *Langmuir*, 1997, **13**, 4767.
544. S.G.Grdadolnik and B.Stanovik, *Magn.Reson.Chem.*, 1997, **35**, 482.
545. Y.Kuroda, H.Ueda, H.Nozawa, and H.Ogoshi, *Tetrahedron Lett.*, 1997, **38**, 7901.
546. T.Ozawa, Y.Isoda, H.Watanabe, T.Yuzuri, H.Suezawa, K.Sakakibara, and M.Hirota, *Magn.Reson.Chem.*, 1997, **35**, 323.
547. A.Ramamoorthy, C.H.Wu, and S.J.Opella, *J.Am.Chem.Soc.*, 1997, **119**, 10479.

548. G.Zheng, L.Wang, J.Hu, X.Zhang, L.Shen, C.Ye, and G.A.Webb, *Magn. Reson.Chem*., 1997, **35**, 606; for L-tryptophan, see G.Zheng, J.Hu, X.Zhang, L.Shen, and C.Ye, *Huaxue Xuebao*, 1997, **55**, 729 (*Chem.Abs*., 1998, **127**, 248386).
549. P.S.Belton and Y.C.Wang, *Mol.Phys*., 1997, **90**, 119.
550. Y.C.Wang, P.S.Belton, and H.R.Tang, *Chem.Phys.Lett*., 1997, **268**, 387.
551. R.H.Havlin, H.Le, D.D.Laws, A.C.de Dios, and E.Oldfield, *J.Am.Chem.Soc*., 1997, **119**, 11951.
552. M.J.Shapiro, J.Chin, R.E.Marti, and M.A.Jarosinski, *Tetrahedron Lett*., 1997, **38**, 1333.
553. M.Jakubcova, A.Meddour, J.-M.Pechine, A.Baklouti, and J.Courtiell, *J.Fluorine Chem*., 1997, **86**, 149.
554. H.Ikeda, M.Nakamura, N.Ise, F.Toda, and A.Ueno, *J.Org.Chem*., 1997, **62**, 1411.
555. S.E.Weinstein, M.S.Vining, and T.J.Wenzel, *Magn.Reson.Chem*., 1997, **35**, 273.
556. M.M.El-Abadelah, M.Z.Nazer, S.S.Sabri, S.M.Khalil, W.Voelter, and M.Geiger, *Z.Naturforsch., B: Chem.Sci*., 1997, **52**, 419.
557. E.Yashima, T.Matsushima, and Y.Okamoto, *J.Am.Chem.Soc*., 1997, **119**, 6345.
558. H.Tamiaki, N.Matsumoto, and H.Tsukube, *Tetrahedron Lett*., 1997, **38**, 4239.
559. V.D.Chivanov, S.A.Aksenov, V.L.Eremenko, I.A.Eremenko, L.I.Grebenik, A.K.Mishnev, S.V.Chivanova, and A.V.Belovol, *Zh.Fiz.Khim*., 1996, **70**, 2154.
560. K.Vekey and G.Czira, *Anal.Chem*., 1997, **69**, 1700.
561. H.Lavanant and Y.Hoppilliard, *J.Mass Spectrom*., 1997, **32**, 1037.
562. Q.Zhan, S.J.Wright, and R.Zenobi, *J.Am.Soc.Mass Spectrom*., 1997, **8**, 525.
563. W.D.Price, R.A.Jockusch, and E.R.Williams, *J.Am.Chem.Soc*., 197, **119**, 11988.
564. Y.She, X.Xiong, Y.Zhou, and S.Liu, *Wuli Huaxue Xuebao*, 1997, **13**, 252 (*Chem.Abs*., 1997, **126**, 212401).
565. B.A.Mansoori, D.A.Volmer, and R.K.Boyd, *Rapid Commun.Mass Spectrom*., 1997, **11**, 1120.
566. A.G.Harrison and T.Yalcin, *Int.J.Mass Spectrom., Ion Processes*, 1997, **165/166**, 339.
567. T.Yalcin, J.Wang, D.Wen, and A.G.Harrison, *J.Am.Soc.Mass Spectrom*., 1997, **8**, 749.
568. M.A.Freitas, R.A.J.O'Hair, and T.D.Williams, *J.Org.Chem*., 1997, **62**, 6112.
569. J.Zhou, S.Hefta, and T.D.Lee, *J.Am.Soc.Mass Spectrom*., 1997, **8**, 1165.
570. X.L.Li and E.de Hoffmann, *J.Am.Soc.Mass Spectrom*., 1997, **8**, 1078.
571. W.B.Fischer and H.-H.Eysel, *J.Mol.Struct*., 197, **415**, 249.
572. K.De Wael and T.Zeegers-Huyskens, *Biopolymers*, 1997, **41**, 205; see also Ref. 529.
573. A.A.Ivanov, E.V.Korolik, R.G.Zhbankov, G.K.Ilych, and N.I.Insarova, in Spectroscopy of Biological Molecules: Modern Trends, Eds P.Carmona, R.Navarro, and A.Hernanz, Kluwer, Dordrecht, 1997, p.19.
574. S.G.Stepanian, I.D.Reva, E.D.Radchenko, M.T.S.Rosado, M.L.T.S.Duarte, R.Fausto, and L.Adamowicz, *J.Phys.Chem.A*, 1998, **102**, 1041.
575. M.T.S.Rosado, M.L.T.S.Duarte, and R.Fausto, *J.Mol.Struct*., 1997, **410**, 343.
576. H.-W.Li, G.-S.Yu, and H.L.Strauss, *J.Phys.Chem.B*, 1998, **102**, 298.
577. M.A.Broda, B.Rzeszotarska, L.Smelka, and M.Rospenk, *J.Pept.Res*., 1997, **50**, 342.
578. Z.S.Klemenskova, V.S.Romanova, V.A.Tsyryapkin, E.F.Kuleshova, Z.Parnes, B.V.Lokshin, and M.E.Volpin, *Vestsi Akad.Navuk Belarusi, Ser.Khim.Navuk*, 1997, 48 (*Chem.Abs*., 1998, **128**, 89099).
579. G.Seiler and R.Schweitzer-Stenner, *J.Am.Chem.Soc*., 1997, **119**, 1720.
580. Y.Yao, W.H.Nelson, P.Hargraves, and J.Zhang, *Appl.Spectrosc*., 1997, **51**, 785.
581. M.Ide, Y.Maeda, and H.Kitano, *J.Phys.Chem.B*, 1997, **101**, 7022.

582. F.Ota, S.Higuchi, Y.Gohshi, K.Furuya, M.Bau, and M.Kyoto, *J.Raman Spectrosc.*, 1997, **28**, 849.
583. J.L.Castro, S.Sanchez-Cortes, J.V.Garcia Ramos, J.C.Otero, and J.I.Marcos, *Biospectroscopy*, 1997, **3**, 449.
584. A.Pawlukojc, K.Bajdor, J.C.Dobrowolski, J.Leciejewicz, and I.Natkaniec, *Spectrochim.Acta, Part A*, 1997, **53A**, 927.
585. M.M.Vorobev, *Z.Naturforsch., C: Biosci.*, 1997, **52**, 227.
586. R.Hulsebosch, J.S.van der Brink, S.A.M.Nieuwenhuis, P.Gast, J.Raap, J.Lugtenburg, and A.J.Hoff, *J.Am.Chem.Soc.*, 1997, **119**, 8685.
587. N.A.Salih, A.Sanderud, E.Sagstuen, O.I.Eid, and A.Lund, *J.Phys.Chem, A*, 1997, **101**, 8214.
588. E.Sagstuen, E.O.Hole, S.R.Haugedal, and W.H.Nelson, *J.Phys.Chem, A*, 1997, **101**, 9763.
589. S.Olsson, E.Lund, and R.Erickson, *Appl.Radiat.Isot.*, 1996, **47**, 1211.
590. G.Castronuovo, V.Elia, and F.Velleca, *J.Chem.Soc., Faraday Trans.*, 1996, **92**, 4215.
591. M.A.Gallardo, T.H.Lilley, H.Lindsell, Y.Lu, S.Otin, and A.J.Ward, *J.Chem.Soc., Faraday Trans.*, 1996, **92**, 4983.
592. J.Fernandez, M.N.Garcia-Lisbona, T.H.Lilley, and H.Lindsell, *J.Chem.Soc., Faraday Trans.*, 1997, **93**, 407.
593. M.K.Koshkbarchi and J.H.Vera, *Ind.Eng.Chem.Res.*, 1997, **36**, 2445.
594. M.K.Koshkbarchi, A.M.Soto-Campos, and J.H.Vera, *J.Solution Chem.*, 1997, **26**, 941.
595. A.M.Soto-Campos, M.K.Koshkbarchi and J.H.Vera, *J.Chem.Thermodyn.*, 1997, **29**, 609.
596. A.M.Soto-Campos, M.K.Koshkbarchi and J.H.Vera, *Biophys.Chem.*, 1997, **67**, 97.
597. J.P.Amend and H.C.Helgeson, *Pure Appl.Chem.*, 1997, **69**, 935.
598. G.I.Timofeeva, E.F.Kuleshova, and V.S.Romanova, *Izv.Akad.Nauk, Ser.Khim.*, 1997, **46**, 472.
599. M.P.Lokhande, S.Mazundar, and S.C.Mehrotra, *Indian J.Biochem.Biophys.*, 1997, **34**, 385.
600. P.H.Rasmussen, B.Jorgensen, and J.Nielsen, *Thermochim.Acta*, 1997, **303**, 23.
601. M.M.Vorobov and A.N.Danilenko, *Izv.Akad.Nauk, Ser.Khim.*, 1996, 2237.
602. H.Kuramochi, H.Noritomi, D.Hoshino, and K.Nagahama, *J.Chem.Eng.Data*, 1997, **42**, 470.
603. M.van Berlo, M.T.Gude, L.A.M.van der Wielen, and K.C.A.M.Luyben, *Ind.Eng.-Chem.Res.*, 1997, **36**, 2474.
604. F.Zhou, K.Kakisaka, T.Ishidao, Y.Iwai, Y.Arai, and T.Furuya, *J.Chem.Eng.Jpn.*, 1997, **30**, 349.
605. Q.-H.Shi, Y.Sun, L.Liu, and S.Bai, *Sep.Sci.Technol.*, 1997, **32**, 2051.
606. M.Rivail da Silva, R.Munos Olivas, O.F.X.Donard, and M.Lamotte, *Appl.Organomet.Chem.*, 1997, **11**, 21.
607. K.Majumdar and S.C.Lahiri, *J.Indian Chem.Soc.*, 1997, **74**, 382.
608. D.Kumar, *J.Indian Chem.Soc.*, 1997, **74**, 610.
609. J.J.Wang, Z.N.Yau, W.B.Liu, and J.S.Lu, *Z.Phys.Chem.*, 1997, **199**, 25.
610. M.Mizuguchi, M.Sakurai, and K.Nitta, *J.Solution Chem.*, 1997, **26**, 579.
611. K.Toko and T.Fukusaka, *Sens.Mater.*, 1997, **9**, 171.
612. H.O.Davies and R.D.Gillard, *Polyhedron*, 1997, **16**, 2145.
613. F.Sanda, M.Nakamura, and T.Endo, *Chem.Lett.*, 1997, 175.
614. I.V.Baskakov and V.L.Voeikov, *Biofizika*, 1995, **40**, 1141.
615. V.L.Voeikov and I.V.Baskakov, *Biofizika*, 1995, **40**, 1150.

616. D.Samuel, T.K.S.Kumar, G.Jayaraman, P.W.Yang, and C.Yu, *Biochem.Mol.Biol.Int.*, 1997, **41**, 235.
617. X.H.Li, R.Wang, and Y.F.Zhao, *Chin.Chem.Lett.*, 1997, **8**, 299 (*Chem.Abs*, 1998, **127**, 91794).
618. X.H.Li and Y.F.Zhao, *Chin.Chem.Lett.*, 1997, **8**, 611 (*Chem.Abs*, 1997, **127**, 230939).
619. M.Matsumoto, H.Yajima, Y.Tezuka, T.Ishii, K.Takahashi, and A.Asai, *Chem.Lett.*, 1997, 193.
620. T.Asahi, M.Takahashi, and J.Kobayashi, *Acta Crystallogr., Sect.A.*, 1997, **A53**, 263.
621. D.D.Frey, *Chem.Eng.Sci.*, 1997, **52**, 1227.
622. G.M.S.El Shafei and C.A.Philip, *J.Colloid Interface Sci.*, 1997, **185**, 140.
623. V.Dominguez, L.Guihard, and Y.Origent, *Chromatographia*, 1997, **44**, 240.
624. A.Garem, G.Daufin, J.L.Maubois, and J.Leonil, *Biotechnol.Bioeng.*, 1997, **54**, 291.
625. T.A.Munro and B.D.Smith, *Chem.Commun.*, 1997, 2167.
626. Z.I.Kuvaeva, L.A.Vodopyanova, and A.V.Mikulich, *Vestsi Akad.Navuk Belarusi, Ser.Khim.Navuk*, 1996, 17 (*Chem.Abs*, 1998, **127**, 34486).
627. P.Wieczorek, *J.Membr.Sci.*, 1997, **127**, 87.
628. P.Wieczorek, J.A.Joensson, and L.Mathiasson, *Anal.Chim.Acta*, 1997, **346**, 191; P.Wieczorek and M.Tomaszewska, *Solvent Extr.Ion Exch.*, 1997, **15**, 879.
629. T.Uragami, *Bunri Gijutsu*, 1996, **26**, 301 (*Chem.Abs*, 1997, **126**, 131014).
630. H.-J.Buschmann and L.Mutihac, *Rev.Roum.Chim.*, 1997, **42**, 121.
631. P.Rao, S.Liu, R.Zhang, G.Chen, Y.Zheng, and B.Shi, *Sepu*, 1997, **15**, 193 (*Chem.Abs.*, 1997, **127**, 132834).
632. S.A.Tweed, B.Loun, and D.S.Hage, *Anal.Chem.*, 1997, **69**, 4790.
633. H.Cheng and T.Shao, *Sepu*, 1997, **15**, 405 (*Chem.Abs.*, 1998, **128**, 1578).
634. D.Totrallardona and C.I.Harris, *J.Chromatogr.A*, 1997, **728**, 383.
635. M.Barboiu, C.Luca, G.Popescu, and L.Cot, *Roum.Biotechnol.Lett.*, 1996, **1**, 77.
636. L.Mutihac, N.Zarna, T.Constantinescu, and R.Mutihac, *Rev.Roum.Chim.*, 1997, **42**, 307.
637. H.Senderowitz, D.Q.McDonald, and W.C.Still, *J.Org.Chem.*, 1997, **62**, 9123.
638. I.Kolossvary, *J.Am.Chem.Soc.*, 1997, **119**, 10233.
639. K.-S.Jeong, J.H.Kim, and Y.P.Hong, *Bull.Korean Chem.Soc.*, 1996, **17**, 1178.
640. W.-J.He, G.-Y.Lu, and H.-W.Hu, *Gaodeng Xuexiao Huaxue Xuebao*, 1997, **18**, 1800 (*Chem.Abs.*, 1998, **128**, 3677).
641. R.J.Pieters, J.Cuntze, M.Bonnet, and F.Diederich, *J.Chem.Soc., Perkin Trans.II*, 1997, 1891.
642. J.L.Sessler, A.Andrievsky, V.Kral, and V.Lynch, *J.Am.Chem.Soc.*, 1997, **119**, 9385.
643. M.Barboiu, C.T.Supuran, A.Scozzafava, F.Briganti, C.Luca, G.Popescu, L.Cot, and N.Hovnanian, *Liebigs Ann./Recl.*, 1997, 1853.
644. Y.Liu, Y.-M.Zhang, A.-D.Qi, R.-T.Chen, K.Yamamoto, T.Wada, and Y.Inoue, *J.Org.Chem.*, 1997, **62**, 1826.
645. Y.Liu, B.Li, B.-H.Han, Y.-M.Li, and R.-T.Chen, *J.Chem.Soc., Perkin Trans. II*, 1997, 1275.
646. Y.Liu, B.-H.Han, A.-D.Qi, and R.-T.Chen, *Bioorg.Chem.*, 1997, **25**, 155; Y.Liu, A.-D.Qi, B.-H.Han, Y.-M.Li, Y.-M.Zhang, and R.-T.Chen, *Chin.Sci.Bull.*, 1997, **42**, 1189.
647. Y.Liu, Y.-M.Zhang, S.-X.Sun, Z.-H.Zhang, and R.-T.Chen, *Huaxue Xuebao*, 1997, **55**, 779 (*Chem.Abs.*, 1998, **127**, 319218).
648. Y.Liu, Y.-M.Zhang, S.-X.Sun, Y.-M.Li, and R.-T.Chen, *J.Chem.Soc., Perkin Trans. II*, 1997, 1609.
649. R.Jin and X.He, *Fenxi Kexue Xuebao*, 1997, **13**, 177 (*Chem.Abs.*, 1998, **127**, 263008).

650. R.Corradini, A.Dossena, G.Galaverna, R.Marchelli, A.Panagia, and G.Sartor, *J.Org.Chem*., 1997, **62**, 6283.
651. I.S.Antipin, I.I.Stoikov, E.M.Pinkhassik, N.A.Fitseva, I.Stibor, and A.I.Konovalov, *Tetrahedron Lett*., 1997, **38**, 5865.
652. R.K.Castellano, B.H.Kim, and J.Rebek, *J.Am.Chem.Soc*., 1997, **119**, 12671.
653. O.dos Santos, A.R.Lajmi, and J.W.Canary, *Tetrahedron Lett*., 1997, **38**, 4383.
654. T.Schrader, *Chem.-Eur.J*., 1997, **3**, 1537.
655. J.-Y.Zheng, K.Konishi, and T.Aida, *Tetrahedron*, 1997, **53**, 9115.
656. A.Perczel, O.Farkas, A.G.Csaszar, and I.G.Csizmadia, *Can.J.Chem*., 1997, **75**, 1120.
657. R.Buesnel, I.H.Hillier, and A.J.Masters, *Mol.Phys*., 1997, **90**, 787; H.M.Sulzbach, G.Vacek, P.R.Schreiner, J.M.Galbraith, P.von R.Schleyer, and H.R.Schaefer, *J.Comput.Chem*., 1997, **18**, 126.
658. C.Aleman, *J.Phys.Chem.B*, 1997, **101**, 5046.
659. S.Shirzian and S.Gronert, *Theochem*., 1997, **387**, 107.
660. C.Aleman and J.Puiggali, *J.Phys.Chem.B*, 1997, **101**, 3441.
661. K.Burgess and C.-Y.Ke, *J.Pept.Res*., 1997, **49**, 201.
662. C.Chipot and A.Pohorille, *J.Phys.Chem.B*, 1998, **102**, 281.
663. M.Ramek, A.-M.Kelterer, and S.Nikolic, *Int.J.Quantum Chem*., 1997, **65**, 1033.
664. A.D.Headley, *Theochem*, 1996, **370**, 147.
665. T.Watanabe, K.Hashimoto, H.Takase, and O.Kikuchi, *Theochem*, 1996, **397**, 113.
666. S.Sirois, E.I.Proynov, D.T.Nguyen, and D.R.Salahub, *J.Chem.Phys*., 1997, **107**, 6770; D.T.Nguyen, A.C.Scheiner, J.W.Andzelm, S.Sirois, D.R.Salahub, and A.T.Hagler, *J.Comput.Chem*., 1997, **18**, 1609.
667. O.Kikuchi, T.Watanabe, Y.Ogawa, H.Takase, and O.Takahashi, *J.Phys.Org.Chem*., 1997, **10**, 145.
668. G.Cativiela, J.I.Garcia, J.A.Mayoral, and L.Salvatella, *Theochem*, 1996, **368**, 57.
669. O.V.Kulikov and P.V.Lapshev, *Izv.Vyssh.Uchebn.Zaved., Khim.Khim.Tekhnol*., 1997, **40**, 53, 5
670. N.Okuyama-Yoshida, M.Nagaoka, and T.Yamabe, *J.Phys.Chem.A*, 1998, **102**, 285.
671. E.Uggerud, *Theor.Chem.Acc*., 1997, **97**, 313.
672. M.T.Rosado, M.L.T.S.Duarte, and R.Fausto, *Phosphorus, Sulfur, Silicon Relat.Elem*., 1996, **116**, 153.
673. S.B.Dixit, R.Bhasin, E.Rajasekaran, and B.Jayaram, *J.Chem.Soc., Faraday Trans*., 1997, **93**, 1105.
674. T.Head-Gordon, J.M.Sorenson, A.Pertsemlidis, and R.M.Glaeser, *Biophys.J*., 1997, **73**, 2106.
675. P.N.Day and R.Pachter, *J.Chem.Phys*., 1997, **107**, 2990.
676. E.Wadielewska, M.Witko, G.Stochel, and Z.Stasicka, *Chem.-Eur.J*., 1997, **3**, 609.
677. S.Hoyan and G.Ohanessian, *J.Am.Chem.Soc*., 1997, **119**, 2016.
678. J.T.L.Navarrete, J.Casado, V.Hernandez, and F.J.Ramirez, *J.Raman Spectrosc*., 1997, **28**, 501; *Theor.Chem.Acc*., 1997, **98**, 5.
679. J.T.L.Navarrete, V.Hernandez, and F.J.Ramirez, *J.Mol.Struct*., 1997, **410**, 353.
680. J.P.Amend and H.C.Helgeson, *J.Chem.Soc., Faraday Trans*., 1997, **93**, 1927.
681. J.Lazovic, *Iugosl.Physiol.Pharmacol.Acta*, 1997, 1997, **33**, 45.
682. A.A.Shevchenko and O.A.Kost, *Biokhimiya*, 1996, **61**, 2092.
683. T.Simonson, C.F.Wong, and A.T.Bruenger, *J.Phys.Chem.A*, 1997, **101**, 1935.
684. N.Rega, M.Cossi, and V.Barone, *J.Am.Chem.Soc*., 1997, **119**, 12962.
685. F.Himo, A.Graeslund, and L.A.Eriksson, *Biophys.J*., 1997, **72**, 1556.
686. S.E.Walden and R.A.Wheeler, *J.Am.Chem.Soc*., 1997, **119**, 3175.

687. P.E.M.Siegbahn, M.R.A.Blomberg, and R.H.Crabtree, *Theor.Chem.Acc.*, 1997, **97**, 289.
688. J.Csapo, Z.Csapo-Kiss, L.Wagner, T.Talos, T.G.Martin, S.Folestad, A.Tivesten, and S.Nemethy, *Anal.Chim.Acta*, 1997, **339**, 99.
689. G.A.Goodfriend, *Geochim.Cosmochim.Acta*, 1997, **61**, 1931.
690. B.J.Johnson and G.H.Miller, *Archaeometry*, 1997, **39**, 265.
691. N.Fujii, Y.Momose, Y.Ishibashi, T.Uemura, M.Takita, and M.Takehana, *Exp.Eye Res.*, 1997, **65**, 99.
692. I.Abe, H.Yanagi, and T.Nakahara, *J.High Resolut.Chromatogr.*, 1997, **20**, 451.
693. G.C.Barrett, in Chemistry and Biochemistry of the Amino Acids, ed.G.C.Barrett, Chapman and Hall, London and New York, 1985, p.354; G.C.Barrett, in Rodd's Chemistry of Carbon Compounds, Second Edition, Second Supplements, ed. M.Sainsbury, Volume ID, Elsevier, Amsterdam, 1993, p. 150.
694. J.M.Antelo, F.Arce, J.Crugeiras, and M.Parajo, *J.Phys.Org.Chem.*, 1997, **10**, 631.
695. R.Gil, J.Casado, and C.Izquierdo, *Int.J.Chem.Kinet.*, 1997, **29**, 495.
696. H.-O.Kim, C.Lum, and M.S.Lee, *Tetrahedron Lett.*, 1997, **38**, 4935.
697. K.E.Holt, G.E.Hutton, C.N.Morfitt, G.Ruecroft, S.J.C.Taylor, P.D.Tiffin, N.Tremayne, and M.Woods, *Tetrahedron Lett.*, 1997, **38**, 8253.
698. H.J.Knolker and T.Braxmeier, *Synlett.*, 1997, 925.
699. M.J.Burk and J.G.Allen, *J.Org.Chem.*, 1997, **62**, 7054.
700. A.R.Katritzky, C.N.Fali, J.Li, D.J.Ager, and I.Prakash, *Synth.Commun.*, 1997, **27**, 1623.
701. R.Valivety, P.Jauregi, I.Gill, and E.Vulfson, *J.Am.Oil Chem.Soc.*, 1997, **74**, 879.
702. R.Liu and L.E.Orgel, *Nature*, 1997, **389**, 52.
703. J.S.Oliver and J.Singh, *J.Org.Chem.*, 1997, **62**, 6436.
704. J.Izdebski and D.Pawlak, *Pol.J.Chem.*, 1997, **71**, 1066.
705. E.F.Evans, N.J.Lewis, I.Kapfer, G.Macdonald, and R.J.K.Taylor, *Synth.Commun.*, 1997, **27**, 1819.
706. N.Thieriet, J.Alsina, E.Giralt, F.Guibe, and F.Albericio, *Tetrahedron Lett.*, 1997, **38**, 7275.
707. D.A.Wellings and E.Atherton, *Methods Enzymol.*, 1997, **289** (Solid-Phase Peptide Synthesis), 44.
708. W.Zeng, P.-O.Regamey, K.Rose, Y.Wang, and E.Bayer, *J.Pept.Res.*, 1997, **49**, 273.
709. W.R.Bowman, M.J.Broadhurst, D.R.Coghlan, and K.A.Lewis, *Tetrahedron Lett.*, 1997, **38**, 6301.
710. K.L.Mlodnosky, H.M.Holmes, V.Q.Lam, and C.E.Berkman, *Tetrahedron Lett.*, 1997, **38**, 8803.
711. J.Roby and N.Voyer, *Tetrahedron Lett.*, 1997, **38**, 191.
712. D.Qiu and R.R.Koganty, *Tetrahedron Lett.*, 1997, **38**, 45.
713. N.Auzeil, G.Dutruc-Rosset, and M.Largeron, *Tetrahedron Lett.*, 1997, **38**, 2283.
714. P.Conti, D.Demont, J.Cals, H.C.J.Ottenheijm, and D.Leysen, *Tetrahedron Lett.*, 1997, **38**, 2915.
715. A.Ravandi, A.Kukcis, N.Shaikh, and G.Jackowski, *Lipids*, 1997, **32**, 989.
716. F.Zaragoza, *Tetrahedron*, 1997, **53**, 3425.
717. C.J.Moody, L.Ferris, D.Haigh, and E.Swann, *Chem.Commun.*, 1997, 2391.
718. L.Yang and K.Chiu, *Tetrahedron Lett.*, 1997, **38**, 7307.
719. W.R.Bowman and D.R.Coghlan, *Tetrahedron*, 1997, **53**, 15787.
720. H.-B.Kraatz, J.Lusztyk, and G.D.Enright, *Inorg.Chem.*, 1997, **36**, 2400.
721. E.Joerss, P.Schuler, C.Maichle-Moessmer, S.Abram, and H.B.Stegmann, *Enantiomer*, 1997, **2**, 5.

722. T.-P.Loh, D.S.-C.Ho, K.-C.Xu, and K.-Y.Sim, *Tetrahedron Lett*., 1997, **38**, 865.
723. T.W.Ku, F.E.Ali, W.E.Bondinell, K.F.Erhard, W.F.Huffman, J.W.Venslavsky, and C.C.-K.Yuan, *Tetrahedron Lett*., 1997, **38**, 3131.
724. E.Nicolas, M.Pujades, J.Bacardit, E.Giralt, and F.Albericio, *Tetrahedron Lett*., 1997, **38**, 2317.
725. J.J.Chruma, D.Sames, and R.Polt, *Tetrahedron Lett*., 1997, **38**, 5085.
726. A.F.Khlebnikov, M.S.Novikov, and R.R.Kostikov, *Synlett*., 1997, 929.
727. K.Wisniewski and A.S.Kolodziejczyk, *Tetrahedron Lett*., 1997, **38**, 483.
728. U.K.Saha and R.Roy, *Tetrahedron Lett*., 1997, **38**, 7697.
729. B.Rigo, P.Gautret, A.Legrand, J.P.Henichart, and D.Couturier, *Synlett*., 1997, 998.
730. The Maillard Reaction: Consequences for the Chemical and Life Sciences, ed. R.Ikan, Wiley, Chichester, 1996.
731. I.A.O'Neil and A.J.Potter, *Tetrahedron Lett*., 1997, **38**, 5731.
732. P.C.Stevenson, G.C.Kite, and M.S.J.Simmonds, *J. Chromatogr. A*, 1997, **766**, 267.
733. S.W.Wright, D.L.Hageman, A.S.Wright, and L.D.McClure, *Tetrahedron Lett*., 1997, **38**, 7345.
734. I.-L.Shih, L.-C.Chiu, C.T.Lai, W.-C.Liaw, and D.-F.Tai, *Biotechnol. Lett*., 1997, **19**, 857.
735. V.F.Pozdev, *Bioorg. Khim*., 1997, **23**, 262.
736. P.Wipf, W.Xu, H.Kim, and H.Takahashi, *Tetrahedron*, 1997, **53**, 16575.
737. W.-C.Chen, M.D.Vera, and M.M.Jouillie, *Tetrahedron Lett*., 1997, **38**, 4025.
738. H.Sajiki, H.Kuno, and K.Hirota, *Tetrahedron Lett*., 1997, **38**, 399.
739. E.Fernandez-Megia, J.M.Iglesias-Pintos, and J.J.Sardina, *J. Org. Chem*., 1997, **62**, 4770.
740. X.Huang, X.Luo, Y.Roupioz, and J.W.Keillor, *J. Org. Chem*., 1997, **62**, 8821.
741. O.B.Wallace, *Tetrahedron Lett*., 1997, **38**, 4939.
742. R.J.Andersen, J.E.Coleman, E.Piers, and D.J.Wallace, *Tetrahedron Lett*., 1997, **38**, 317.
743. C.Pothion, M.Paris, A.Heitz, L.Rocheblave, F.Rouch, J.-A.Fehrentz, and J.Martinez, *Tetrahedron Lett*., 1997, **38**, 7749; J.-A.Fehrentz, M.Paris, A.Heitz, J.Valek, F.Winternitz, and J.Martinez, *J. Org. Chem*., 1997, **62**, 6792.
744. J.D.Armstrong, J.L.Keller, J.Lynch, T.Liu, F.W.Hartner, N.Ohtake, S.Okada, Y.Imai, O.Okamoto, R.Ushijima, S.Nakagawa, and R.P.Volante, *Tetrahedron Lett*., 1997, **38**, 3203.
745. H.H.Wasserman and A.K.Petersen, *Tetrahedron Lett*., 1997, **38**, 953.
746. P.Chen, P.T.W.Cheng, S.H.Spergel, R.Zahler, X.Wang, J.Thottathil, J.C.Barrish, and R.P.Polniaszek, *Tetrahedron Lett*., 1997, **38**, 3175.
747. A.Albeck and G.I.Estreicher, *Tetrahedron*, 1997, **53**, 5325.
748. J.Branalt, I.Kvarnstrom, B.Classon, B.Samuelsson, U.Nillroth, U.H.Danielson, A.Karlen, and A.Hallberg, *Tetrahedron Lett*., 1997, **38**, 3483.
749. R.N.Patel, A.Banerjee, C.G.McNamee, D.B.Brzozowski, and L.J.Szarka, *Tetrahedron: Asymmetry*, 1997, **8**, 2547.
750. J.Podlech and M.R.Linder, *J. Org. Chem*., 1997, **62**, 5873.
751. F.Benedetti, S.Miertus, S.Norbedo, A.Tossi, and P.Zlatoidzky, *J. Org. Chem*., 1997, **62**, 9348.
752. T.K.Chakraborty and S.Dutta, *Synth. Commun*., 1997, **27**, 4163.
753. J.S.Oliver and A.K.Oyelere, *Tetrahedron Lett*., 1997, **38**, 4005.
754. J.Martin, M.-C.Lasne, J.-C.Plaquevent, and L.Duhamel, *Tetrahedron Lett*., 1997, **38**, 7181.
755. D.A.Evans, P.H.Carter, C.J.Dinsmore, J.C.Barrow, J.L.Katz, and D.W.Kung,

Tetrahedron Lett., 1997, **38**, 4535 (using $NaNO_2$ – aq AcOH for nitrosation; use of nitric oxide for the same purpose, applied to secondary amides, is described by T.Itoh, K.Nagata, Y.Matsuya, M.Miyazaki, and A.Ohsawa, *Tetrahedron Lett*., 1997, **38**, 5017).
756. A.R.Haight, T.L.Stuk, J.A.Menzia, and T.A.Robbins, *Tetrahedron Lett*., 1997, **38**, 4191.
757. A.Dondini and D.Perrone, *Synthesis*, 1997, 527; erratum, *ibid*, p. 1512.
758. J.L.Toujas, E.Jost, and M.Vaultier, *Bull.Soc.Chim.Fr*., 1997, **134**, 713.
759. A.McCluskey, D.M.Mayer, and D.J.Young, *Tetrahedron Lett*., 1997, **38**, 5217.
760. Y.N.Bubnov, M.A.Misharin, and A.V.Ignatenko, *Tetrahedron Lett*., 1997, **38**, 6259.
761. D.Gryko, Z.Urbanczyk-Lipkowska, and J.Jurczak, *Tetrahedron*, 1997, **53**, 13373.
762. J.M.Concellon, P.L.Bernad, and J.A.Perez-Andres, *J.Org.Chem*., 1997, **62**, 8902.
763. R.Vanderesse, L.David, V.Grand, M.Marraud, J.P.Mangeot, and A.Aubry, *Tetrahedron Lett*., 1997, **38**, 2669.
764. M.Adia, N.Henaff, and A.Whiting, *Tetrahedron Lett*., 1997, **38**, 3101.
765. A.K.Ghosh, K.A.Hussain, and S.Fidanze, *J.Org.Chem*., 1997, **62**, 6080.
766. R.Kreuder, T.Rein, and O.Reiser, *Tetrahedron Lett*., 1997, **38**, 9035.
767. T.Kawano, K.Negoro, and I.Ueda, *Tetrahedron Lett*., 1997, **38**, 8219.
768. J.S.Panek and P.Liu, *Tetrahedron Lett*., 1997, **38**, 5127.
769. L.A.Paquette, T.M.Mitzel, M.B.Isaac, C.F.Crasto, and W.W.Schomer, *J.Org.Chem*., 1997, **62**, 4293; erratum, see p. 8960.
770. A.F.Parsons and R.M.Pettifer, *Tetrahedron Lett*., 1997, **38**, 5907.
771. S.J.E.Mulders, A.J.Brouwer, and R.M.J.Liskamp, *Tetrahedron Lett*., 1997, **38**, 3085.
772. S.Dupre, M.Fontana, G.Pitari, and D.Cavallini, *Adv.Exp.Med.Biol*., 1996, **403**, 3.
773. I.G.Jones, W.Jones, and M.North, *SynLett*., 1997, 63.
774. P.F.Vogt, J.-G.Hansel, and M.J.Miller, *Tetrahedron Lett*., 1997, **38**, 2803.
775. W.J.Hoekstra, M.N.Greco, S.C.Yabut, B.L.Hulshizer, and B.E.Maryanoff, *Tetrahedron Lett*., 1997, **38**, 2629.
776. M.Ohba, H.Kubo, T.Fujii, H.Ishibashi, M.V.Sargent, and D.Arbain, *Tetrahedron Lett*., 1997, **38**, 6697.
777. R.Kluger, R.W.Loo, and V.Mazza, *J.Am.Chem.Soc*., 1997, **119**, 12089.
778. K.Goto and R.Ueoka, *Nippon Kagaku Kaishi*, 1997, 127 (*Chem.Abs*., 1997, **126**, 251362); K.Goto and R.Ueoka, *Yuki Gosei Kogaku Kyokaishi*, 1997, **55**, 803 *Chem.Abs*., 1998, **127**, 248371).
779. M.C.Cleij, F.Mancin, P.Scrimin, P.Tecilla, and U.Tonellato, *Tetrahedron*, 1997, **53**, 357.
780. J.W.Suggs and R.M.Pires, *Tetrahedron Lett*., 1997, **38**, 2227.
781. F.Tinardon, R.Lamrini, P.Lacan, M.Desage, and A.Francina, *Rapid Commun.Mass Spectrom*., 1997, **11**, 1373.
782. J.Bujdak and B.M.Rode, *J.Mol.Evol*., 1997, **45**, 457.
783. B.M.Rode, A.H.Eder, and Y.Yongyai, *Inorg.Chim.Acta*, 1997, **254**, 309.
784. D.Jia, J.Zhuang, and X.Xin, *Huaxue Yanjiu Yingyong*, 1997, **9**, 245 (*Chem.Abs*., 1997, **127**, 210820).
785. A.R.Hill and L.E.Orgel, *Origins Life Evol.Biosphere*, 1996, **26**, 539.
786. K.I.Zamaraev, V.N.Romannikov, R.I.Salganik, W.A.Wlassoff, and V.V.Khramtsov, *Origins Life Evol.Biosphere*, 1997, **27**, 325.
787. V.A.Basiuk and R.Navarro-Gonzalez, *J.Chromatogr., A*, 1997, **776**, 255.
788. M.B.Simakov, E.A.Kuzicheva, N.Y.Dodonova, and A.E.Antropov, *Adv.Space Res*., 1997, **19**, 1063.

789. V.V.Novikov and A.S.Lysitsin, *Biofizika*, 1996, **41**, 1163; *Idem*, *Biofizika*, 1997, **42**, 1003.
790. J.J.Farmer, A.B.Attygalle, S.R.Smedley, T.Eisner, and J.Meinwald, *Tetrahedron Lett*., 1997, **38**, 2787.
791. A.Nowak and B.Steffan, *Liebigs Ann./Recl*. 1997, 1817.
792. R.A.Aitken, K.Ali, and S.T.E.Mesher, *Tetrahedron Lett*., 1997, **38**, 4179.
793. M.Falorni, G.Giacomelli, F.Nieddu, and M.Taddei, *Tetrahedron Lett*., 1997, **38**, 4663.
794. M.Kawase, S.Saito, H.Kikuchi, and H.Miyamae, *Heterocycles*, 1997, **45**, 2185.
795. R.Zamora, M.Alaiz, and F.J.Hidalgo, *Biochemistry*, 1997, **36**, 15765.
796. T.Ishida, Y.In, C.Hayashi, R.Manabe, and A.Wakahara, *Bull.Chem.Soc.Jpn*., 1997, **70**, 2375.
797. S.W.Kim, S.Y.Ahn, J.S.Koh, J.H.Lee, S.Ro, and H.Y.Cho, *Tetrahedron Lett*., 1997, **38**, 4603.
798. B.Mo, J.Li, and S.Liang, *Anal.Biochem*., 1997, **249**, 207.
799. J.Wang, Y.Okada, W.Li, T.Yokoi, and J.Zhu, *J.Chem.Soc., Perkin Trans.I*, 1997, 621.
800. K.Iseki, Y.Kuroki, and Y.Kobayashi, *Tetrahedron Lett*., 1997, **38**, 7209.
801. J.-P.Joly and G.Schroder, *Tetrahedron Lett*., 1997, **38**, 8197.
802. M.E.Volpin, V.S.Romanova, and Z.N.Parnes, *Mol.Cryst.Liq.Cryst.Sci.Technol., Sect.C*, 1996, **7**, 53.
803. J.-H.Xu, Y.-L.Li, D.-G.Zheng, J.-K.Yang, Z.Mao, and D.-B.Zhu, *Tetrahedron Lett*., 1997, **38**, 6613.
804. C.J.Easton, S.K.Eichinger, and M.J.Pitt, *Tetrahedron*, 1997, **53**, 5609.
805. M.M.Alauddin, J.D.Fissekis, and P.S.Conti, *Nucl.Med.Biol*., 1997, **24**, 771.
806. F.C.Ross, N.P.Botting, and P.D.Leeson, *Tetrahedron*, 1997, **53**, 15761.
807. C.Haefele, C.Bonfils, and Y.Sauvaire, *Phytochemistry*, 1997, **44**, 563.
808. A.Ozaki, T.Shibasaki, and H.Mori, *Baiosaiensu to Indasutori*, 1998, **56**, 11 (*Chem.Abs*., 1998, **128**, 114052).
809. J.Hiebl, H.Kollmann, F.Rovenszky, and K.Winkler, *Bioorg.Med.Chem.Lett.,* 1997, **7**, 2963.
810. G.T.Crisp and M.G.Gebauer, *J.Organomet.Chem*., 1997, **532**, 83.
811. G.T.Crisp and M.J.Millan, *Tetrahedron*, 1998, **54**, 637, 649.
812. M.J.Burk, J.G.Allen, W.P.Kiesman, and K.M.Stoffan, *Tetrahedron Lett*., 1997, **38**, 1309.
813. M.K.Gurjar and N.K.Tripathy, *Tetrahedron Lett*., 1997, **38**, 2163.
814. K.El Abdioui, J.Martinez, P.Viallefont, and Y.Vidal, *Bull.Soc.Chim.Belg*., 1997, **106**, 425.
815. F.P.J.T.Rutges and H.E.Schoemaker, *Tetrahedron Lett*., 1997, **38**, 677.
816. S.E.Gibson, V.C.Gibson, and S.P.Keen, *Chem.Commun*., 1997, 1107.
817. K.Goodall and A.F.Parsons, *Tetrahedron Lett*., 1997, **38**, 491.
818. M.Oba, T.Terauchi, J.Hashimoto, T.Tanaka, and K.Nishiyama, *Tetrahedron Lett*., 1997, **38**, 5515.
819. S.Cacchi, G.Fabrizi, C.Gallina, and P.Pace, *Synlett*., 1997, 54.
820. A.Avenoza, C.Cativiela, M.A.Fernandez-Recio, and J.M.Peregrina, *Synthesis*, 1997, 165.
821. C.A.Hutton, *Tetrahedron Lett*., 1997, **38**, 5899.
822. R.F.W.Jackson, D.Turner, and M.H.Block, *J.Chem.Soc., Perkin Trans.I*, 1997, 865.
823. R.F.W.Jackson, D.Turner, and M.H.Block, *Synlett*., 1997, 789.
824. S.Gair, R.F.W.Jackson, and P.A.Brown, *Tetrahedron Lett*., 1997, **38**, 3059; erratum, p. 3795.

825. M.Skof, J.Svete, and B.Stanovnik, *J.Heterocycl.Chem.*, 1997, **34**, 853.
826. N.M.Howarth and L.P.G.Wakelin, *J.Org.Chem.*, 1997, **62**, 5441.
827. C.J.Easton, A.J.Ivory, and C.A.Smith, *J.Chem.Soc., Perkin Trans.II*, 1997, 503.
828. T.Nakazawa, T.Suzuki, and M.Ishii, *Tetrahedron Lett.*, 1997, **38**, 8951.
829. A.M.P.Koskinen, J.Schwerdtfeger, and M.Edmonds, *Tetrahedron Lett.*, 1997, **38**, 5399.
830. W.W.Epstein and Z.Wang, *Chem.Commun.*, 1997, 863.
831. C.Dugave and A.Menez, *Tetrahedron: Asymmetry*, 1997, **8**, 1453.
832. E.M.Stocking, J.N.Schwarz, H.Senn, M.Salzmann, and L.A.Silks, *J.Chem.Soc., Perkin Trans.I*, 1997, 2443.
833. C.Somlai, B.Penke, and A.Peter, *J.Prakt.Chem./Chem.-Ztg.*, 1997, **339**, 464.
834. M.A.Walker, K.P.Kaplita, T.Chen, and D.H.King, *Synlett.*, 1997, 169.
835. H.-H.Lee, H.Yamaguchi, and H.Senda, *Spectrosc.Lett.*, 1997, **30**, 685.
836. A.G.Dybenko, V.P.Romanova, and V.V.Shilin, *Ukr.Khim.Zh.*, 1996, **62**, 52 (*Chem.Abs.*, 1997, **126**, 212389).
837. E.Diez-Barra, A.de la Hoz, and P.Sanchez-Verdu, *An.Quim.Int.Ed.*, 1997, **93**, 145.
838. K.G.I.Nilsson, G.Ljunger, and P.M.Melin, *Biotechnol.Lett.*, 1997, **19**, 889.
839. G.Antoni, H.Omura, M.Bergstrom, Y.Furuya, R.Moulder, A.Roberto, A.Sundin, Y.Watanabe, and B.Langstrom, *Nucl.Med.Biol.*, 1997, **24**, 595.
840. T.Laib, J.Chastanet, and J.Zhu, *Tetrahedron Lett.*, 1997, **38**, 1771.
841. P.Lloyd-Williams, A.Sanchez, N.Carulla, T.Ochoa, and E.Giralt, *Tetrahedron*, 1997, **53**, 3369; P.Lloyd-Williams, N.Carulla, and E.Giralt, *Tetrahedron Lett.*, 1997, **38**, 299.
842. J.-N.Denis, S.Tchertchian, and Y.Vallee, *Synth.Commun.*, 1997, **27**, 2345.
843. A.Avenoza, C.Cativiela, J.M.Peregrina, and M.M.Zurbano, *Tetrahedron: Asymmetry*, 1997, **8**, 863. See Ref 757, and A.Avenoza, C.Cativiela, F.Corzana, and M.M.Zurbano, *Synthesis*, 1997, 1146, for new syntheses of the Garner aldehyde.
844. J.S.R.Kumar and A.Datta, *Tetrahedron Lett.*, 1997, **38**, 473.
845. K.C.Nicolaou, K.Koide, J.Xu, and M.H.Izraelewicz, *Tetrahedron Lett.*, 1997, **38**, 3671.
846. M.Shimizu, I.Wakioka, and T.Fujisawa, *Tetrahedron Lett.*, 1997, **38**, 6027.
847. S.Knapp and Y.Dong, *Tetrahedron Lett.*, 1997, **38**, 3813.
848. A.V.Rama Rao, M.K.Gurjar, P.Lakshmipathi, M.M.Reddy, M.Nagarajan, S.Pal, B.V.N.B.S.Sarma, and N.K.Tripathy, *Tetrahedron Lett.*, 1997, **38**, 7433.
849. G.T.Crisp, Y.-L.Jiang, P.J.Pullman, and C.De Savi, *Tetrahedron*, 1997, **53**, 17489.
850. H.Gruza, K.Kiciak, A.Krasinski, and J.Jurczak, *Tetrahedron; Asymmetry*, 1997, **8**, 2627.
851. P.Merino, A.Lanaspa, F.L.Merchan, and T.Tejero, *Tetrahedron Lett.*, 1997, **38**, 1813.
852. W.Tuckmantel, A.P.Kozikowski, S.Wang, S.Pchenichkin, and J.T.Wroblewski, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 601.
853. J.R.Bellettini and M.J.Miller, *Tetrahedron Lett.*, 1997, **38**, 167.
854. F.B.Charvillon and R.Amouroux, *Synth.Commun.*, 1997, **27**, 395.
855. G.Guillena, I.Mico, C.Najera, J.Ezquerra, and C.Pedregal, *An.Quim.Int.Ed.*, 1996, **92**, 362.
856. C.M.Moody and D.W.Young, *J.Chem.Soc., Perkin Trans.I*, 1997, 3519.
857. E.Coudert, F.Acher, and R.Azerad, *Synthesis*, 1997, 863.
858. I.Baussanne, O.Schwardt, J.Royer, M.Pichon, B.Figadere, and A.Cave, *Tetrahedron Lett.*, 1997, **38**, 2259.
859. S.K.Panday and N.Langlois, *Synth.Commun.*, 1997, **27**, 1373.

860. S.Fehn and K.Burger, *Tetrahedron: Asymmetry*, 1997, **8**, 2001.
861. Y.Bousquet, P.C.Anderson, T.Bogri, J.-S.Duceppe, L.Grenier, and I.Guse, *Tetrahedron*, 1997, **53**, 15671.
862. H.Li, T.Sakamoto, and Y.Kikugawa, *Tetrahedron Lett*., 1997, **38**, 6677.
863. H.-D.Arndt, K.Polborn, and U.Koert, *Tetrahedron Lett*., 1997, **38**, 3879.
864. P.W.H.Chan, I.F.Cottrell, and M.G.Moloney, *Tetrahedron Lett*., 1997, **38**, 5891; N.Langlois, O.Calvez, and M.-O.Radom, *Tetrahedron Lett*., 1997, **38**, 8037.
865. C.Herdeis, A.Aschenbrenner, A.Kirfel, and F.Schwabenlander, *Tetrahedron: Asymmetry*, 1997, **8**, 2421.
866. A.N.Bowler, P.M.Doyle, P.B.Hitchcock, and D.W.Young, *Tetrahedron*, 1997, **53**, 10545.
867. H.Zhang, M.T.Fletcher, J.W.Avery, and W.Kitching, *Tetrahedron Lett*., 1997, **38**, 3477.
868. L.Zhang, G.S.Kauffman, J.A.Pesti, and J.Yin, *J.Org.Chem*., 1997, **62**, 6918.
869. E.Fernandez-Megia and F.J.Sardina, *Tetrahedron Lett*., 1997, **38**, 673.
870. M.D.Andrews, K.A.O'Callaghan, and J.C.Vederas, *Tetrahedron*, 1997, **53**, 8295.
871. R.M.Werner, O.Shokek, and J.T.Davis, *J.Org.Chem*., 1997, **62**, 8243.
872. K.-M.Cheung and P.M.Shoolingin-Jordan, *Tetrahedron*, 1997, **53**, 15807.
873. F.Burkhart, M.Hoffmann, and H.Kessler, *Angew.Chem.Int.Ed*., 1997, **36**, 1191.
874. G.Zvilichovsky and V.Gurvich, *J.Chem.Soc., Perkin Trans.I*, 1997, 1069.
875. H.-O.Kim and M.Kahn, *Tetrahedron Lett*., 1997, **38**, 6483.
876. K.Wisniewski and A.S.Kolodziejczyk, *Org.Prep.Proced.Int*., 1997, **29**, 338.
877. J.B.Ducep, B.Heintzelmann, K.Jund, B.Lesur, M.Schleimer, and P.R.Zimmermann, *Tetrahedron: Asymmetry*, 1997, **8**, 327.
878. M.Falorni, A.Porcheddu, and G.Giacomelli, *Tetrahedron: Asymmetry*, 1997, **8**, 1633.
879. H.Baumgartner and A.C.O'Sullivan, *Tetrahedron*, 1997, **53**, 2775.
880. T.Yamazaki, K.Komatsu, H.Umemiya, Y.Hashimoto, K.Shudo, and H.Kagechika, *Tetrahedron Lett*., 1997, **38**, 8363.
881. G.M.Dubowchik and S.Radia, *Tetrahedron Lett*., 1997, **38**, 5257.
882. M.Prabhakaram, Q.Cheng, M.S.Feather, and B.J.Ortwerth, *Amino Acids*, 1997, **12**, 225.
883. J.R.Requena, M.X.Fu, M.U.Ahmed, A.J.Jenkins, T.J.Lyons, J.W.Baynes, and S.R.Thorpe, *Biochem.J*., 1997, **322**, 317; K.Uchida, K.Sakai, K.Itakura, T.Osawa, and S.Toyokuni, *Arch.Biochem.Biophys*., 1997, **346**, 45.
884. K.Itakura, T.Osawa, and K.Uchida, *J.Org.Chem*., 1998, **63**, 185.
885. I.N.Shipanova, M.A.Glomb, and R.H.Nagaraj, *Arch.Biochem.Biophys*., 1997, **344**, 29.
886. H.Miel and S.Rault, *Tetrahedron Lett*., 1997, **38**, 7865.
887. R.O.Duthaler and A.Hafner, *New Methods Drug Res*., 1995, **4**, 103.
888. R.Wang, X.Qiao, Y.Li, and F.Liu, *Huaxue Shiji*, 1996, **18**, 357 (*Chem.Abs*., 1997, **126**, 171860).
889. T.Yomiyama and K.Fujimori, *Bioorg.Med.Chem.Lett*., 1997, **7**, 175 (S-nitroso-L-cysteine); M.Le, H.Zhang, and G.E.Means, *Bioorg.Med.Chem.Lett*., 1997, **7**, 1393 (S-nitrosated dithiols); J.K.J.Park and P.Kostka, *Anal.Biochem*., 1997, **249**, 61 (fluorimetric analysis).
890. A.R.Butler and P.Rhodes, *Anal.Biochem*., 1997, **249**, 1.
891. N.Bainbridge, A.R.Butler, and C.H.Goerbitz, *J.Chem.Soc., Perkin Trans.II*, 1997, 351.
892. P.H.Beloso and D.L.H.Williams, *Chem.Commun*., 1997, 89.

893. C.Boullais, M.Riva, and J.-P.Noel, *J.Labelled Compd.Radiopharm.*, 1997, **39**, 621.
894. P.Schwenkkraus, S.Merkle, and H.H.Otto, *Liebigs Ann./Recl.*, 1997, 1261; D.Glasl, G.Rins, and H.H.Otto, *Helv.Chim.Acta*, 1997, **80**, 671.
895. X.-M.Shen and G.Dryhurst, *Bioorg.Chem.*, 1996, **24**, 340.
896. A.G.Griesbeck, J.Hirt, K.Peters, E.-M.Peters, and H.G.von Schnering, *Chem.-Eur.J.*, 1996, **2**, 1388.
897. M.Benrahmoune, M.Ghassiqh, and Z.Abendinzadeh, *J.Chim.Phys., Phys.-Chim.-Biol.*, 1997, **94**, 257.
898. K.Pandian and S.S.Narayanan, *Asian J.Chem.*, 1997, **9**, 859 (erratum, *ibid.*, 1998, **10**, 212).
899. S.Chowdhury and S.Roy, *Tetrahedron Lett.*, 1997, **38**, 2149.
900. M.Jonsson and H.-B.Kraatz, *J.Chem.Soc., Perkin Trans.II*, 1997, 2673.
901. H.C.Potgieter, J.Bubbink, S.Bissbort, M.J.Bester, J.H.Spies, and W.J.H.Vermaak, *Anal.Biochem.*, 1997, **248**, 86.
902. J.S.R.Kumar and A.Datta, *Tetrahedron Lett.*, 1997, **38**, 6779.
903. P.Singh, C.R.Hurrell, J.B.C.Findlay, and C.W.G.Fishwick, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 715.
904. M.Tepper, O.Stelzer, T.Hausler, and W.S.Sheldrick, *Tetrahedron Lett.*, 1997, **38**, 2257.
905. M.N.Qabar, J.Urban, and M.Kalin, *Tetrahedron*, 1997, **53**, 11171.
906. E.Morera and G.Ortar, *Synlett.*, 1997, 1403.
907. Y.Okada, N.Shintomi, Y.Kondo, T.Yokoi, S.Joshi, and W.Li, *Chem.Pharm.Bull.*, 1997, **45**, 1860.
908. K.Rosenthal, A.Karlstrom and A.Unden, *Tetrahedron Lett.*, 1997, **38**, 1075.
909. E.-K.Kim, H.Choi, and E.-S.Lee, *Yakhak Hoechi*, 1997, **41**, 588.
910. A.A.El-Mohty, A.S.El-Wetery, M.T.El-Kolaly, and M.Raieh, *J.Radioanal.Nucl.-Chem.*, 1996, **214**, 133.
911. B.Kayser, J.Altman, and W.Beck, *Tetrahedron*, 1997, **53**, 2475.
912. T.N.Das, *J.Phys.Chem.A*, 1998, **102**, 426.
913. O.T.Dejesus, C.J.Endres, S.E.Shelton, R.J.Nickles, and J.E.Holden, *J.Nucl.Med.*, 1997, **38**, 630.
914. M.E.Jung and T.I.Lazarova, *J.Org.Chem.*, 1997, **62**, 1553.
915. A.R.Butler, T.J.Rutherford, D.M.Short, and J.H.Ridd, *J.Chem.Soc., Chem. Commun.*, 1997, 669.
916. M.E.Jung and L.S.Starkey, *Tetrahedron*, 1997, **53**, 8815.
917. Z.Guo, G.M.Salamonczyk, K.Han, K.Machiya, and C.J.Sih, *J.Org.Chem.*, 1997, **62**, 6700.
918. N.V.Bell, W.R.Bowman, P.F.Coe, A.T.Turner, and D.Whybrow, *Can.J.Chem.*, 1997, **75**, 873.
919. G.A.Salamonczyk, V.B.Oza, and C.J.Sih, *Tetrahedron Lett.*, 1997, **38**, 6965.
920. J.E.Baldwin, M.R.Spyvee, and R.C.Whitehead, *Tetrahedron Lett.*, 1997, **38**, 2771.
921. B.Matusczak, *Pharmazie*, 1996, **51**, 862.
922. Y.Okada, J.Wang, T.Yamamoto, T.Yokoi, and Y.Mu, *Chem.Pharm.Bull.*, 1997, **45**, 452.
923. A.Kimbouguila, S.Boucida, F.Guibe, and A.Loffet, *Tetrahedron*, 1997, **53**, 12525.
924. R.Jain, L.A.Cohen, N.A.El-Kadi, and M.M.King, *Tetrahedron*, 1997, **53**, 2365.
925. R.Zamora and F.J.Hidalgo, *Grasas Aceites*, 1996, **47**, 326.
926. J.H.Jones and V.L.Walker, *J.Pept.Sci.*, 1997, **3**, 391.
927. X.Huang, R.Xu, M.D.Hawley, and K.J.Kramer, *Bioorg.Chem.*, 1997, **25**, 179.
928. P.Yu, T.Wang, F.Yu, and J.M.Cook, *Tetrahedron Lett.*, 1997, **38**, 6819.

929. P.Yu and J.M.Cook, *Tetrahedron Lett*., 1997, **38**, 8799.
930. H.Wang and A.Ganesan, *Tetrahedron Lett*., 1997, **38**, 4327.
931. U.Anthoni, C.Christopherson, P.H.Nielsen and E.J.Pedersen, *Acta Chem.Scand*., 1997, **51**, 407.
932. C.C.McComas, E.J.Gilbert, and D.L.Van Vranken, *J.Org.Chem*., 1997, **62**, 8600.
933. T.J.Simat and H.Steinhart, *J.Agric.Food Chem*., 1998, **46**, 490.
934. B.Van Wickern, B.Mueller, T.J.Simat and H.Steinhart, *J.Chromatogr. A*, 1997, **786**, 57.
935. L.P.Candeias, P.Wardman, and R.P.Mason, *Biophys.Chem*., 1997, **67**, 229.
936. H.Goerner and D.N.Nikogosyan, *J.Photochem.Photobiol.B*, 1997, **39**, 84.
937. E.V.Khoroshinova, Y.A.Repeev, and D.N.Nikogosyan, *Dokl.Akad.Nauk*, 1997, **352**, 643.
938. D.N.Nikogosyan and H.Goerner, *Biol.Chem*., 1997, **378**, 1349.
939. Z.Stuglik and J.Sadlo, *Appl.Radiat.Isot*., 1996, **47**, 1219.
940. Z.P.Zagorski and G.K.Przybytniak, *Nukleonika*, 1997, **42**, 373.
941. H.Hidaka, S.Horikoshi, K.Ajisaka, J.Zhao, and N.Serpone, *J.Photochem.Photobiol.A*, 1997, **108**, 197.
942. L.Gan, J.Jiang, W.Zhang, Y.Huang, Y.Su, and C.Huang, *Proc.Electrochem.Soc*., 1997, **97-14**, 271.
943. W.Zhang, Y.Su, L.Gan, J.Jiang, and C.Huang, *Chem.Lett*., 1997, 1007.
944. R.Navarro-Gonzalez, M.Akaboshi, A.Romero, and C.Ponnamperuma, *J.Biol.Phys*., 1996, **22**, 87.
945. Q.Peng, K.Berg, J.Moan, M.Kongshaug, and J.M.Nesland, *Photochem.Photobiol*., 1997, **65**, 235.
946. A.Colwell, A.Hamer, A.Blumsohn, and R.Eastell, *Eur.J.Clin.Invest*., 1996, **26**, 1107.
947. W.Wiczk, L.Lankiewicz, C.Czaplewski, S.Oldziej, K.Stachowiak, A.Michniewicz, and A.Liwo, *J.Photochem.Photobiol*., 1996, **101**, 171.
948. L.Fabbrizzi, G.Francese, M.Licchelli, A.Perotti, and A.Taglietti, *Chem.Commun*., 1997, 581.
949. R.Huang, *Shengwu Huaxue Yu Shengwu Wuli Jinzhan*, 1997, **24**, 60 (*Chem.Abs*., 1998, **127**, 331708).
950. D.Burdi, B.M.Aveline, P.D.Wood, J.Stubbs, and R.W.Redmond, *J.Am.Chem.Soc*., 1997, **119**, 6457.
951. S.Criado, S.G.Bertolotti, A.T.Soltermann, and N.A.Garcia, *J.Photochem.Photobiol.B*, 1996, **38**, 107.
952. J.M.Wessels, C.S.Foote, W.E.Ford, and M.A.J.Rogers, *Photochem.Photobiol*., 1997, **65**, 96.
953. L.Ambrosone, G.D'Errico, and R.Ragone, *Spectrochim.Acta, Part A*, 1997, **53A**, 1615.
954. J.Q.Wu, A.Ozarowski, and A.H.Maki, *J.Phys.Chem.A*, 1997, **101**, 6177.
955. C.Alpert, N.Ramdev, D.George, and J.Loscalzo, *Anal.Biochem*., 1997, **245**, 1.
956. A.J.Smith, *Methods Enzymol*., 1997, **289** (Solid Phase Peptide Synthesis), 419.
957. W.G.Engelhart, in *Microwave-Enhanced Chemistry*, eds. H.M.Kingston and S.J.Haswell, American Chemical Society, Washington, 1997, p. 613.
958. I.Fermo, E.De Vecchi, C.Arcelloni, A.D'Angelo, and R.Paroni, *Haematologica*, 1997, **82**, 246; R.Schreiner, B.Goebel-Schreiner, C.Durst, R.Casper, and S.Walch, *Clin.Lab*., 1997,.**43**, 1121.
959. J.Moller, L.Christensen, and K.Rasmussen, *Scand.J.Clin.Lab.Invest*., 1997, **57**, 613.
960. I.Molnar-Perl, J.*Chromatogr.A*, 1997, **763**, 1.
961. H.Kataoka, S.Matsumura, H.Koizumi, and M.Makita, *J.Chromatogr.A*, 1997, **758**, 167.

962. H.Kataoka, *Biomed.Chromatogr.*, 1997, **11**, 154.
963. B.C.Blount and M.W.Duncan, *Anal.Biochem.*, 1997, **244**, 270.
964. R.M.Castro, M.T.D.Carbo, V.P.Martinez, J.V.G.Adelantado, and F.B.Reig, *J.Chromatogr.A*, 1997, **778**, 373.
965. U.Matthiesen and P.Schadewaldt, *Isotopenpraxis*, 1994, **30**, 219.
966. S.A.Macko, M.E.Uhle, M.H.Engel, and V.Andrusevich, *Anal.Chem.*, 1997, **69**, 926.
967. M.W.Duncan and A.Poljak, *Anal.Chem.*, 1998, **70**, 890.
968. P.Dallakian and H.Budzikiewicz, *J.Chromatogr.A*, 1997, **787**, 195.
969. P.Cao and M.Moini, *J.Chromatogr.A*, 1997, **759**, 111.
970. J.Pietzsch, U.Julius, and M.Hanefeld, *Clin.Chem.*, 1997, **43**, 2001; *Rapid Commun. Mass Spectrom.*, 1997, **11**, 1835.
971. R.Anacardio, M.G.Cantalini, F.De Angelis, and M.Gentile, *J.Mass Spectrom.*, 1997, **32**, 388.
972. S.L.Hazen, J.R.Crowley, D.M.Mueller, and J.W.Heinecke, *Free Radical Biol.Med.*, 1997, **23**, 909.
973. G.C.Kite and M.J.Hughes, *Phytochem.Anal.*, 1997, **8**, 294.
974. M.Pan, T.J.Mabry, P.Cao, and M.Moini, *J.Chromatogr.A*, 1997, **787**, 288.
975. I.Abe, Y.Nakao, and T.Nakahara, *Chem.Lett.*, 1997, 629.
976. B.Fransson and U.Ragnarsson, *Chem.Lett.*, 1997, 779.
977. M.Candito, P.Bedoucha, M.H.Mahague, G.Scarini, and M.Chatel, *J.Chromatogr.B: Biomed.Appl.*, 1997, **692**, 213.
978. J.Le Boucher, C.Charret, C.Coudray-Lucas, J.Giboudeau, and L.Cynober, *Clin. Chem.*, 1997, **43**, 1421.
979. A.Kitakawa, Y.Yamanishi, and T.Yonemoto, *Ind.Eng.Chem.Res.*, 1997, **36**, 3809.
980. H.J.Chaves-das-Neves and Z.Braga-Morais, *An.Quim.Int.Ed.*, 1997, **93**, 98.
981. R.Bhushan and J.Martens, *Biomed.Chromatogr.*, 1997, **11**, 280.
982. R.Bhushan, J.Martens, S.Wallbaum, S.Joshi, and V.Parshad, *Biomed.Chromatogr.*, 1997, **11**, 286.
983. N.Baudry, B.Mallet, P.J.Lejeune, L.Vinet, and J.L.Franc, *J.Endocrinol.*, 1997, **153**, 99; A.de la Vieja, M.Calero, P.Santisteban, and L.Lamas, *J.Chromatogr.B: Biomed. Appl.*, 1997, **688**, 143.
984. M.Dunnett and R.C.Harris, *J.Chromatogr.B: Biomed.Appl.*, 1997, **688**, 47.
985. M.Abdelrahim, E.Morris, J.Carrer, S.Facchina, A.White, and A.Verma, *J.Chromatogr.B: Biomed.Appl.*, 1997, **696**, 175.
986. B.Daneshvar, H.Frandsen, L.O.Dragsted, L.E.Knudsen, and H.Autrup, *Pharmacol.Toxicol.*, 1997, **81**, 205.
987. P.Spacek, H.Hulejova, and M.Adam, *J.Chromatogr.B: Biomed.Appl.*, 1997, **689**, 404; *Klin.Biochem.Metab.*, 1996, **4**, 45 (*Chem.Abs.*, 1997, **126**, 235433); *J.Liq.Chromatogr.Relat.Technol.*, 1997, **20**, 1921; P.Bjellerup, *Scand.J.Clin.Lab.Invest., Suppl.*, 1997, **57**, 80 (*Chem.Abs.*, 1998, **127**, 14962); G.N.Kent, *Ibid.*, p.73 (*Chem.Abs.*, 1998, **127**, 15049); A.Dessauer, *ibid.*, p. 84 (*Chem.Abs.*, 1998, **127**, 15050).
988. G.Lamprecht, *J.Chromatogr.B: Biomed.Appl.*,1997, **691**, 297.
989. I.Fermo, C.Arcelloni, E.Casari, and R.Paroni, *Clin.Chem.*, 1997, **43**, 2186; K.Nakatsuka, T.Miki, K.Sekiya, H.Kawakami, Y.Hirota, M.Miura, Y.Obi, Y.Nishizawa, and H.Morii, *J.Bone Miner.Metab.*, 1997, **15**, 153.
990. Y.Sun, W.Yang, Y.Zhang, X.Feng, and J.Yuan, *Sepu*, 1997, **15**, 235 (*Chem.Abs.*, 1998, **127**, 132865).
991. K.Okada, A.Kondo, O.Ishikawa, Y.Miyachi, and Y.Kuboki, *Photomed.Photobiol.*, 1996, **18**, 79; A.Kondo, O.Ishikawa, K.Okada, Y.Miyachi, S.Abe, and Y.Kuboki, *Anal.Biochem.*, 1997, **252**, 255.

992. B.Flath, B.Rolinski, and A.A.Roscher, *J.Chromatogr.B: Biomed.Appl.*, 1997, **694**, 227.
993. M.J.Nozal, J.L.Bernal, L.Toribio, P.Marinero, O.Moral, L.Manzanas, and E.Rodriguez, *J.Chromatogr.A*, 1997, **778**, 347.
994. P.A.Biondi, L.M.Chiesa, M.R.Stovelli, and P.Renon, *J.Chromatogr.Sci.*, 1997, **35**, 509.
995. F.Blandini, E.Martignoni, C.Pacchetti, S.Desiderio, D.Rivellini, and G.Nappi, *J.Chromatogr.B: Biomed.Appl.*, 1997, **700**, 278.
996. C.K.Wise, C.A.Cooney, S.F.Ali, and C.A.Poirier, *J.Chromatogr.B: Biomed.Appl.*, 1997, **696**, 145.
997. S.Yu, K.Sugahara, J.Zhang, T.Ageta, H.Kodama, M.Fontana, and S.Dupre, *J.Chromatogr.B: Biomed.Appl.*, 1997, **698**, 301.
998. T.Yokokura, T.Shirasaki, K.Kohda, and Y.Mochizuki, *Chromatography*, 1997, **18**, 132.
999. S.Bird, P.C.Uden, J.F.Tyson, E.Block, and E.Denoyer, *J.Anal.At.Spectrom.*, 1997, **12**, 785.
1000. B.Anderstam, K.Katzarski, and J.Bergstrom, *J.Am.Soc.Nephrol.*, 1997, **8**, 1437.
1001. C.Kroll and J.Hartmann, *Labor Praxis*, 1997, **21**, 62, 65.
1002. M.Pugniere, H.Mattras, B.Castro, and A.Previero, *J.Chromatogr.A*, 1997, **767**, 69.
1003. K.J.Hunter and A.H.Fairlamb, *Methods Mol.Biol.*, 1998, **79** (Polyamine Protocols), 125.
1004. C.Philippe-Bourgeois, H.Levesque, and B.Maitrot, *Ann.Biol.Clin.*, 1997, **55**, 619; N.Jacob, L.Guillaume, L.Garcon, and M.-J.Foglietti, *Ann.Biol.Clin.*, 1997, **55**, 583.
1005. P.-H.Qiu, H.S.Zhang, G.-L.Xu, and J.-K.Cheng, *Chem.Res.Chin.Univ.*, 1997, **13**, 14 (*Chem.Abs.*, 1997, **126**, 311548).
1006. J.M.You, X.J.Fan, and Q.You, *Chin.Chem.Lett.*, 1997, **8**, 875 (*Chem.Abs.*, 1998, **128**, 35020).
1007. N.Kuroda, Y.Ohyana, K.Nakashima, K.Arizono, and S.Akiyama, *J.Fluoresc.*, 1997, **7**, 239S.
1008. W.W.You, R.P.Haugland, D.K.Ryan, and R.P.Haugland, *Anal.Biochem.*, 1997, **244**, 277.
1009. D.Mou, *Sepu*, 1997, **15**, 319 (*Chem.Abs.*, 1998, **127**, 202330).
1010. G.Shang, Y.Jiang, H.Tang, Y.Fan, S.Wang, G.Dong, and J.Wang, *Sepu*, 1997, **15**, 474 (*Chem.Abs.*, 1998, **128**, 125453).
1011. J.Meyer, N.Richter, and M.Hecker, *Anal.Biochem.*, 1997, **247**, 11.
1012. P.Tsiboli, G.Konstantinidis, Y.Skendros, A.Katsani, and T.Choli-Papadopoulou, *Amino Acids*, 1997, **13**, 13.
1013. Z.M.Habbal and A.Sakr, *Clin.Chim.Acta*, 1997, **264**, 239.
1014. I.N.Krasova, I.V.Kolmakova, and L.A.Kartsova, *Zh.Anal.Khim.*, 1997, **52**, 693.
1015. N.M.Pokrasen, I.A.Shvadchin, V.A.Deer, V.I.Davydov, S.G.Koryakov, and S.S.Stavitskaya, *Klin.Lab.Diagn.*, 1997, 18; J.R.B.Rodriguez, G.G.Reina, and J.J.S.Rodriguez, *Biomed.Chromatogr.*, 1997, **11**, 335.
1016. C.A.Costa, G.C.Trivelato, M.Demasi, and E.J.H.Bechara, *J.Chromatogr.B: Biomed.Appl.*, 1997, **695**, 245.
1017. G.P.Palace and C.H.Phoebe, *Am.Biotechnol.Lab.*, 1997, **15**, 74,76; *Anal.Biochem.*, 1997, **244**, 393.
1018. N.Shindo, S.Nojimo, T.Fujimura, H.Taka, R.Mineki, and K.Murayama, *Anal.Biochem.*, 1997, **249**, 79; D.L.Crimmins and R.Cherian, *Idem*, 1997, **244**, 407.
1019. C.van Wandelen and S.A.Cohen, *J.Chromatogr.A*, 1997, **763**, 11.
1020. M.Reverter, T.Lundh, and J.E.Lindberg, *J.Chromatogr.B: Biomed.Appl.*, 1997, **696**, 1.

1021. M.Kamo, L.-P.Chow, and A.Tsugita, *Res.Commun.Biochem.Cell Mol.Biol.*, 1997, **1**, 61 (*Chem.Abs.*, 1997, **126**, 168720).
1022. Y.Kurosu, T.Iwata, A.Tsuji, and M.Maeda, *J.Chromatogr.A*, 1997, **787**, 261.
1023. Y.Kurosu, K.Murayama, N.Shindo, Y.Shisa, Y.Satou, and N.Ishioka, *J.Chromatogr.A*, 1997, **771**, 311.
1024. D.Tong, R.L.Moritz, J.S.Eddes, G.E.Reid, R.K.Rasmussen, D.S.Dorow, and R.J.Simpson, *J.Protein Chem.*, 1997, **16**, 425.
1025. N.Chauvaux, W.Van Dongen, E.L.Esmans, and H.A.van Onckelen, *J.Chromatogr.A*, 1997, **775**, 143.
1026. A.Ducret, E.J.Bures, and R.Aebersold, *J.Protein Chem.*, 1997, **16**, 323.
1027. H.Matsunaga, T.Santa, T.Iida, T.Fukushima, H.Homma, and K.Imai, *Analyst*, 1997, **122**, 931.
1028. K.Fujii, Y.Ikai, T.Mayumi, H.Oka, M.Suzuki, and K.Harada, *Anal.Chem.*, 1997, **69**, 3346; K.Fujii, Y.Ikai, H.Oka, M.Suzuki, and K.Harada, *Ibid.*, p.5146.
1029. T.Suzuki, T.Watanabe, and T.Toyo'oka, *Anal.Chim.Acta*, 1997, **352**, 357.
1030. T.Fukushima, *Chromatography*, 1997, **18**, 1 (*Chem.Abs.*, 1998, **127**, 62682); Y.Imai, *Ikagaku Oyo Kenkyu Zaidan Kenkyu Hokoku*, 1996, **14**, 189 (*Chem.Abs.*, 1998, **127**, 62687).
1031. Q.-H.Wan, P.N.Shaw, M.C.Davies, and D.A.Barrett, *J.Chromatogr.A*, 1997, **765**, 187.
1032. E.Peyrin, Y.C.Guillaume, and C.Guinchard, *Anal.Chem.*, 1997, **69**, 4979.
1033. T.Miyazawa, H.Minowa, K.Imagawa, and T.Yamada, *Anal.Lett.*, 1997, **30**, 867.
1034. Q.-H.Wan, P.N.Shaw, M.C.Davies, and D.A.Barrett, *J.Chromatogr.A*, 1997, **786**, 249.
1035. M.Laemmerhofer, P.Di Eugenio, I.Molnar, and W.Lindner, *J.Chromatogr.B: Biomed.Appl.*, 1997, **689**, 123.
1036. F.Gasparrini, D.Misiti, W.C.Still, C.Villani, and H.Wennemers, *J.Org.Chem.*, 1997, **62**, 8221.
1037. F.Gasparrini, I.D'Acquarica, C.Villani, C.Cimarelli, and G.Palmieri, *Biomed.Chromatogr.*, 1997, **11**, 317.
1038. J.-M.Lin, T.Nakagama, K.Uchiyama, and T.Hobo, *Biomed.Chromatogr.*, 1997, **11**, 298.
1039. Y.Okamoto and E.Yashima, in Chromatographic Separations Based on Molecular Recognition, ed. K.Jinno, Wiley, New York, 1997, p. 239.
1040. S.A.C.Wren, *J.Chromatogr.A*, 1997, **768**, 153.
1041. Y.Deng, W.Maruyama, M.Kawai, P.Dostert, and M.Naoi, *Prog.HPLC – HPCE*, 1997, **6**, 301.
1042. S.M.Han, *Biomed.Chromatogr.*, 1997, **11**, 259.
1043. Handbook of Capillary Electrophoresis, Second Edition, ed. J.P.Landers, CRC, Boca Raton, Florida, 1997; Handbook of Capillary Electrophoresis Applications, Eds. H.Shintani and J.Polonsky, Blackie, London, 1997 (*e.g.* applications to amino acid analysis, L.J.Brunner, p. 149).
1044. R.Lehmann, W.Voelter, and H.M.Liebich, *J.Chromatogr.B: Biomed.Appl.*, 1997, **697**, 3; E.Lickl, *Oesterr.-Chem.Ztg.*, 1997, **98**, 128.
1045. T.-S.Hsi, J.-N.Liu, and K.-Y.Kuo, *J.Chin.Chem.Soc.*, 1997, **44**, 101.
1046. Z.Wang and E.S.Yeung, *J.Chromatogr.B: Biomed.Appl.*, 1997, **695**, 59.
1047. G.M.Robinson and M.R.Smyth, *Analyst*, 1997, **122**, 797.
1048. K.C.Panak, S.A.Giorgieri, L.E.Diaz, and O.A.Ruiz, *Electrophoresis*, 1997, **18**, 2047.
1049. H.Chen, Y.Xu, and M.P.C.Ip, *J.Liq.Chromatogr., Relat.Technol.*, 1997, **20**, 2475.
1050. S.Cladrowa-Runge and A.Rizzi, *J.Chromatogr.A*, 1997, **759**, 157.

1051. J.Zhou, J.Liao, X.Qian, and F.Dong, *Sepu*, 1997, **15**, 159 (*Chem.Abs.*, 1998, **127**, 297348).
1052. Q.Chu, B.T.Evans, and M.G.Zeece, *J.Chromatogr.B: Biomed.Appl.*, 1997, **692**, 293.
1053. S.Tucci, P.Rada, M.J.Sepulveda, and L.Hernandez, *J.Chromatogr.B: Biomed.Appl.*, 1997, **694**, 343.
1054. A.Tivesten and S.Folestad, *Electrophoresis*, 1997, **18**, 970.
1055. L.A.Dawson, J.M.Stow, and A.M.Palmer, *J.Chromatogr.B: Biomed.Appl.*, 1997, **694**, 455.
1056. S.Oguri, K.Yokoi, and Y.Motohase, *J.Chromatogr.A*, 1997, **787**, 253.
1057. S.H.Kang, J.-W.Kim, and D.S.Chung, *J.Pharm.Biomed.Anal.*, 1997, **15**, 1435.
1058. D.J.Pietrzyk, S.Chen, and B.Chanthawat, *J.Chromatogr.A*, 1997, **775**, 321.
1059. W.H.Church, C.S.Lee, and K.M.Dvanchak, *J.Chromatogr.B: Biomed.Appl.*, 1997, **700**, 253.
1060. I.D.Ireland, D.F.Lewis, X.-F.Li, A.Reuborg, S.Kwong, M.Chen, and N.J.Dovichi, *J.Protein Chem.*, 1997, **16**, 491.
1061. T.Schmitt, *Progr.HPLC-HPCE*, 1997, **5** (CE in Biotechnology and Environmental Analysis), 383.
1062. S.Cladrowa-Runge and A.Rizzi, *J.Chromatogr.A*, 1997, **759**, 167.
1063. I.E.Valko, H.Siren, and M.-L.Riekkola, *Electrophoresis*, 1997, **18**, 919.
1064. M.Tsunoda, M.Kato, T.Fukushima, T.Santa, H.Homma, and K.Imai, *Chromatography*, 1997, **18**, 21 (*Chem.Abs.*, 1998, **127**, 62753); M.Tsunoda, M.Kato, T.Fukushima, T.Santa, H.Homma, K.Imai, H.Yanai, and T.Soga, *ibid.*, 1997, **18**, 310.
1065. K.De Silva and T.Kuwana, *Biomed.Chromatogr.*, 1997, **11**, 230.
1066. A.Tivesten, A.Lundqvist, and S.Folestad, *Chromatographia*, 1997, **44**, 623.
1067. J.-M.Lin, T.Nakagama, K.Uchiyama, and T.Hobo, *J.Pharm.Biomed.Anal.*, 1997, **15**, 1351.
1068. L.B.Creemers, D.C.Jansen, A.van Veen-Reurings, T.van den Bos, and V.Everts, *BioTechniques*, 1997, **22**, 656.
1069. G.Zhang and A.W.Bown, *Phytochemistry*, 1997, **44**, 1007.
1070. W.J.C.Geerts, A.Jonker, L.Boon, A.J.Meijer, R.Charles, C.J.F.Van Noorden, and W.H.Lamers, *J.Histochem.Cytochem.*, 1997, **45**, 1217.
1071. P.D.Shaw, G.Ping, S.L.Daly, C.Cha, J.E.Cronan, K.L.Rinehart, and S.K.Farrand, *Proc.Natl.Acad.Sci.USA*, 1997, **94**, 6036.
1072. M.-L.Feng, Y.-W.Huang, Z.-L.Gong, and Z.-J.Zhang, *Gaodeng Xuexiao Huaxue Xuebao*, 1996, **17**, 1859 (*Chem.Abs.*, 1997, **126**, 248413).
1073. G.A.Rivas and B.Maestroni, *Anal.Lett.*, 1997, **30**, 489.
1074. C.Bala, M.Rujoi, S.Fleschin, and V.Magearu, *Roum.Biotechnol.Lett.*, 1997, **2**, 365.
1075. M.Sawai, A.Saito, K.Inoue, Y.Ohta, and K.Yoda, *J.Adv.Sci.*, 1997, **9**, 93.
1076. S.H.Chough, J.H.Kim, S.Y.Kim, S.B.Chun, K.C.Nam, S.D.Cho, and O.J.Choi, *Proc.-Electrochem.Soc.*, 1997, **97-19**, 874.
1077. J.R.Woodward and R.B.Spokane, *Am.Biotechnol.Lab.*, 1997, **15**, 82; M.B.Madaras, R.B.Spokane, J.M.Johnson, and J.R.Woodward, *Anal.Chem.*, 1997, **69**, 3674.
1078. N.Kiba, A.Itagaki, and M.Furusawa, *Talanta*, 1997, **44**, 131.
1079. P.Akhtar, C.O.Too, and G.G.Wallace, *Anal.Chim.Acta*, 1997, **339**, 201.
1080. P.Akhtar, C.O.Too, and G.G.Wallace, *Anal.Chim.Acta*, 1997, **339**, 211.
1081. T.Huang, A.Warsinke, T.Kuwana, and F.W.Scheller, *Anal.Chem.*, 1998, **70**, 991.
1082. C.Jeffries, N.Passo, K.Baronian, and L.Gorton, *Biosens.Bioelectron.*, 1997, **12**, 225.
1083. A.L.Simonian, E.I.Rainina, P.F.Fitzpatrick, and J.R.Wild, *Biosens.Bioelectron.*, 1997, **12**, 363.
1084. O.Niwa, R.Kurita, T.Horiuchi, and K.Torimitsu, *Anal.Chem.*, 1998, **70**, 89.

2
Peptide Synthesis

BY DONALD T. ELMORE

1 Introduction

As in the previous Report,[1] new techniques are becoming less numerous, but reviews and applications of peptide synthesis continue to increase. Some reviews[2–8] relate to several sections of this Report whereas others are cognate to particular sections as follows;- Section 2.1,[9] Section 2.3,[10] Section 2.4,[11,12] Section 2.5,[13–19] Section 2.6,[20–49] Section 2.7,[50–53] Section 3.1,[54–57] Section 3.4,[58–60] Section 3.5,[61–71] Section 3.6,[72] Section 3.7,[73,74] Section 3.8,[75] Section 3.9[76–83] and Section 3.10.[84–88] Readers are reminded that references to symposia papers and patent literature are not included in this Report.

2 Methods

2.1 Amino-group Protection – *N*-Acetyl- and *N*-benzoyl derivatives of amino-acid esters can be converted into the corresponding *N*-Boc derivatives in two steps.[89] The substrate is first reacted with 2 equivalents of Boc_2O and 0.2 equivalents of 4-dimethyl-aminopyridine preferably in tetrahydrofuran to give the *N*-acyl-*N*-Boc derivative. The *N*-Ac or *N*-Bz group is then removed with LiOH or N_2H_4. If the latter (4 equivalents) is used in MeOH, the two steps can be effected as a one-pot reaction. Experiments designed to replace *N*-Ac by *N*-Z were only partially successful. *N*-Boc groups can be removed using BF_3-Et_2O and molecular sieves in CH_2Cl_2 at room temperature.[90] The base-labile 2-(4′-nitrophenyl-sulfonyl)ethoxy-carbonyl (Nsc) group can be used as an alternative to Fmoc as witnessed by SPPS of several models including the inevitable ACP(65–74).[91] *N*-Boc- and *N*-4-methoxybenzyloxy-carbonyl derivatives of amino acids can be prepared[92] from the corresponding benzotriazole derivatives (**1**). Improved syntheses of the Boc and Fmoc derivatives of 4-(2′-aminoethyl)-6-dibenzo-furanpropionic acid (**2**) have been described.[93] This amino acid nucleates β-sheet folding in a synthetic peptide. Difficulties experienced while using the Fmoc group have prompted Carpino[94] to seek a different reagent where deprotection is simultaneously accompanied by a scavenging process. Appropriate location of a urethane group relative to a Michael acceptor sufficed (Scheme 1). A sulfonyl group proved to be satisfactory for X and experience with three candidate groups focused attention on the 1,1-dioxobenzo[*b*]thiophene-2-ylmethoxycarbonyl (Bsmoc) group (**3**).

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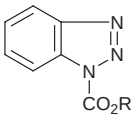
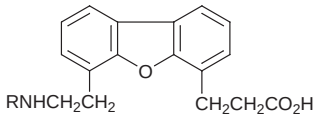
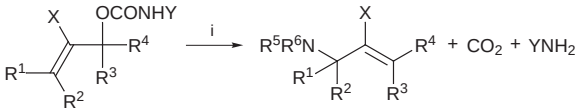


(**1**) R = Bu^t, 4-MeOBzI



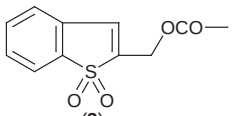
(**2**) R = Boc, Fmoc



(X = electron–attracting group; Y = amino acid or peptide residue)

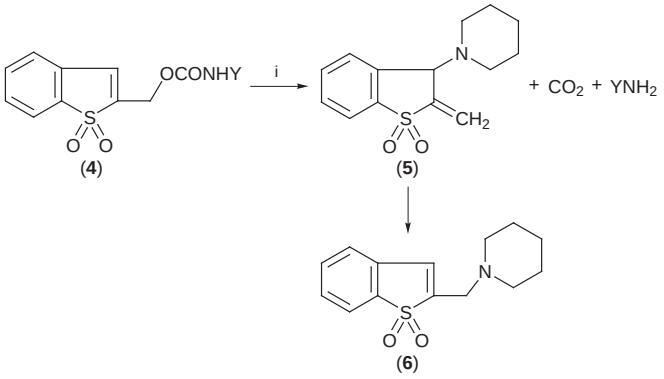
Reagent: i, R^5R^6NH

Scheme 1



(**3**)

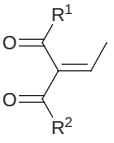
Deprotection of a Bsmoc derivative (**4**) with piperidine is outlined in Scheme 2. The labile intermediate (**5**) spontaneously rearranges to the stable product (**6**) in a short time. Some Bsmoc-derivatives were noncrystalline. The use of the related 1,1–dioxonaphtho[1,2–*b*]thiophene-2–ylmethoxycarbonyl (Nsmoc) derivatives avoided this slight difficulty. The 2–benzoyl-2–ethoxycarbonylvinyl group (**7**) (R^1 = OEt, R^2 = Ph) has been examined for amino-group protection.[95] Deprotection is achieved by reaction with $NH_2OH.HCl$ or $N_2H_4.HCl$ which produces the hydrochloride of a peptide ester and respectively (**8**) or (**9**). Some variation of the substituents R^1 and R^2 is possible. The 2–picolinoyl group is a potential candidate for protection of α-amino groups[96] since it can be removed by

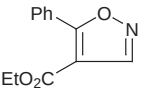


(**4**) (**5**) (**6**)

Reagent: i, piperidine

Scheme 2


(7)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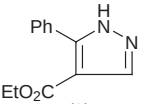
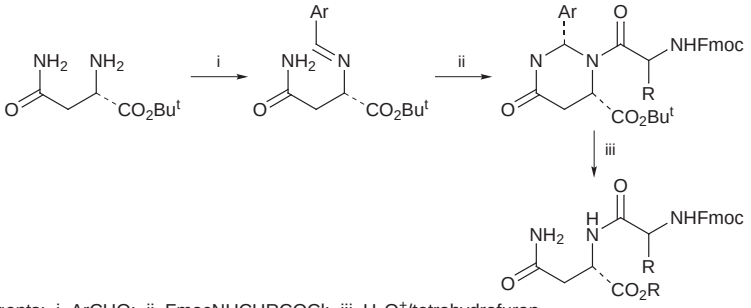

(9)

electrochemical reduction in aqueous MeOH at pH 2 without noticeable enantiomerization at any stage. When using *N*-Alloc protection at the *N*-terminus and an *O*-dimethylallyl ester at the *C*-terminus, the former can be removed by $Pd(OAc)_2$/triphenylphosphino trisulfonate sodium salt without affecting the latter.[97] *N*-Alloc protection of α-amino groups gives satisfactory results in SPPS using $Pd(PPh_3)_4$ in the presence of $PhSiH_3$ as a scavenger for the allyl system.[98] A tandem *N*-terminal deprotection – coupling protocol was developed to avoid formation of diketopiperazine where this was a danger. As a development of the use of the Dde group for *N*-terminal protection, 2-biotinoyl- and 2-hexanoyldimedone were prepared.[99] The former, for example, could be attached to a resin-bound peptide. After detachment, the peptide derivative could be purified by affinity chromatography on an avidin-agarose column. The free peptide was then released by elution with 5% aqueous N_2H_4. Dipeptide surrogates containing a tetrahydropyrimidinone derived from asparagine have been prepared (Scheme 3)



Reagents: i, ArCHO; ii, FmocNHCHRCOCl; iii, H_3O^+/tetrahydrofuran

Scheme 3

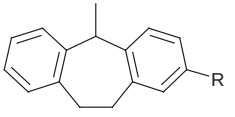
and used in SPPS.[100] The heterocycle protecting the two *N*-atoms of Asn could then be cleaved by mild aqueous acid hydrolysis. *N*-Alkylamino acids with a substituent in the ω-position of the alkyl group are accessible by reductive alkylation of amino acids and the Boc and Fmoc derivatives of these can be prepared using temporary trimethylsilyl protection.[101] Incorporation of a trifluoromethylamino acid into the *C*-terminal position of a peptide has been effected using *N*-Teoc derivatives of α-CF_3-amino acids.[102] The monomethoxytrityl group has been used to protect the ε-amino group of Lys residues in the synthesis of complex prodrugs of anticancer compounds.[103] It is cleanly removed by Cl_2CHCO_2H and $ClCH_2CO_2H$ in presence of anisole. In the synthesis of methyl ketones of peptides containing *C*-terminal Lys, amino groups were protected with

the Z-group and only hydrogenation was satisfactory for subsequent removal of these groups.[104]

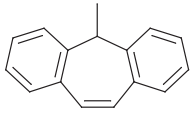
2.2 Carboxy-group Protection – t-Butyl esters of amino acids can be prepared in a one-pot reaction using anhydrous $MgSO_4$ and a catalytic quantity of concentrated H_2SO_4.[105] Hydroxyamino acids are converted into the t-butyl ether by this method. t-Butyl 2-(trifluoroacetylamino)carboxylates, which are accessible by the alkylation of trifluoroacetamide with t-butyl 2-bromocarboxylates, are selectively hydrolysed to the hydrochlorides of the t-butyl esters of amino acids under liquid-liquid phase transfer catalytic conditions using CH_2Cl_2 or Et_2O, aqueous 20% KOH, 10% triethylbenzylammonium chloride at 25–40 °C.[106] $MgBr_2$-Et_2O in CH_2Cl_2, which had previously been shown to cleave β-(trimethylsilyl)ethoxymethyl esters of carboxylic acids, has been found to function similarly with derivatives of amino acids and peptides.[107] This mild method could be useful especially when orthogonal protection of carboxy groups is required. 4–Hydroxyphenacyl esters of simple peptides release the free peptide and 4–hydroxyphenylacetic acid under the influence of a photo-trigger.[108] γ-2-Adamantyl glutamate has been found suitable in peptide synthesis.[109] The ester group is removed using $MeSO_3H$, M-CF_3SO_3H/PhSMe in CF_3CO_2H or HF.

2.3 Side-chain Protection – *O*-Alkylation of Ser derivatives can be achieved with various alkyl bromides or iodides with finely divided ground KOH and Aliquat 337 at room temperature.[110] The tris(trimethylsilyl)silyl (sisyl) group is of potential interest for the protection of side-chain hydroxyl groups although it has not been applied to peptide synthesis yet.[111] It is resistant to fluoride but labile to photolysis with a medium pressure Hg lamp. The Na^+ salt of Thr or the Li^+ salt of Ser react with $BF_3.Et_2O$ in tetrahydrofuran to give a 2,2–difluoro-1,3,2-oxazaborolidin-5-one in which the NH_2 and CO_2H groups are simultaneously blocked, allowing formation of the t-butyl ether by reaction with $Me_2C{=}CH_2$ with BF_3–H_3PO_4 as catalyst.[112] H-Ser(Bu^t)-OH is liberated by mild hydrolysis. The hydroxyl group of Tyr can be protected by the 2-Adoc group by Schotten-Baumann type acylation of the Cu complex of the amino acid.[113] The 2-Adoc group is stable to mild acid treatment but detached by reaction with M-CF_3SO_3H/thioanisole and HF. The 2,4-dimethylpent-3-yloxycarbonyl (Doc) group is another new group for the protection of the Tyr hydroxy group.[114] It is stable to piperidine but completely cleaved by HF.

New protecting groups for the side chain of arginine have been reported.[115,116] They (**10**, **11**) are based on the dibenzo[*a*,*d*]cycloheptene structure and are acid-labile, although **10b** is too labile to be useful synthetically. It is notable that Trp



(**10**) a, R = H; b, R = OMe

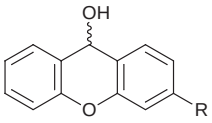


(**11**)

residues need not be protected from alkylation by carbocations if protecting groups are removed at ~6 °C.[115]

If the imidazole ring of His residues is protected by the 2,4-dinitrophenyl group, removal of Fmoc groups by 20% piperidine in $CHONMe_2$ partially deprotects the heterocycle.[117] The imidazole group of His can be protected by allylation.[118] Boc-His-OMe can be converted into the N^{τ}-trityl derivative which provides a route to the N^{π}-allyl derivative. Alternatively, Boc-His-OMe reacts with excess $CH_2{=}CHCH_2Br$ to give the bis-allyl derivative as the hydrobromide. Surprisingly, reaction of the latter with $Et_2NH/NaHCO_3$ in the presence of a catalytic amount of $Pd(PPh_3)_4$ or $PhSiH_3/NaHCO_3$ removed the more hindered N^{π}-allyl group and the resultant N^{τ}-derivative could be converted into the N^{τ}-Bom compound. Reaction of Boc-His(N^{τ}-Boc)-OMe with 2–adamantyloxymethyl chloride (2–Adom-Cl) followed by saponification afforded Boc-His(N^{π}-Adom)-His-OH.[119] This can be used for peptide synthesis without fear of enantiomerization and the process is assisted by the enhanced solubility of intermediates and products in organic solvents. The 2–Adom group is stable to CF_3CO_2H, M-NaOH and 20% piperidine/$CHONMe_2$ but is easily removed by M-CF_3SO_3H/thioanisole.

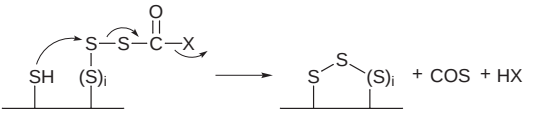
Novel xanthenyl groups (Xan and 2-Moxan, **12**) have been used for thiol group protection.[120] Cysteine or other thiol-containing amino acid is reacted with xanthydrol and CF_3CO_2H as catalyst. Both groups are stable under conditions used to remove Fmoc groups, but are cleaved by I_2 or $Tl(OCOCF_3)_3$. An unexpected lability of the Acm group has been observed.[121] After solid-phase assembly of H-Asn-Gly-Gly-Cys(Acm)-Glu(OBu^t)-Gln-Tyr(Bu^t)-Cys(Acm)-Ser(Bu^t)-Asp(OBu^t)-resin, attempted deprotection and cleavage with various reagents containing CF_3CO_2H resulted in cleavage of some Acm groups and some alkylation of the Tyr residue ortho to the OH group. Formation of both byproducts could be avoided by using more dilute cleavage/deprotection reagents and by including a scavenger such as phenol.



(**12**) R = H, OMe

2.4 Disulfide Bond Formation – In a comparative study of methods of forming disulfide bonds by oxidising reduced oxytocin, the best yield was obtained using CF_3CO_2H/Me_2SO (5:1).[122] Other similar comparative studies have been carried out[123,124] and the latter paper recommends treatment of the bis-Acm peptide with $Hg(OAc)_2$ followed by treatment with H_2O_2. It is clear that no method for forming disulfide bonds is universally superior to other procedures. A more ambitious project involved the synthesis of a 32–residue peptide obtained from amaranth seeds.[125] Of several reagents used to remove Acm groups, $Hg(OAc)_2$ was best. Cyclopsychotride, a 31-residue cyclic peptide from the tropical plant

Psychotria longibes, containing three intrachain disulfide bonds, has been synthesized using orthogonal methodology for forming the correct disulfide bonds.[126] Methods for forming disulfide and trisulfide bridges in peptides have been thoroughly reinvestigated.[127] The sulfur bridges were found to be best formed by directed methods (Scheme 4) while the peptide was still attached to the resin used for SPPS. Peptides containing two residues of Se-(4-methoxybenzyl)selenocysteine appropriately spaced in the peptide chain have been synthesized.[128] Removal of the 4-methoxybenzyl groups with piperidine had to be carried out in the minimum time in order to suppress β-elimination of 4-$MeOC_6H_4CH_2SeOH$ and loss of chirality. Formation of an intramolecular diselenide bridge was unsatisfactory except with Me_2SO/CF_3CO_2H.

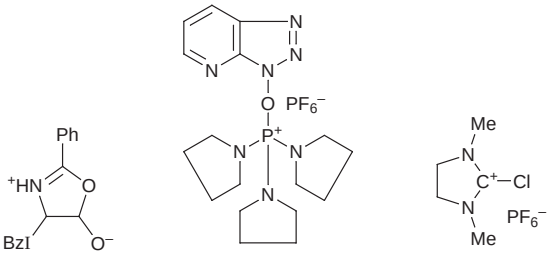


Scheme 4

2.5 Peptide Bond Formation – Several carbodiimides have been compared for their efficiency in forming peptide bonds.[129,130] The use of additives such as HOBt, HOSu and *N*-hydroxy-5-norbornene-2,3-dicarboximide to enhance peptide bond formation in coupling reactions mediated by *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbo-diimide in aqueous solution has been studied.[131] Only 0.1 moles of additive is sufficient. The formation of the mesionic compound, 4-benzyl-2-phenyl-1,3-oxazolium-5-olate (**13**), has been observed during the carbodiimide-mediated synthesis of Bz-Phe-Leu-OMe.[132] This compound can also be obtained by tautomeric transformation of 4-benzyl-2-phenyl-5(4H)-oxazolone. A series of N^{π}-Bpoc amino acid pentafluorophenyl esters including several with bulky side chains has been synthesized.[133] A series of 4-sulfotetrafluorophenyl esters of amino acids has been described.[134] They are highly reactive and hydrophilic compounds and can be used in aqueous solution.

Urethane-protected *N*-carboxyanhydrides (UNCAs) are uniquely suited for the study of enantiomerization during peptide bond formation since only the direct abstraction of proton from the α-carbon atom is available. Such a study has been carried out[135] and the enantiomers were separated by chiral HPLC. The influence of the nature of tertiary amine used, the side chain structure of the UNCA and the nature of the solvent were studied. The coupling of Cys in SPPS with the side chain protected with Acm, Trt, Tmob or Xan using coupling agents such as BOP, HBTU, HATU, or PyAOP gives unacceptable levels of enantiomerization especially if a preactivation period is used.[136] By avoiding a preactivation period, use of smaller quantities of weaker bases and change of solvent from $CHONMe_2$ to CH_2Cl_2, the loss of chirality was greatly diminished.

Phosphonium derivatives of 1-hydroxy-7-azabenzotriazole (HOAt) such as PyAOP (**14**) are useful for the synthesis of peptides of hindered amino acids, difficult short sequences and cyclic peptides.[137] An advantage of phosphonium



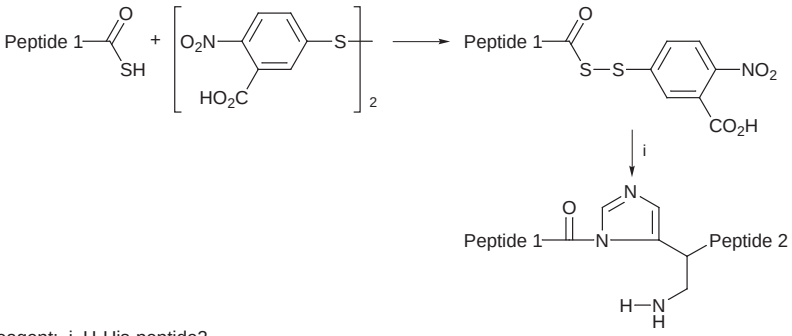
(**13**) (**14**) (**15**)

reagents relative to uronium salts is the absence of the detrimental side reaction at the *N*-terminus which blocks further chain assembly. In the assembly of the notorious ACP(65–74), PyAOP/HOAt gave better yields than PyAOP alone or with HOBt and better than PyBOP with or without HOBt. Interestingly, four new organophosphorus coupling reagents have been described.[138] Tetramethylfluoro-formamidinium is reported to be an excellent reagent for the formation of acyl fluorides in peptide synthesis.[139] 2-Chloro-1,3-dimethylimidazolidium hexafluorophosphate (**15**) in the presence of HOAt or HODhbt is a suitable reagent for synthesizing peptaibol peptides.[140] Alamethicin F-30 (containing 8 Aib residues) and trichovirin I4A (containing 5 Aib residues) were synthesized using this coupling agent.

In the Kent method of coupling peptide segments, one of which has a *C*-terminal thioester group while the other has an *N*-terminal Cys residue, the undesirable removal of an Acm group is suppressed when AgCl is used as an activator of the thioester moiety.[141] It was further found that addition of an excess of a thiol had considerable influence on the rate of coupling of two peptides by the thioester method.[142] The highly reactive 3-nitro-4-carboxybenzyl α-thioester is converted into the less reactive thiobenzyl ester by addition of $C_6H_5CH_2SH$. Conversely, a weakly reactive peptide α-thioester (e.g. $-COSCH_2CO_2H$) is converted into a more reactive ester by the addition of C_6H_5SH. This paper also describes the synthesis of a barnase analogue by forming a Gly^{48}-Cys^{49} bond. Barnase contains a Lys residue at position 49 so it was somewhat surprising that no attempt was made to convert Cys^{49} in the analogue into a thialysine residue. The related method of Kemp and Tam has been extended by having *N*-terminal His in the *C*-terminal fragment.[143] The imidazole ring is postulated to afford anchimeric assistance in the coupling step (Scheme 5).

There are two reports of physical aspects of peptide bond formation. The use of sonication in peptide synthesis has been described.[144] From the rate enhancement of peptide synthesis induced by increased pressure, the activation volume, $\Delta V^{\ddagger}$, can be computed. Values reported[145] are strongly negative, indicating an association with development of charges proceeds in a rate-determining transition state.

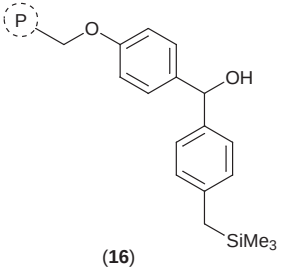
2.6 Peptide Synthesis on Macromolecular Supports and Methods of Combinatorial Synthesis – Most of the research activity on new methodology is focused on this



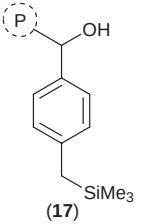
Reagent: i, H-His-peptide2

Scheme 5

section of the report. Some new supports and new linkers have been devised. 3-Nitrobenzophenone oxime resin is claimed to be more stable than the isomeric Kaiser resin and to be more reactive.[146] 4-Bromomethyl-3-nitrobenzamidobenzylpolystyrene resin has been used to synthesize Leuprolide, an analogue of LHRH.[147] Sepharose™ to which 1,3–diaminopropane and *p*-[(R,S)-α-[1-(9H-fluorenyl-9-yl)methoxyformamido]-2,4-di-methoxybenzyl]phenoxyacetic acid had been linked in turn was used to synthesize ACP(65–74) using Fmoc chemistry[148] with satisfactory results. 4-Hydroxy-2-methoxybenzaldehyde was coupled to Merrifield resin and the product was reduced with $NaBH_4$ affording a new synthesis of SASRIN™ resin.[149] The use of two high-loading resins has been described.[150,151] 4-Hydroxymethylbenzoic acid can be attached as a stable linker to cellulose for peptide synthesis by the SPOT technique.[152] Two new fluoride-labile linkers (**16,17**) have been designed.[153] Peptide detachment can be achieved

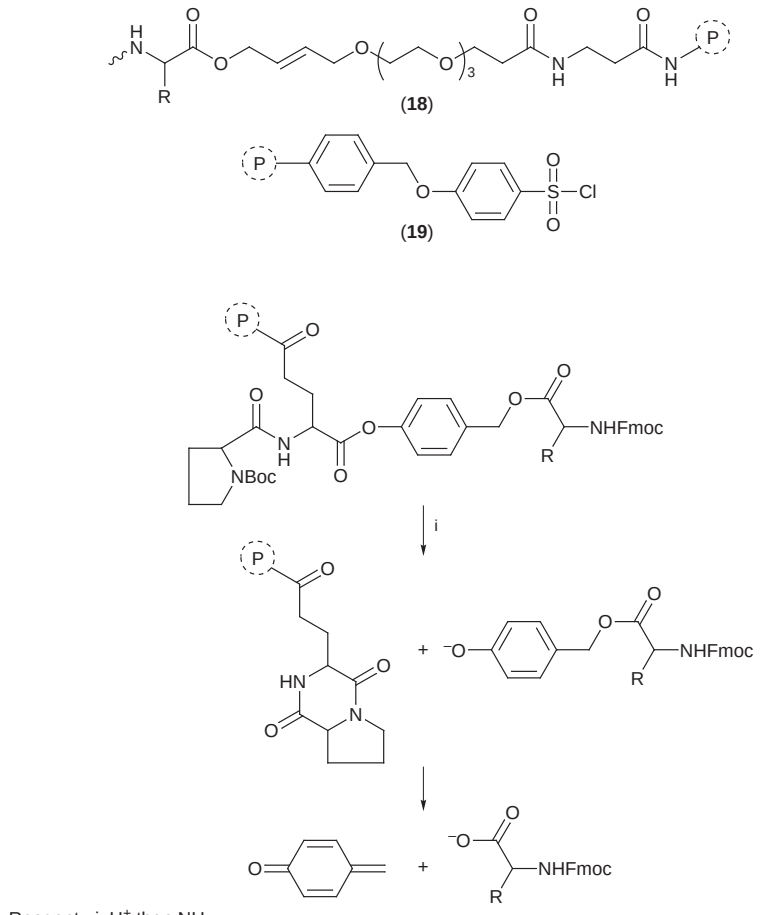


(**16**)



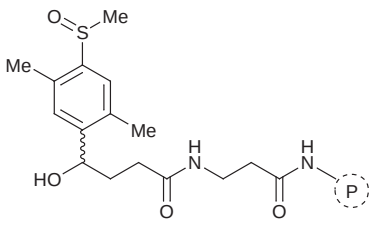
(**17**)

under basic or neutral conditions. An improved allylic linker (**18**) has been developed which contains a flexible portion that permits access of Pd(0) complex during the detachment step.[154] A new support (**19**) for the synthesis of Arg peptides is covalently bound to the guanidino group and consequently assembly can proceed from either end.[155] Detachment is best achieved with HF since other acids tend to give some product still bearing the linker. An ingenious linker containing a safety catch which is operated when a Boc group is removed by acid

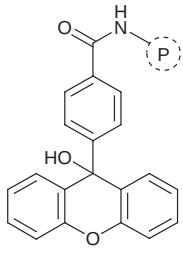


Scheme 6

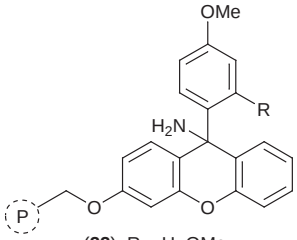
is depicted in Scheme 6.[156] The 4-methylsulfinylbenzyl (Msob) group, which is rather stable to acid, has been used for side-chain protection and incorporated into linkers (e.g. **20**).[157] The acid stability of the Msob group vanishes when the sulfoxide group is reduced so that the peptide is detached from the resin and Msob-based protecting groups are removed. Fmoc protection can be used during SPPS. Some new xanthene derivatives (**21,22**) have been used as linkers for the SPPS of amides.[158,159] New tritylamine-based linkers (**23**) have been developed for the same purpose.[160] Secondary amides of peptides can be prepared using 2-methoxy-4-benzyloxypolystyrene aldehyde resin.[161] Peptide hydroxamic acids can be prepared by assembling a peptide on 4-methylbenzhydrylamine resin followed by reaction with NH_2OBzl and then deprotection by HF.[162] Aminoxy-trityl[163] and -2-chloro-trityl[164] supports have been used for the synthesis of



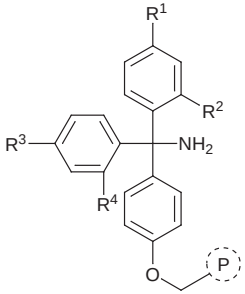
(**20**)



(**21**)



(**22**) R = H, OMe



(**23**) R^1 = H, OMe; R^2 = H, OMe; R^3 = H, OMe; R^4 = H, OMe

peptide hydroxamic acids. Alternatively, protected *N*-alkylhydroxylamines can be attached to a resin and peptide assembly using Fmoc chemistry affords peptide *N*-alkylhydroxamic acids.[165] Peptide aldehydes have been obtained by a multipin method using a methacrylic acid/dimethylacrylamide polymer grafted on to polyethylene pins.[166] An alternative method starts from an αβ-unsaturated δ-amino acid which is the C-terminal residue in SPPS; on completion of assembly, the peptide aldehyde is liberated by ozonolysis.[167] Two new photolabile linkers attached to polymeric supports have been prepared[168,169] and these offer another method of detaching products of solid-phase syntheses under mild conditions. The generation of *C*-terminal thioesters is of some interest. By using a linker containing a thiol group, SPPS generates a thioester which can be cleaved with alcohols, amines and organometallic reagents affording esters, amides, ketones, aldehydes and alcohols.[170] Thiolesters have been prepared by coupling $HS(CH_2)_2CO_2R$ after detachment from a 2-chlorotrityl resin.[171] If a hydroxymethyl resin is treated with disuccinimidyl carbonate an unsymmetrical carbonate ester is formed. This will react with the free hydroxyl group in the side chain of Ser, Thr or Tyr and a peptide can be assembled and converted into a homodetic cyclic peptide while still attached thus avoiding an intermolecular coupling at the point of cyclization.[172]

Cs salts of Boc amino acids react rapidly with chloromethyl polystyrene in the presence of a catalytic amount of dibenzo-18-crown-6 in CH_3CONMe_2 and Fmoc amino acids can be similarly coupled to Wang resin.[173] A problem has been encountered in the attachment of glycosylated Ser and Cys derivatives.[174]

Unacceptable levels of enantiomerization have been found with several coupling methods and best results were obtained using 2′,4′,6′-mesitylenesulfonyl-3-nitro-1,2,4-triazolide. An amino acid attached by its carboxy group to a resin can be reacted with 2–nitrobenzenesulfonyl chloride in presence of $Pr^{i}_{2}NEt$, the product *N*-methylated under Mitsunobu conditions and the protecting group removed with PhSNa in $CHONMe_2$. The published paper reports the introduction of an Fmoc group and the detachment of the protected *N*-methyl amino acid,[175] but this procedure could be the first stage of the SPPS of a peptide with a *C*-terminal *N*-methylamino acid. A similar method can be used to methylate particular amino acids in peptide undergoing SPPS.[176] Six analogues of a thrombin receptor agonist hexapeptide were synthesized in which a different amino acid was methylated in each case. The peptides contained Arg(Pmc) and some reaction with the side chain occurred. This was best avoided by assembling the related peptide containing Orn and guanidinating it after assembly. The *N*-terminal amino acid of a peptide attached to a resin can be alkylated by first forming a Schiff base with an aromatic aldehyde then reacting the protected peptide with an alkyl halide in the presence of a base to effect alkylation.[177] The peptide can be either further extended or detached as required.

Attachment of the α-amino group of the intended *N*-terminal amino acid to a support has not been widely used because of the high risk of loss of chirality in the product. SPPS of mini-libraries, however, has been reported.[178] A more extensive and ambitious study has been made of inverse SPPS.[179] Careful choice of linker and coupling conditions together with strategic backbone protection enabled some peptides of high optical purity to be synthesized. A new method for evaluating conductimetric data during SPPS has been described.[180] A neural network algorithm is used and this can predict the final yield by analysing the conductivity signal over the first 5 min.

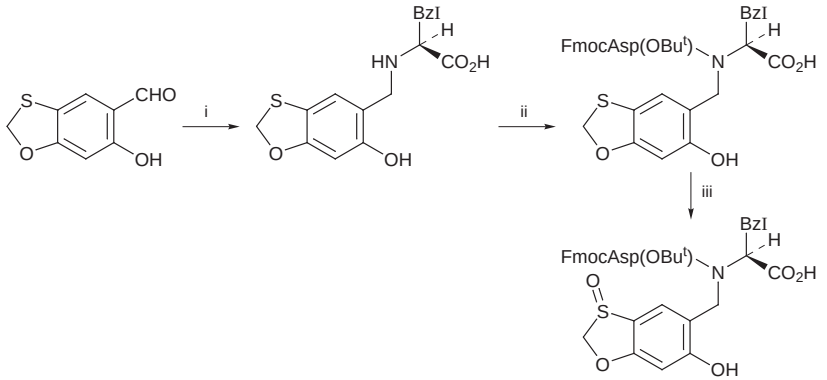
Azido acids, obtained by α-bromination of alkyl acids followed by reaction with NaN_3, can be converted into acid chlorides which can then be used in SPPS. The azido group is reduced to amino by dithiothreitol.[181] Another interesting variation of conventional SPPS involves the synthesis of β-peptides using the Arndt-Eistert method of homologation of a peptide attached to an inert support.[182] SPPS of peptides containing the ψ[CH_2NH] moiety can be complicated by double alkylation especially if the pseudopeptide group is followed by Gly when double alkylation can occur. This can be circumvented by protection of the secondary amine with the 2,4-dimethoxybenzyl group.[183] Deprotection is effected using M CF_3SO_3H/PhSMe/CF_3CO_2H. SPPS of peptides containing sulfated Tyr residues can be complicated by loss of the sulfate group at the deprotection and detachment stages. A two-stage process is now recommended in which the peptide is detached from the support using CH_3CO_2H/CF_3CH_2OH/CH_2Cl_2 (1:1:3, 25 °C, 45 min.) followed by 90% CF_3CO_2H for removal of protecting groups.[184] In the synthesis of peptides containing 4-benzoylphenylalanine, some dithioketal was formed during the acidolytic detachment of product in the presence of dithiols as scavengers.[185] This side reaction can be avoided by using dithiothreitol as scavenger. The release of peptides that are attached to hydrophilic supports by the Dpr(Phoc) linker can be facilitated by adding Ca^{2+}

ions[186], thus reducing the need for high OH^- concentrations. Resin-bound peptides can be cleaved from Kaiser oxime resin using an oxygen nucleophile (H_2O, CH_3OH, BzlOH) in the presence of 1,8–diaza-bicyclo[5,4,0]undec-7-ene (DBU).[187] Protecting groups are retained.

Difficult solid-phase syntheses continue to attract attention. ACP(65–74) has been prepared by SPPS at 60 °C using diisopropyl carbodiimide with no problems arising from peptide aggregation.[188] A search was also made for an optimized synthesis of unsulfated CCK12. More syntheses along these lines are desirable to determine if aggregation is regularly circumvented and to ensure that enantiomerization is not an alternative difficulty. A detailed study of the synthesis of H-Ala-Arg-$(Ala)_6$-Lys-OH has been made.[189] Aggregation interfered with removal of Fmoc group, a problem that was alleviated by using DBU. Inefficient coupling in the later steps was overcome by double coupling. The authors recommend using absorbance measurements to monitor deprotection and to detect the onset of aggregation. Similar problems were encountered in the synthesis of H-$(Ala)_6$-Lys-OH.[190] The authors used near-infrared Fourier transform spectroscopy to monitor the progress of reaction. Yet another ploy to detect aggregation of a peptide chain involves the incorporation of 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid in the peptide so that chain aggregation can be detected by EPR spectroscopy.[191]

Two papers[192,193] describe the synthesis of N^{π}-Fmoc-N^{π}-(2-hydroxy-4-methoxybenzyl)amino acids and their incorporation into peptides by SPPS. 4,5-Dihydro-8-methoxy-1,4-benzoxazepin-2(3H)-one is reported to be an intermediate in the coupling step.[193] Several difficult sequences were successfully synthesized. Using the longer known bis-Fmoc-Hmb amino acids, a 34-residue sequence from the rat bradykinin receptor fragment has been synthesized.[194] Although reversible alkylation of the *C*-terminal amide bond of a protected peptide segment with a 2-hydroxy-4-methoxybenzyl group dramatically suppresses enantiomerization during subsequent coupling, formation of a 4,5-dihydro-8-methoxy-1,4-benzoxazepin-2(3H)-one during activation greatly decreases the rate of coupling[195] (cf. ref. 193). A new safety-catch protecting group, 6-hydroxy-5-methyl-1,3-benzoxathiolyl-, has been designed and found to be satisfactory. Scheme 7 outlines its synthesis and use. The Fmoc group can be removed by piperidine/$CHONMe_2$ (1:4) and the protecting group is labilized for acidic hydrolysis by reduction of the sulfoxide group when assembly is complete (cf. ref. 157). Despite the plethora of resins, linkers, protecting groups and coupling methods available, not every synthetic protocol yields high yields of pure peptides. A neat method of peptide purification has been designed.[196] A removable handle, e.g. $BocNH(CH_2)_2SO_2(CH_2)_2OCO$-, is attached to the free amino group of the finished peptide. The Boc group is removed and replaced by a group that is complementary (not complimentary as in the paper!) to another group which is covalently attached to a suitable chromatographic support to permit affinity chromato-graphy to be carried out. After purification, treatment with base detaches the desired peptide with complete removal of the disposable handle.

The design and synthetic methodology in preparing combinatorial libraries of



Reagents: i, L-H-Phe-OH, KOH, $NaBH_4$ in aq. EtOH; ii, [FmocAsp(OBut)$_2$O, Na_2CO_3 in aq. dioxan; iii, 3-chloroperoxybenzoic acid in CH_2Cl_2

Scheme 7

peptides continues to attract much attention. Even small peptides present problems of low yield.[197] As expected, coupling rates depend on the nature of both incoming and immobilized amino acids, and some potential library members may be absent. Increasing the amount of incoming amino acid does not always cure the problem. In preparing a library of potential epitopes, ELISA binding studies showed that a flexible linker is better than a rigid moiety.[198] The diversity of libraries can be optimized using a genetic algorithm.[199] The design of cyclic scaffolds for peptide combinatorial libraries has been described.[200] The optimization of one-pot syntheses of peptide libraries has been studied.[201] The peptides were identified by sequencing, MALDI-TOF spectroscopy and amino-acid analysis. An apparatus for the SPPS of libraries of 10 mg quantities has been designed and constructed.[202]

A library of peptides containing δ-substituted derivatives of Orn has been assembled.[203] A diketopiperazine with four carboxylic acid groups has been used as a template for a peptide library.[204] A variety of *C*-terminal amide groups has been used in assembling a library.[205] Diversification of structure has been achieved by synthesizing peptomers, a new term to describe peptide-peptoid hybrids.[206] In the first reported example of double combinatorial chemistry, a library of compounds based on two *O*-alkylated Tyr residues joined by a variety of aromatic dibasic acids has been assembled.[207] Combinatorial synthesis of peptides usually aims to produce lead compounds with specified properties. For example, a library of resin-bound peptides related to the active site of isopenicillin *N*-synthase (IPNS) has been produced to study their binding of transition element ions.[208] A library of 1152 peptidomimetics based on the β-turn has been tested for affinity towards HCO-Met-Leu-Phe-OH receptor.[209] A library of 64 linear and 256 cyclic pseudopeptides containing the ψ[CH_2NH] moiety and related to enkephalin has been constructed.[210] It is important to stress that combinatorial techniques are used to search for non-peptide compounds with desirable pharmacological properties for the obvious reason that they should be

resistant to the ubiquitous proteolytic enzymes. Since this Report is concerned with peptide synthesis, only one example of this approach is cited here. Antagonists of the GpIIb/IIIa system are important and peptidic compounds are mentioned in Section 3.1 under RGD peptides. Nonpeptidic antagonists have been sought using combinatorial synthesis.[211]

This section concludes with a very brief mention of analytical methods used to determine what combinatorial synthesis has produced. The methods can include tagging during synthesis, sequence analysis and the use of physical methods. Fluorescent confocal microscopy is a very sensitive and nondestructive tagging method.[212] Laser optical encoding can also be used.[213] Multiple fluorophores can be used for tagging since spectral deconvolution is feasible.[214] For libraries with restricted diversity generated by the split-mix methodology, the amino acids on a selected single bead identified by precolumn derivatization correlate directly with the sequence of a peptide thereby permitting rapid sequencing without the need to undertake encoding strategies.[215] If SPPS is used in the reverse sense by coupling the *N*-terminal residue to the support, the Edman method of sequencing cannot be used and an alternative method ("methionine scanning") has been devised.[216] A small amount of Met is incorporated in each coupling step and single beads can be treated with CNBr in aqueous CF_3CO_2H for 24 h to effect cleavage at Met residues and electrospray mass spectroscopy gives a ladder of peptides which permits determination of sequence. A photolytic mass laddering method for characterization of oligomers on single resin beads has been described.[217] If PhN=C=O is used as a terminating label for ladder sequencing of peptides, the method is complicated by the loss of label in the gas phase on the time scale of a Fourier-transform mass spectrometry experiment. The use of *N*-carboethoxyphthalimide as a labelling agent, however, is satisfactory because the derivatives are stabilized by their cyclic structure.[218] Other physical methods can be used to assess the results of SPPS. If Fmoc chemistry is used and the product assessed using time of flight-secondary ion mass spectrometry, the Fmoc group gives an ion with $m/z = 165$. This should be absent after deprotection.[219] This provides a sensitive test for the presence of a truncated species. A UNIX-based programme is available for quality control of peptide libraries using mass spectrometrical data as input.[220] A combinatorial search algorithm in combination with partial-least squares models of QSAR has been used in the synthesis of a library of peptides aimed at production of analogues of an antibacterial peptide.[221] The result of the analysis showed that hydrophobicity and amphipathicity were the most important physical features of the active molecules. A model experiment has been used to screen a library of peptides as potential substrates of glutathione-*S*-transferase with a double technique consisting of electrospray ionization – Fourier-transform ion cyclotron resonance mass spectrometry.[222] Monitoring of Fmoc/SPPS by matrix-assisted laser desorption/ionization mass spectrometry of product after detachment from beads has been reported.[223] Diffuse reflectance infrared Fourier transform spectroscopy has been used with supports that do not contain carbonyl groups as these would interfere with the method.[224] The analysis is done without detachment of the peptide(s) from the resin and requires only 30 sec. Finally, homonuclear and heteronuclear

magic-angle spinning NMR spectroscopy has been used during the SPPS of a 10-residue peptide.[225] Difficulties due to peptide-chain aggregation could be resolved by titrating the immobilized peptide with Me_2SO which increased the mobility of peptide and narrowed the NMR peaks. Some of these last few methods were used in the SPPS of single peptides, but they could probably be applied to the assembly of libraries.

2.7 Enzyme-mediated Synthesis and Semi-synthesis – Crosslinked crystals of subtilisin display high catalytic synthetic activity with an enhanced stability.[226]

Proteinases have been used to synthesize esters of amino acids from ethanol,[227] secondary alcohols[228] and glycerol.[229] The last are good substrates for trypsin-catalysed peptide synthesis. A considerable amount of work has been done on peptide synthesis in different systems. Acetylated dipeptide amides have been made in reversed micelles using α-chymotrypsin.[230,231] By careful study of the conditions, yields of 85% were obtained. Further studies have been made of syntheses in one-phase systems composed of water and a polar solvent.[232,233] His, Tyr or Met could be attached to the *C*-terminus of the nonapeptide, microperoxidase-9 using trypsin in 50% aqueous $CHONMe_2$. The effect of co-solvent on yield and kinetics was investigated using carboxypeptidase Y.[234] Highest yields were obtained in 50% aqueous Me_2SO. When corrections were applied for enzyme denaturation and the concentration of amino component was fixed, the mechanism appeared to be Random Bi Bi. The industrial proteinase, neutrase, when codeposited with sorbitol on a polyamide, gave very high yield of dipeptide derivatives in a system composed of MeCN and 4%(v/v) buffer.[233] The synthesis of Bz-Arg-Gly-NH_2 using trypsin modified with carboxymethyldextran was studied in a biphasic system containing dextran and polyethyleneglycol (PEG).[235] Good yields were obtained because the product was more soluble in the PEG phase, whereas the enzyme was mainly in the other phase. Fundamental thermodynamic aspects of synthesis in biphasic systems have been studied.[236] Mass transfer provides the driving force for synthesis. The overall synthesis equilibrium is characterized by an equilibrium constant that is independent of pH, phase volume ratio and reactant partition coefficients. The catalytic activity of subtilisin Carlsberg and α-chymotrypsin in anhydrous solvents is dramatically enhanced on addition of dry MeOH, but EtOH or Pr^nOH have no effect.[237] It was concluded that MeOH activates the enzyme by a solvation process. When MeOH and H_2O are added together, kinetic data suggest that these act cooperatively. The use of various anhydrous solvents in the transesterification of CF_3CO-DL-Phe-OCH_2CF_3 by Pr^nOH using subtilisin affects velocity and enantioselectivity differently.[238] Surface-active agents complex with proteinases and the complexes are soluble in hydrophobic and hydrophilic solvents. α-Chymotrypsin and subtilisin in a form that is soluble in organic solvents are effective catalysts for peptide synthesis.[239–242] k_{cat}/K_m Is considerably higher than for suspended forms of the enzymes.[239] An alternative model of subtilisin in organic solvents proposes the existence of four forms of the enzyme, native, a denatured form that is renaturable in octane or water, a denatured form that is renatured only in water and an irreversibly denatured form.[242] A wider study of the structure and activity

of enzymes complexed with surface-active agents in organic solvents is to be expected. Further studies have been made of the protocol in which one or both substrates are undissolved.[243,244] Conditions are particularly favourable if the product precipitates. The key variable for a high yield is the ratio between aminolysis and hydrolysis and so a high nucleophile concentration is advisable. Optimal conditions afford an almost quantitative yield. The use of frozen aqueous solution as a medium for peptide synthesis with proteinase catalysis displays important differences from homogeneous systems. Di- and tri-peptide esters without *N*-α-protection can be used as acyl donors in presence of α-chymotrypsin[245] and yields can be very high. It is important, however, to note that enzyme specificity can be modified. At room temperature with this enzyme, amino acids with positively charged side chains are unacceptable at the $P_{2'}$, position but are in frozen solution.

In contrast to chemical synthesis of peptides, it has been shown that enzymic synthesis can be effected from the *N*- to the *C*-terminus.[246] Z-Lys-Tyr-OEt was coupled with Arg-OPr using α-chymotrypsin and the product was coupled with Ser-OBzl using clostripain to give Z-Lys-Tyr-Arg-Ser-OBzl; hydrogenolysis afforded the free tetrapeptide. Subtilisin BPN′ can be used to couple nucleophiles such as β-amino acid derivatives, peptide mimetics or amino acids bearing protected phosphoester groups to suitable acyl donors.[247] Thus, peptide derivatives with an amino alcohol or amino aldehyde at the *C*-terminus are easily accessible.[248]

Some aspects of proteinase specificity are relevant to peptide synthesis. Peptides containing α-CF_3 groups are absolutely resistant to chymotrypsin so presumably cannot be synthesized using this enzyme.[249] Esters of Bz-Phe-OH can be used as acyl donors in syntheses catalysed by trypsin.[250] The products are resistant to tryptic hydrolysis thus providing high yields. Peptides derived from L-t-leucine or L-neopentylglycine have been obtained in syntheses catalysed by thermolysin or chymotrypsin.[251] The use of inverse substrates of trypsin is well established. 4-Guanidinophenyl and 4-guanidinomethylphenyl esters of αα-dialkyl amino acids couple with amino acid 4-nitroanilides in high yield in presence of bovine trypsin or preferably *S.griseus* trypsin.[252] Fragment coupling has been described using inverse substrates.[253] For example, coupling of the 4-guanidinophenyl ester of Boc-Phe-Gly-Gly-OH and H-Ala-Phe-Ala-Ala-Gly-OH gave yields of 83–98% of octapeptide. This type of approach to the coupling of peptides will surely be more fully exploited in the future.

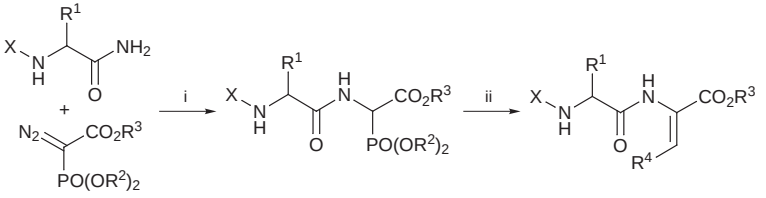
Modified forms of proteinases have been long been used and there have been some further examples. Acetylated trypsin gives very high yields of Bz-Arg-Leu-NH_2 due to a decreased K_m and increased k_{cat} compared to native trypsin.[254] Methyltrypsin has been used for coupling alkyl and 4-guanidinophenyl esters as acyl donors.[255] Thiolsubtilisin has been freed from subtilisin by affinity chromatography on bacitracin-Sepharose and used to synthesis peptides from reactive esters as acyl donors.[256] By using conditions that are favourable for both enzymic synthesis and hydrolysis, a library of peptides results[257] and this could become an alternative approach to combinatorial synthesis. There have been a few more examples of the use of enzymes for introducing or removing blocking groups. 2-

Methoxyethyl esters are easily deprotected by lipase and their use is favoured for the synthesis of simple peptides and glycopeptides.[258] Alternatively, heptyl esters have been used in the synthesis of phosphopeptides since their deprotection by lipase avoids possible dephosphorylation by β-elimination.[259,260] In contrast, the phenylacetyl group can be introduced on to the α-amino group using penicillin G acylase in biphasic conditions.[261]

This section concludes with a miscellaneous selection of enzyme-catalysed syntheses of peptides. Small fragments of CCK have been synthesized.[262,263] Enzyme-catalysed semisynthesis of analogues of calcitonin[264] and of human insulin from porcine insulin[265] have been reported. Finally, a combination of chemical and enzymic methods have been used for the synthesis of fluorescently labelled *N-Ras* lipopeptides[266] and a phosphorylated glycopeptide fragment of mammalian RNA polymerase II.[267]

2.8 Miscellaneous Reactions Related to Peptide Synthesis – A stereoselective synthesis of dipeptides involves the *C*-alkylation of a 2-ethoxypyrazin-4-one followed by ring-opening hydrolysis.[268]

A new synthesis of dehydrodipeptides comprises a two-step process consisting of a rhodium(II)-catalysed reaction of a diazophosphonoacetate and a protected amino acid amide to give an intermediate phosphonate which then undergoes a Wadsworth-Emmons reaction with aldehydes (Scheme 8).[269] A further new



Reagents: i, $Rh^{(II)}$; ii, DBU, R^4CHO, in CH_2Cl_2

Scheme 8

reaction starts with a di- or tri-peptide containing iodoalanine at the *C*-terminus.[270] These can be converted into organozinc derivatives which react with nucleophiles either under Pd catalysis or by transmetallation to Zn/Cu reagent to give peptides containing an unnatural amino acid. The 2-iodoalanine peptides were prepared from the corresponding Ser peptide using $Me(PhO)_3P^+I^-$. This sequence of reactions offers an intriguing possibility of building double combinatorial libraries[207] by varying the amino acids used in the first step and then reacting with a combination of nucleophiles in the second step. Coupling of $BocNHCH_2CH_2OH$ and $Tos\text{-}CHRCO_2Bzl$ via the Mitsunobu reaction using PPh_3 and EtOCON=NCOOEt gives $Boc\text{-}Gly\psi[CH_2N(Tos)]CHRCO_2Bzl$.[271] The last step in the splicing of two proteins is commonly either an O→N or S→N acyl rearrangement of an ester or thioester. Kinetic measurements of rates of such rearrangements in depsipeptides showed that the increase of pH accelerated reaction but that the process had a low dependence on temperature and hence a

low energy of activation.[272] The last group of papers in this section may herald a new approach to peptide synthesis. A synthetic 33-residue peptide, which has a coiled-coil structural motif, efficiently catalyses the coupling of shorter peptide fragments with high sequence and diastereoselectivity.[273] A 35-residue peptide:

Ac-ELYALEK-ELGALEK-ELACLEK-ELGALEK-ELYALEK-$CONH_2$

was found to adopt a coiled-coil structure that was pH-dependent.[274] The helical content was maximal at pH 4.0, presumably as a result of protonation of the side chains of the Glu residues. In this state, it catalysed its own formation from the *N*-terminal heptadecapeptide and the *C*-terminal octadecapeptide. There was no evidence that the two smaller peptides associated with each other in the absence of the largest peptide. A similar situation obtained in the self-replication of a 32-residue peptide resembling a leucine zipper sequence from the *N*-terminal heptadecapeptide and the *C*-terminal pentapeptide fragments.[275] A further auto-catalytic synthesis of a peptide was demonstrated in a flow reaction simulating submarine hydrothermal vents.[276]

3 Appendix: A List of Syntheses Reported Mainly in 1997

Peptide/Protein	*Ref.*
3.1 Natural Peptides, Proteins and Partial Sequences	
Agouti protein	
C-Terminal 41-residue fragment	277
β-Amyloid	
SPPS of β-amyloid(1–42)	278
Angiotensin	
Angiotensin analogues	279–283
Angiotensin II receptor ligands	284
Antibacterial peptides	
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References

1. D.T. Elmore, Specialist Periodical Report: Amino Acids, Peptides and Proteins, 1998, **29**, 126.
2. R.H. Angeletti, L.F. Bonewald and G.B. Fields, *Methods Enzymol.,* 1997, **289**, 697.
3. T.J. Deming, *Adv.Mater*., 1997, **9**, 299.
4. D. Sadat-Aalaee, *Protein-Based Mater*., 1997, 3.
5. T. Kappes and H. Waldmann, *Liebig's Ann./Recl.*, 1997, 803.
6. V. Slavinskaya, G. Cipens, A. Strautina, E. Korchagova, D. Sile, I. Liepina, M. Katkevics and R. Vegners, *Latv.Kim.Z*., 1996, 3, 56.
7. V.A. Slavinskaya, G. Cipens, A. Strautina, E.Kh. Korchagova, I. Liepina, M. Katkevics and R. Vegners, *Latv.Kim.Z*., 1997, 3, 51.
8. R. Matsueda, *Phosphorus, Sulfur Silicon Relat.Elem.*, 1997, **120 & 121**, 387.
9. M. Noda and K. Takeda, *Shimadzu Hyoron*, 1997, **54**, 49.
10. B. Sauerbrei, T. Kappes and H. Waldmann, *Top.Curr.Chem*., 1997, **186**, 65.
11. I. Annis, B. Hargittai and G. Barany, *Methods Enzymol*., 1997, **289**, 198.
12. K. Akaji, *Rev.Heteroat.Chem*., 1997, **16**, 85.
13. T.W. Muir, P.E. Dawson and S.B.H. Kent, *Methods Enzymol*., 1997, **289**, 266.
14. M.A. Walker, *Angew.Chem., Int.Ed*., 1997, **36**, 1069.
15. J.M. Humphrey and R.A. Chamberlin, *Chem.Rev*.,1997, **97**, 2243.
16. T. Kawakami, *Tanpakushitsu Kogaku Kiso Kenyu Senta Dayori*, 1997, **18**, 34.
17. M.J. O'Donnell, I.A. Esikova, A. Mi, D.F. Shullenberger and S. Wu, *A.C.S. Symp.Ser*., 1997, **659**, 124.
18. F. Albericio and L.A. Carpino, *Methods Enzymol*., 1997, **289**, 104.
19. I. Ojima and F. Delaloge, *Chem.Soc.Rev*., 1997, **26**, 377.
20. J.L. Fauchère, J.M. Henlin and J.A. Boutin, *Analusis*, 1997, **25**, 97.
21. A. Furka, *Comb.Pept.Nonpept.Libr*., 1996, 111.
22. W. Keilholz and S. Stevanovic, *Comb.Pept.Nonpeptid.Libr*., 1996, 287.
23. K.S. Lam and M. Lebl, *Comb.Pept.Nonpeptid.Libr*., 1996, 173.
24. J.L. Fauchère, J.M. Henlin and J.A. Boutin, *Canad.J.Physiol.Pharmacol*., 1997, **75**, 683.
25. R. Frank, S. Hoffmann, M. Kiess, H. Lahmann, W. Tegge, C. Behn and H. Gausepohl, *Comb.Pept.Nonpeptid,Libr*., 1996, 363.
26. A.G. Beck-Sickinger and G. Jung, *Comb.Pept.Nonpeptid.Libr*., 1996, 79.
27. D.V. Patel, *Ann.Rep.Comb.Chem.Mol.Diversity*, 1997, **1**, 78.
28. J.L. Fauchère, *Adv.Mol.Cell Biol*., 1996, **15B**, 569.
29. Z.-G. Zhao and K.S. Lam, *Ann.Rep.Comb.Chem.Mol.Diversity*, 1997, **1**, 192.
30. K. Kudo, *Seisan Kenkyu*, 1997, **49**, 131.
31. M. Meldal, *Methods Enzymol*., 1997, **289**, 83.
32. M.F. Songster and G. Barany, *Methods Enzymol*., 1997, **289**, 126.
33. C.A. Guy and G.B. Fields, *Methods Enzymol*., 1997, **289**, 67.
34. D.A. Wellings and E. Atherton, *Methods Enzymol*., 1997, **289**, 44.
35. B. Merrifield, *Methods Enzymol*., 1997, **289**, 3.
36. P. Alewood, D. Alewood, L. Miranda, S. Love, W. Meutermans and D. Wilson, *Methods Enzymol*., 1997, 289, 14.
37. J.M. Stewart, *Methods Enzymol*., 1997, **289**, 29.
38. N.K. Terrett, M. Gardner, D.W. Gordon, R.J. Kobylecki and J. Steele, *Chem. Eur.J*., 1997, **3**, 1917.
39. F. Albericio, P. Lloyd-Williams and E. Giralt, *Methods Enzymol*., 1997, **289**, 313.
40. M. Lebl and V. Krchnák, *Methods Enzymol.*, 1997, **289**, 336.

41. G. Barany, F. Albericio, S.A. Kates and M. Kempe, *ACS Symp.Ser*., 1997, **680**, 239.
42. R. Frank, *Immunol.Methods Man*., 1997, **2**, 777.
43. A. Holm, S. Ostergaard, R.S. Hodges, D. Husband and S. Buus, *Immunol. Methods Man*., 1997, **2**, 847.
44. A.M. Bray, *Immunol.Methods Man*., 1997, **2**, 809.
45. R. Frank, *Immunol.Methods Man*., 1997, **2**, 783.
46. G.A. Grant, M.W. Crankshaw and J. Gorka, *Methods Enzymol*., 1997, **289**, 395.
47. S. Zhu, *Beijing Daxue Xuebao, Ziran Kexueban*, 1997, **33**, 534.
48. L.S. Richter, D.C. Spellmeyer, E.J. Martin, G.M. Figliozzi and R.N. Zuckermann, *Comb.Pept.Nonpept.Libr*., 1996, 327.
49. K.H. Wiesmuller, S. Feiertag, B. Fleckenstein, S. Kienle, D. Stall, M. Herrman and G. Jung, *Comb.Pept.Nonpept.Libr*., 1996, 203.
50. N.J. Turner, *Curr.Org.Chem*., 1997, **1**, 21.
51. H.-D. Jakubke, U. Eichhorn, S. Gerisch, M. Hansler, K. Salchert, G. Ullmann and D. Ullmann, *NATO ASI Ser., Ser.C.,* 1996, **478**, 53.
52. M.D. Grim, *Pharm.Manuf.Int*., 1997, 49.
53. A.C. Braisted, J.K. Judice and J.A. Wells, *Methods Enzymol*., 1997, **289**, 298.
54. R. Haubner, D. Finsinger and H. Kessler, *Angew.Chem., Int.Ed*., 1997, **36**, 1374.
55. B. Kutscher, M. Bernd, T. Beckers, E.E. Polymeropoulos and J. Engel, *Angew.-Chem., Int.Ed*., 1997, **36**, 2149.
56. D. Konopi′ska, *J.Pept.Res*., 1997, **49**, 457.
57. I. Clark-Lewis, L. Vo, P. Owen and J. Anderson, *Methods Enzymol*., 1997, **287**, 233.
58. J.S. Nowick, I.Q. Lee, G. Mackin, M. Pairish, A.J. Shaka, E.M. Smith and J.W. Ziller, *NATO ASI Ser., Ser.C*., 1996, **478**, 111.
59. J.S. Nowick, E.M. Smith and M. Pairish, *Chem.Soc.Rev*., 1996, **25**, 401.
60. A.G. Beck-Singer, *Methods Mol.Biol*., 1997, **73**, 61.
61. J. Kihlberg and M. Elofsson, *Curr.Med.Chem*., 1997, **4**, 85.
62. S.J. Danishefsky and J.Y. Roberge, *Glycopept.Relat.Compd*., 1997, 245.
63. D.G. Large and I.J. Bradshaw, *Glycopept.Relat.Compd*., 1997, 295.
64. S. Peters, M. Meldal and K. Bock, *Front.Nat.Prod.Res*., 1996, **1**, 352.
65. H. Kunz and M. Schultz, *Glycopept.Relat.Compd.*, 1997, 23.
66. Y. Ichikawa, *Glycopept.Relat.Compd.*, 1997, 79.
67. J.W. Perich, *Methods Enzymol*., 1997, **289**, 221.
68. R.T. Lee and Y.C. Lee, *New Compr.Biochem*., 1997, **29b**, 601.
69. M. Meldal and P.M. St. Hilaire, *Curr.Opin.Chem.Biol*., 1997, **1**, 552.
70. M. Okada, K. Aoi and K. Tsutsumiuchi, *Proc.Jpn.Acad*., 1997, **73B**, 205.
71. K. Haneda, *Noguchi Kenkyusho Jiho*, 1997, 40.
72. J.W. Perich, *Methods Enzymol*., 1997, **289**, 245.
73. S.J. Rodda, G. Trebbick and M. Geysen, *Comb.Pept.Nonpeptid.Libr*., 1996, 303.
74. S.J. Rodda, G. Tribbick and N.J. Maeji, *Immunochem.1,* 1997, 121.
75. K.L. Dueholm and P.E. Nielsen, *New J.Chem*., 1997, **21**, 19.
76. J.M. Conlon, *Methods Mol.Biol*., 1997, **73**, 231.
77. M.P. Doyle and M.A. McKervey, *J.Chem.Soc., Chem.Commun*., 1997, 983.
78. F.R. Naider and J.M. Becker, *Biopolymers*, 1997, **43**, 3.
79. T.P. Singh and P. Kaur, *Prog.Biophys.Mol.Biol*., 1996, **66**, 141.
80. J.G. Tirrell, D.A. Tirrell, M.J. Fournier and T.L. Mason, *Protein-Based Mater*., 1997, 61.
81. D.H.R. Barton and J.A. Ferreira, *Phosphorus, Sulfur Silicon Relat.Elem*., 1997, **120 & 121**, 1.
82. A.M. Felix, *ACS Symp.Ser*., 1997, **680**, 218.

83. D. Seebach and J.L. Matthews, *J.Chem.Soc., Chem.Commun*., 1997, 2015.
84. Y. Ma and Y. Ito, *Anal.Chim.Acta*, 1997, **352**, 411.
85. M. Koller and H. Eckert, *Anal.Chim.Acta*, 1997, **352**, 31.
86. K. Štulík, V. Pacáková, J. Suchánková and H.A. Claessens, *Anal.Chim. Acta*, 1997, **352**, 1.
87. G.B. Irvine, *Anal.Chim.Acta*, 1997, **352**, 387.
88. A. Peter and G. Toth, *Anal.Chim.Acta*, 1997, **352**, 335.
89. M.J. Burk and J.G. Allen, *J.Org.Chem*., 1997, **62**, 7054.
90. E.F. Evans, N.J. Lewis, I. Kapfer, G. Macdonald and R.J.K. Taylor, *Synth. Commun*., 1997, **27**, 1819.
91. A.N. Sabirov, Y.-D. Kim, H.-J. Kim and V.V. Samukov, *Protein Pept.Lett*., 1997, **4**, 307.
92. A.R. Katritzky, C.N. Fali, J. Li, D.J. Ager and I. Prakash, *Synth. Commun*., 1997, **27**, 1623.
93. H. Bekele, C.L. Nesloney, K.W. McWilliams, N.M. Zacharias, P. Chitnumsub and J.W. Kelly, *J.Org.Chem*., 1997, **62**, 2259.
94. L.A. Carpino, M. Philbin, M. Ismail, G.A. Truran, E.M.E. Mansour, S. Iguchi, D. Ionescu, A. El-Faham, C. Riemer, R. Warrass and M.S. Weiss, *J.Amer.Chem.Soc*., 1997, **119**, 9915.
95. J. Svete, M. Aljaž-Rožič and B. Stanovnik, *J.Heterocycl.Chem*., 1997, **34**, 177.
96. N. Auzeil, G. Dutruc-Rosset and M. Largeron, *Tetrahedron Lett*., 1997, **38**, 2283.
97. S. Lemaire-Audoire, M. Savignac, E. Blart, J.-M. Bernard and J.-P. Genêt, *Tetrahedron* Lett., 1997, **38**, 2955.
98. N. Thieriet, J. Alsina, E. Giralt, F. Guibé and F. Albericio, *Tetrahedron Lett*., 1997, **38**, 7275.
99. B. Kellam, W.C. Chen, S.R. Chhabbra and B.W. Bycroft, *Tetrahedron Lett*., 1997, **38**, 5391.
100. J.P. Konopelski, L.K. Filonova and M.M. Olmstead, *J.Amer.Chem.Soc*., 1997, **119**, 4305.
101. G. Bitan, D. Muller, R. Kasher, E.V. Gluhov and C. Gilon, *J.Chem.Soc., Perkin Trans.1*, 1997, 1501.
102. W. Hollweck, N. Sewald, T. Michel and K. Burger, *Liebig's Ann./Recl*., 1997, 2549.
103. G.M. Dubowchik and S. Radia, *Tetrahedron Lett*., 1997, **38**, 5257.
104. K. Midura-Nowaczek, *Monatsh.Chem*., 1997, **128**, 207.
105. S.W. Wright, D.L. Hageman, A.S. Wright and L.D. McClure, *Tetrahedron Lett*., 1997, **38**, 7345.
106. D. Albanese, F. Corcella, D. Landini, A. Maia and M. Penso, *J.Chem.Soc., Perkin Trans.1*, 1997, 247.
107. W.-C. Chen, M.D. Vera and M.M. Joullié, *Tetrahedron Lett*., 1997, **38**, 4025.
108. R.S. Givens, A. Jung, C.-H. Park, J. Weber and W. Bartlett, *J.Amer.Chem. Soc*., 1997, **119**, 8369.
109. Y. Okada and Y. Mu, *Chem.Pharm.Bull*., 1997, **45**, 88.
110. E. Diez-Barra, A. De La Hoz and P. Sanchez-Verdu, *An.Quim., Int.Ed*., 1997, **93**, 145.
111. M.A. Brook, C. Gottardo, S. Balduzzi and M. Mohamed, *Tetrahedron Lett*., 1997, **38**, 6997.
112. J. Wang, Y. Okada, W. Li, T. Yokoi and J. Zhu, *J.Chem.Soc., Perkin Trans.1*, 1997, 621.
113. Y. Okada, N. Shintomi, Y. Kondo, T. Yokoi, S. Joshi and W. Li, *Chem. Pharm.Bull*., 1997, **45**, 1860.

114. K. Rosenthal, A. Karlström and A. Undén, *Tetrahedron Lett*., 1997, **38**, 1075.
115. M. Noda and M. Kiffe, *J.Pept.Res*., 1997, **50**, 329.
116. M. Noda and M. Kiffe, *Shimadzu Hyoron*, 1997, **54**, 223.
117. H.E. Garay, L.J. Gonzalez, L.J. Cruz, R.C. Estrada and O. Reyes, *Biotecnol.Apl*., 1997, **14**, 193.
118. A.M. Kimbonguila, S. Boucida, F. Guibé and A. Loffet, *Tetrahedron*, 1997, **53**, 12525.
119. Y. Okada, J. Wang, T. Yamamoto, T. Yokoi and Y. Mu, *Chem.Pharm.Bull*., 1997, **45**, 452.
120. Y. Han and G. Barany, *J.Org.Chem*., 1997, **62**, 3841.
121. M. Engebretsen, E. Agner, J. Sandosham and P.M. Fischer, *J.Pept.Res*., 1997, **49**, 341.
122. J.C. Spetzler and M. Meldal, *Lett.Pept.Sci*., 1997, **3**, 327.
123. J. Eichler and R.A. Houghten, *Protein Pept.Lett.*, 1997, **4**, 157.
124. E.V. Kudryavtseva, M.V. Sidorova, M.V. Ovchinnikov, Zh.D. Bespalova and V.N. Bushuev, *J.Pept.Res*., 1997, **49**, 52.
125. V. Lozanov, C. Guarnaccia, A. Patthy, S. Foti and S. Pongor, *J.Pept. Res*., 1997, **50**, 65.
126. J.P. Tam and Y.-A. Lu, *Tetrahedron Lett*., 1997, **38**, 5599.
127. L. Chen, I. Zoulíková, J. Slaninová and G. Barany, *J.Med.Chem*., 1997, **40**, 864.
128. D. Besse and L. Moroder, *J.Pept.Sci*., 1997, **3**, 442.
129. J. Izdebski, A. Orlowska, M. Pachulska and E. Witkowska, *Pol.J.Chem*., 1997, **71**, 903.
130. J. Izdebski and D. Kunce, *J.Pept.Sci*., 1997, **3**, 141.
131. S. Nozaki, *Chem.Lett*., 1997, 1.
132. M. Slebioda, *Pol.J.Chem*., 1997, **71**, 1045.
133. R.I. Carey, L.W. Bordas, R.A. Slaughter, B.C. Meadows, J.L. Wadsworth, H. Huang, J.J. Smith and E Furusjö, *J.Pept.Res*., 1997, **49**, 570.
134. V.N. Medvedkin, V.F. Zabolotskikh, E.A. Permyakov, Yu.V. Mitin, M.N. Sorokina and L.V. Klimenko, *Bioorg.Khim*., 1995, **21**, 684.
135. T.T. Romoff and M. Goodman, *J.Pept.Res*., 1997, **49**, 281.
136. Y. Han, F. Albericio and G. Barany, *J.Org.Chem*., 1997, **62**, 4307.
137. F. Albericio, M. Cases, J. Alsina, S.A. Triolo, L.A. Carpino and S.A. Kates, *Tetrahedron Lett*., 1997, **38**, 4853.
138. Y.-H. Ye, C.-X. Fan, D.-Y. Zhang, H.-B. Xie, X.-L. Hao and G.-L. Tian, *Gaodeng Xuexiao Huaxue Xuebao*, 1997, **18**, 1086.
139. T. Vojkovsky and B. Drake, *Org.Prep.Proced.Int*., 1997, **29**, 497.
140. K. Akaji, Y. Tamai and Y. Kiso, *Tetrahedron*, 1997, **53**, 567.
141. T. Kawakami and S. Aimoto, *Chem.Lett*., 1997, 1157.
142. P.E. Dawson, M. Churchill, M.R. Ghadiri and S.B.H. Kent, *J.Amer.Chem. Soc*., 1997, **119**, 4325.
143. L. Zhang and J.P. Tam, *Tetrahedron Lett*., 1997, **38**, 3.
144. M.V. Anuradha and B. Ravindranath, *Tetrahedron*, 1997, **53**, 1123.
145. F.G. Klärner, U. Kalthof and J. Gante, *J.Prakt.Chem./Chem.-Ztg*., 1997, **339**, 359.
146. D.-H. Park, K.-W. Roh and Y.-S. Lee, *Bull.Korean Chem.Soc*., 1997, **18**, 3.
147. E. Nicolás, J. Clemente, T. Ferrer, F. Albericio and E. Giralt, *Tetrahedron*, 1997, **53**, 3179.
148. W. Tegge and R. Frank, *J.Pept.Res*., 1997, **49**, 355.
149. A.R. Katritzky, D. Toader, K. Watson and J.S. Kiely, *Tetrahedron Lett*., 1997, **38**, 7849.

150. V. Swali, N.J. Wells, G.J. Langley and M. Bradley, *J.Org.Chem*., 1997, **62**, 4902.
151. E. Bikshapathy and R. Nagaraj, *Proc.-Indian Acad.Sci.*,*Chem.Sci*., 1997, **109**, 319.
152. R. Volkmer-Engert, B. Hoffmann and J. Schneider-Mergener, *Tetrahedron Lett*., 1997, **38**, 1029.
153. A. Routledge, H.T. Stock, S.L. Flitsch and N.J. Turner, *Tetrahedron Lett*., 1997, **38**, 8287.
154. O. Seitz and H. Kunz, *J.Org.Chem*., 1997, **62**, 813.
155. H.M. Zhong, M.N. Greco and B.E. Maryanoff, *J.Org.Chem*., 1997, **62**, 9326.
156. B. Atrash and M. Bradley, *J.Chem.Soc., Chem.Commun*., 1997, 1397.
157. T. Kimura, T. Fukui, S. Tanaka, K. Akaji and Y. Kiso, *Chem.Pharm.Bull*., 1997, **45**, 18.
158. B. Henkel, W. Zeng and E. Bayer, *Tetrahedron Lett*., 1997, **38**, 3511.
159. M. Meisenbach, H. Echner, and W. Voelter, *J.Chem.Soc., Chem.Commun*., 1997, 849.
160. M. Meisenbach and W. Voelter, *Chem.Lett*., 1997, 1265.
161. D. Sarantakis and J.J. Bicksler, *Tetrahedron Lett*., 1997, **38**, 7325.
162. J.J. Chen and A.F. Spatola, *Tetrahedron Lett*., 1997, **38**, 1511.
163. U. Bauer, W.-B. Ho and A.M.P. Koskinen, *Tetrahedron Lett*., 1997, **38**, 7233.
164. S.L. Mellor, C. McGuire and W.C. Chan, *Tetrahedron Lett*., 1997, **38**, 3311.
165. S.L. Mellor and W.C. Chan, *J.Chem.Soc., Chem.Commun*., 1997, 2005.
166. N.J. Ede and A.M. Bray, *Tetrahedron Lett*., 1997, **38**, 7119.
167. C. Pothion, M. Paris, A. Heitz, L. Rocheblave, F. Rouch, J.-A. Fehrentz and J. Martinez, *Tetrahedron Lett*., 1997, **38**, 7749.
168. D.L. Whitehouse, S.N. Savinov and D.J. Austin, *Tetahedron Lett*., 1997, **38**, 7851.
169. R. Rodebaugh, B. Fraser-Reid and H.M. Geysen, *Tetrahedron Lett*., 1997, **38**, 7653.
170. I. Vlattas, J. Dellureficio, R. Dunn, I.I. Sytwu and J. Stanton, *Tetrahedron Lett*., 1997, **38**, 7321.
171. S. Futaki, K. Sogawa, J. Maruyama, T. Asahara and M. Niwa, *Tetrahedron Lett*., 1997, **38**, 6237.
172. J. Alsina, C. Chiva, M. Ortiz, F. Rababnal, E. Giralt and F. Albericio, *Tetrahedron Lett*, 1997, **38**, 883.
173. S. Sharma and S. Pasha, *Bioorg.Med.Chem.Lett*., 1997, **7**, 2077.
174. E. Harth-Fritschy and D. Cantacuzène, *J.Pept.Res*., 1997, **50**, 415.
175. L. Yang and K. Chiu, *Tetrahedron Lett*., 1997, **38**, 7307.
176. S.C. Miller and T.S. Scanlan, *J.Amer.Chem.Soc*., 1997, **119**, 2301.
177. W.L. Scott, C. Zhou, Z. Fang and M.J. O'Donnell, *Tetrahedron Lett*., 1997, **38**, 3695.
178. W.J. Hoekstra, M.N. Greco, S.C. Yabut, B.L. Hulshizer and B.E. Maryanoff, *Tetrahedron Lett*., 1997, **38**, 2629.
179. B. Henkel, L. Zhang and E. Bayer, *Liebig's Ann./Recl*., 1997, 2161.
180. A. Bagno, S. Bicciato, M. Dettin and C. Di Bello, *J.Pept.Res*., 1997, **50**, 231.
181. M. Meldal, M.A. Juliano and A.M. Jansson, *Tetrahedron Lett*., 1997, **38**, 2531.
182. R.E. Marti, K.H. Bleicher and K.W. Bair, *Tetrahedron Lett*., 1997, **38**, 6145.
183. Y. Sasaki and J. Abe, *Chem.Pharm.Bull*., 1997, **45**, 13.
184. K. Kitagawa, C. Aida, H. Fujiwara, T. Yagami and S. Futaki, *Tetrahedron Lett.*, 1997, **38**, 599.
185. M. Breslav, J. Becker and F. Naider, *Tetrahedron Lett*., 1997, **38**, 2219.
186. R. Pascal and R. Sola, *Tetrahedron Lett*., 1997, **38**, 4549.
187. A. Pichette, N. Voyer, R. Larouche and J.-C. Meillon, *Tetrahedron Lett*., 1997, **126**, 1279.

188. L.M. Varanda and M.T. Miranda, *J.Pept.Res.*, 1997, **50**, 102.
189. M. Dettin, S. Pegoraro, P. Rovero, S. Bicciato, A. Bagno and C. di Bello, *J.Pept.Res.*, 1997, **49**, 103.
190. J. Ryttersgaard, B.D. Larsen, A. Holm, D.H. Christensen and O.F.Nielsen, *Spectrochim.Acta*, 1997, **53A**, 91.
191. E.M. Cilli, R. Marchetto, S. Schreier and C.R. Nakaie, *Tetrahedron Lett.,* 1997, **38**, 517.
192. W. Zeng, P.-O. Regamey, K. Rose, Y. Wang and E. Bayer, *J.Pept.Res.*, 1997, **49**, 273.
193. E. Nicolás, M. Pujades, J. Bacardit, E. Giralt and F. Albericio, *Tetrahedron Lett.*, 1997, **38**, 2317.
194. E. Oliviera, A. Miranda, F. Albericio, D. Andreu, A.C.M. Paiva, C.R. Nakaie and M. Tominaga, *J.Pept.Res.*, 1997, **49**, 300.
195. J. Offer, T. Johnson and M. Quibell, *Tetrahedron Lett.*, 1997, **38**, 9047.
196. L.E. Canne, R.L. Winston and S.B.H. Kent, *Tetrahedron Lett.*, 1997, **38**, 3361.
197. J.A. Boutin, I. Gesson, J.-M. Henlin, S. Bertin, P.-H. Lambert, J.-P. Volland and J.-L. Fauchère, *Mol.Diversity*, 1997, **3**, 43.
198. Z. Yu and Y.-H. Chu, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 95.
199. R.D. Brown and Y.C. Martin, *J.Med.Chem.*, 1997, **40**, 2304.
200. I. Sanders, M.C. Allen, I.M. Matthews and M.M. Campbell, *Bioorg.Med. Chem.-Lett.*, 1997, **7**, 2603.
201. L.W. Herman, G. Tarr and S.A. Kates, *Mol.Diversity*, 1997, **2**, 147.
202. D. Gani, M. Akhtar, F.E.K. Kroll, C.F.M. Smith and D. Stones, *Tetrahedron* Lett., 1997, **38**, 8577.
203. T. Lescrinier, R. Busson, H. De Winter, C. Hendrix, G. Jansen, C. Pannecouque, J. Rozenski, A. Van Aerschot and P. Herdewijn, *J.Pept. Res.*, 1997, **49**, 183.
204. M. Falorni, G. Giacomelli, F. Nieddu and M. Taddei, *Tetrahedron Lett.*, 1997, **38**, 4663.
205. M. Davies and M. Bradley, *Angew.Chem., Int.Ed.*, 1997, **36**, 1097.
206. S. Østergaard and A. Holm, *Mol.Diversity*, 1997, **3**, 17.
207. J. Nielsen and F.R. Jensen, *Tetrahedron Lett.*, 1997, **38**, 2011.
208. N. Shibata, J.E. Baldwin and M.E. Wood, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 413.
209. A.A. Virgilio, A.A. Bray, W. Zhang, L. Trinh, M. Snyder, M.M. Morrissey and J.A. Ellman, *Tetrahedron*, 1997, **53**, 6635.
210. J.J. Wen and A.F. Spatola, *J.Pept.Res.*, 1997, **49**, 3.
211. T. Harada, J. Katada, A. Tachiki, T. Asari, K. Iijima, I. Uno, I. Ojima and Y. Hayashi, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 209.
212. B.J. Egner, S. Rana, H. Smith, N. Bouloc, J.G. Frey, W.S. Brocklesby and M. Bradley, *J.Chem.Soc., Chem.Commun.*, 1997, 735.
213. X.-Y. Xiao, C. Zhao, H. Potash and M.P. Nova, *Angew.Chem., Int.Ed.*, 1997, **36**, 780.
214. R.H. Scott and S. Balasubramanian, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 1567.
215. H. Weinberger, E. Lichte, C. Griesinger and B. Kutscher, *Arch.Pharm.*, 1997, **330**, 109.
216. M. Davies and M. Bradley, *Tetrahedron Lett.*, 1997, **38**, 8565.
217. K. Burgess, C.I. Martinez, D.H. Russell, H. Shin and A.J. Zhang, *J.Org. Chem.*, 1997, **62**, 5662.
218. S. Schurch, J.R. Scott and C.L. Wilkins, *Int.J.Mass Spectrom.Ion Processes*, 1997, **169-170**, 39.
219. C. Drouot, C. Enjalbal, P. Fulcrand, J. Martinez, J.-L. Aubagnac, R. Combarieu and Y. de Puydt, *Tetrahedron Lett.*, 1997, **38**, 2455.

220. J. Brunjes, J.W. Metzger and G. Jung, *Comb.Pept.Nonpeptid.Libr*., 1996, 511.
221. R.P. Mee, T.R. Auton and P.J. Morgan, *J.Pept.Res*., 1997, **49**, 89.
222. M. Wigger, J.P. Nawrocki, C.H. Watson, J.R. Eyler and S.A. Benner, *Rapid Commun.Mass Spectrom*., 1997, **11**, 1749.
223. G. Talbo, J.D. Wade, N. Dawson, M. Manoussios and G.W. Tregear, *Lett. Pept.Sci*., 1997, **4**, 121.
224. T.Y. Chan, R. Chen, M.J. Sofia, B.C. Smith and D. Glennon, *Tetrahedron Lett*., 1997, **38**, 2821.
225. C. Dhalluin, C. Boutillon, A. Tartar and G. Lippens, *J.Amer.Chem.Soc*., 1997, **119**, 10494.
226. Y.-F. Wang, K. Yakovlevsky, B. Zhang and A.L. Margolin, *J.Org.Chem*., 1997, **62**, 3488.
227. K. Braun and P. Kuhl, *Pharmazie*, 1997, **52**, 203.
228. I.-L. Shih, L.-C. Chiu, C.-T. Lai, W.-C. Liaw and D.-F. Tai, *Biotechnol. Lett*., 1997, **19**, 857.
229. Y.V. Mitin, K. Braun and P. Kuhl, *Biotechnol.Bioeng*., 1997, **54**, 287.
230. A.S. Feliciano, J.M.S. Cabral and D.M.F. Prazeres, *Enzyme Microb. Technol*., 1997, **21**, 284.
231. A.S. Feliciano, J.M.S. Cabral and D.M.F. Prazeres, *Biocatal. Biotransform*., 1997, **14**, 219.
232. D.W. Low, G. Yang, J.R. Winkler and H.B. Gray, *J.Amer.Chem.Soc*., 1997, **119**, 4094.
233. P. Clapés, E. Pera and J.L. Torres, *Biotechnol.Lett*., 1997, **19**, 1023.
234. S. Kunugi, N. Suzuki, S. Nishimoto, T. Morisawa and M. Yoshida, *Biocatal.Biotransform*., 1997, **14**, 205.
235. Y. Maeda, H. Ito, R. Izumida and H. Kitano, *Polym.Bull*., 1997, **38**, 49.
236. I.P. Ivanov, V. Kasche and D.D. Petkov, *Biocatal.Biotransform*., 1997, **14**, 181.
237. G.A. Hutcheon, M.C. Parker, A. James and B.D. Moore, *J.Chem.Soc., Chem. Commun*., 1997, 931.
238. K. Kawashiro, H. Sugahara, S. Sugiyama and H. Hayashi, *Biotechnol. Bioeng*., 1997, **53**, 26.
239. M.V. Sergeeva, V.M. Paradkar and J.S. Dordick, *Enzyme Microb.Technol*., 1997, **20**, 623.
240. I.V. Getun, I.Yu. Filippova, E.N. Lysogorskaya, E.S. Oksenoit, V.V. Anisimova, S.V. Kolobanova, A.V. Bacheva and V.M. Stepanov, *Bioorg.Med. Chem.Lett*., 1997, **7**, 2691.
241. K. Abe, T. Kawazoe, S.-y. Okazaki, M. Goto and F. Nakashio, *Biotechnol. Tech*., 1997, **11**, 25.
242. P.P. Wangikar, P.C. Michels, D.S. Clark and J.S. Dordick, *J.Amer.Chem. Soc*., 1997, **119**, 70.
243. U. Eichhorn, K. Beck-Piotraschke, R. Schaaf and H.-D. Jakubke, *J.Pept. Sci*., 1997, **3**, 261.
244. U. Eichhorn, A.S. Bommarius, K. Drauz and H.-D. Jakubke, *J.Pept.Sci*., 1997, **3**, 245.
245. S. Gerisch and H.-D. Jakubke, *J.Pept.Sci*., 1997, **3**, 93.
246. F. Bordusa, D. Ullmann and H.-D. Jakubke, *Angew.Chem., Int.Ed*., 1997, **36**, 1099.
247. W.J. Moree, P. Sears, K. Kawashiro, K. Witte and C.-H. Wong, *J.Amer. Chem.Soc*. 1997, **119**, 3942.
248. J.V. Potetinova, T.L. Voyushina and V.M. Stepanov, *Bioorg.Med.Chem. Lett*., 1997, **7**, 705.

249. B. Koksch, N. Sewald, H.-J. Hofmann, K. Burger and H.-D. Jakubke, *J.Pept.Sci.*, 1997, **3**, 157.
250. I.P. Ivanov, V.M. Yomtova and D.D. Petkov, *Biocatal.Biotransform.*, 1997, **14**, 195.
251. G. Krix, U. Eichhorn, H.-D. Jakubke and M.-R. Kula, *Enzyme Microb. Technol.*, 1997, **21**, 252.
252. H. Sekizaki, K. Itoh, E. Toyota and K. Tanizawa, *Tetrahedron Lett.*, 1997, **38**, 1777.
253. F. Bordusa, D. Ullmann, C. Elsner and H.-D. Jakubke, *Angew.Chem., Int. Ed.*, 1997, **36**, 2473.
254. A. Murphy and C.O. Fagain, *Biotechnol.Tech.*, 1997, **11**, 13.
255. K. Itoh, H. Sekizaki, E. Toyota and K. Tanizawa, *Bioorg.Chem.*, 1997, **25**, 307.
256. S.V. Kolobanova, E.N. Lysogorskaya, I.Yu. Filippova, V.V. Anisimova, E.S. Oksenoit and V.M. Stepanov, *Biochemistry (Moscow)*, 1997, **62**, 329.
257. P.G. Swann, R.A. Casanova, A. Desai, M.M. Frauenhoff, M. Urbancic, U. Slomczynska, A.J. Hopfinger, G.C. Le Breton and D.L. Venton, *Biopolymers*, 1996, **40**, 617.
258. M. Gewehr and H. Kunz, *Synthesis*, 1997, 1499.
259. D. Sebastian, A. Heuser, S. Schulze and H. Waldmann, *Synthesis*, 1997, 1098.
260. D. Sebastian and H. Waldmann, *Tetrahedron Lett.*, 1997, **38**, 2927.
261. M. Fite, M. Capellas, M.D. Benaiges, G. Caminal, P. Clapés and G. Alvaro, *Biocatal.Biotransform.*, 1997, **14**, 317.
262. M. Capellas, G. Caminal, G. Gonzalez, J. Lopez-Santin and P. Clapes, *Biotechnol.-Bioeng.*, 1997, **56**, 456.
263. S. Ruiz, J.A. Feliu, G. Caminal, G. Alvaro and J. Lopez-Santin, *Biotechnol.Prog.*, 1997, **13**, 783.
264. V. Ceřovský, E. Wünsch and J. Brass, *Europ.J.Biochem.*, 1997, **247**, 231.
265. J. Yang, Z. Lin and J. Yu, *Shanghai Jiaotong Daxue Xuebao*, 1997, **31**, 96.
266. H. Waldmann, M. Schelhaas, E. Nägele, J. Kuhlmann, A. Wittinghofer, H. Schroeder and J.R. Silvius, *Angew.Chem., Int.Ed.*, 1997, **36**, 2238.
267. T. Pohl and H. Waldmann, *J.Amer.Chem.Soc.*, 1997, **119**, 6702.
268. V. Favero, G. Porzi and S. Sandri, *Tetrahedron: Asymmetry*, 1997, **8**, 599.
269. C.J. Moody, L. Ferris, D. Haigh and E. Swann, *J.Chem.Soc., Chem.Commun.*, 1997, 2391.
270. M.J. Dunn and R.F.W. Jackson, *Tetrahedron*, 1997, **53**, 13905.
271. K. Wisniewski, S. Joswig, B. Falkiewicz and A. Kolodziejczyk, *Pol.J. Chem.*, 1997, **71**, 1506.
272. Y. Shao and H. Paulus, *J.Pept.Res.*, 1997, **50**, 193.
273. K. Severin, D.H. Lee, A.J. Kennan and M.R. Ghadiri, *Nature*, 1997, **389**, 706.
274. S. Yao, I. Ghosh, R. Zutshi and J. Chmielewski, *J.Amer.Chem.Soc.*, 1997, **119**, 10559.
275. K. Severin, D.H. Lee, J.A. Martinez and M.R. Ghadiri, *Chem.-Eur.J.*, 1997, **3**, 1017.
276. E. Imai, H. Honda, K. Hatori and K. Matsuno, *Viva Origini*, 1997, **25**, 289.
277. J. Bódi, H. Süli-Vargha, K. Ludányi, K. Vékey and G. Orosz, *Tetrahedron Lett.*, 1997, **38**, 3293.
278. M.V. Sidorova, A.S. Molokoedov, M.V. Ovchinnikov, Zh.D. Bespalova and V.N. Bushuev, *Bioorg.Khim.*, 1997, **23**, 46.
279. J. Ancans, G.J. Moore, P.J. Roumelioti and J.M. Matsoukas, *Epitheor. Klin.Farmakol.Farmakokinet., Int.Ed.*, 1997, **11**, 108.
280. J. Sueiras-Diaz, D.M. Jones, M. Szelke, B.J. Leckie, S.R. Beattie, C. Beattie and J.J. Morton, *J.Pept.Res.*, 1997, **50**, 239.

281. C. Kuhn, G. Lindeberg, A. Gogoll, A. Hallberg and B. Schmidt, *Tetrahedron,* 1997, **53**, 12497.
282. L. Maletnská, W. Neugebauer, J. Pérodin, M. Lefebvre and E. Escher, *J. Med. Chem.*, 1997, **40**, 3271.
283. S.L. Chambers, R. Ronald, J.M. Hanesworth, D.H. Kinder and J.W. Harding, *Peptides*, 1997, **18**, 505.
284. B. Schmidt, S. Lindman, W. Tong, G. Lindeberg, A. Gogoll, Z. Lai, M. Thoernwall, B. Synnergren, A. Nilsson, C.J. Welch, M. Sohtell, C. Westerlund, F. Nyberg, A. Karlen and A. Hallberg, *J. Med. Chem.*, 1997, **40**, 903.
285. A. Ogrel, W. Bloemhoff, J. Lugtenburg and J. Raap, *Liebig's Ann./Recl.*, 1997, 41.
286. A. Ogrel, W. Bloemhoff, J. Lugtenburg and J. Raap, *J. Pept. Sci.*, 1997, **3**, 193.
287. M. Liakopoulou-Kyriakides, C. Pachatouridis, L. Ekateriniadou and V.P. Papageorgiou, *Amino Acids*, 1997, **13**, 155.
288. R. Christen, T. Jancic, Z.H. Zhou, M.W.W. Adams, J.M. Tomich and E.T. Smith, *J. Inorg. Biochem.*, 1997, **65**, 53.
289. J. Hu and M.J. Miller, *J. Amer. Chem. Soc.*, 1997, **119**, 3462.
290. A. Iwahori, Y. Hirota, R. Sampe, S. Miyano and N. Numao, *Biol. Pharm. Bull.*, 1997, **20**, 267.
291. M.K. Lee, D.G. Lee, S.Y. Shin, S.G. Lee, J.H. Kang and K.-S. Hahm, *J. Microbiol. (Seoul)*, 1996, **34**, 274.
292. D. Andreu and F.J. Garcia, *Lett. Pept. Sci.*, 1997, **4**, 41.
293. M. Tamaki, S. Komiya, S. Akabori and I. Muramatsu, *J. Chem. Soc., Perkin Trans.1*, 1997, 2045.
294. D. Boumrah, M.M. Campbell, S. Fenner and R.G. Kinsman, *Tetrahedron*, 1997, **53**, 6977.
295. D. Gatos, S. Patrianakou, O. Hatzi and K. Barlos, *Lett. Pept. Sci.*, 1997, **4**, 177.
296. T. Haack, Y.M. Sánchez, M.-J. González and E. Giralt, *J. Pept. Sci.*, 1997, **3**, 299.
297. A. Nagy, P. Armatis, R.-Z. Cai, K. Szepeshazi, G. Halmos and A.V. Schally, *Proc. Natl. Acad. Sci., USA*, 1997, **94**, 652.
298. M. Llinares, C. Devin, J. Azay, G. Berge, J.A. Fehrentz and J. Martinez, *Eur. J. Med. Chem.*, 1997, **32**, 767.
299. A. Safavy, M.B. Khazaeli, H. Qin and D.J. Buchasbaum, *Cancer*, 1997, **80**, 2354.
300. M. Gobbo, L. Biondi, F. Cavaggion, F. Filira, T. Piek, P. Mantel and R. Rocchi, *J. Pept. Res.*, 1997, **50**, 336.
301. A. Prahl, T. Wierzba, M. Wszedybyl, W. Juzwa and B. Lammek, *Pol. J. Chem.*, 1997, **71**, 915.
302. A. Prahl, T. Wierzba, P. Winklewski, P. Musial, W. Juzwa and B. Lammek, *Pol. J. Chem.*, 1997, **71**, 929.
303. M. Tancredi, C. Galoppini, S. Meini, L. Quartara, C.A. Maggi and P. Rovero, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2661.
304. L.J. Suva, M.S. Flannery, M.P. Caulfield, D.M. Findlay, H. Juppner, S.R. Goldring, M. Rosenblatt and M. Chorev, *J. Pharmacol. Exp. Ther.*, 1997, **283**, 876.
305. J. Li, J.E. Matsuura, D.J.J. Waugh, T.E. Adrian, P.W. Abel, M.C. Manning and D.D. Smith, *J. Med. Chem.*, 1997, **40**, 3071.
306. S.Y. Shin, M.K. Lee, K.L. Kim and K.-S. Hahm, *J. Pept. Res.*, 1997, **50**, 279.
307. D.G. Lee, J.-H. Park, S.Y. Shin, S.G. Lee, M.K. Lee, K.L. Kim and K.-S. Hahm, *Biochem. Mol. Biol. Int.*, 1997, **43**, 489.
308. S. Vunnam, P. Juvvadi and R.B. Merrifield, *J. Pept. Res.*, 1997, **49**, 59.
309. P. Lucietto, G. Fossati, H.L. Ball, P. Giuliani and P. Mascagni, *J. Pept. Res.*, 1997, **49**, 308.

310. M.E. Houston, L. Harvath and J.F. Honek, *Bioorg.Med.Chem.Lett*., 1997, **7**, 3007.
311. M. Boggian, G. Vertuani, G. Cavicchioni, R. Pecoraro, A. Scatturin and S. Spisani, *Farmaco*, 1997, **52**, 439.
312. I. Torrini, M.P. Paradisi, G.P. Zecchini, G. Lucente, E. Gavuzzo, F. Mazza, G. Pochetti, S. Traniello and S. Spisani, *Biopolymers*, 1997, **42**, 415.
313. K. Shiosaki, C.W. Lin, H. Kopecka, B. Bianchi, T. Miller, M. Stashko and D. Witte, *J.Med.Chem*., 1997, **40**, 1169.
314. S. Herrero, M.L. Suárez-Gea, R. González-Muñiz, M.T. García-López, R. Herranz, S. Ballaz, A. Barber, A. Fortuño and J. Del Río, *Bioorg.Med. Chem.Lett*., 1997, **7**, 855.
315. M.E. Pierson, J.M. Comstock, R.D. Simmons, F. Kaiser, R. Julien, J. Zongrone and J.D. Rosamond, *J.Med.Chem*., 1997, **40**, 4302.
316. J.-C. Califano, L. Goullieux, M. Amblard, J.-A. Fehrentz, N. Bernad, G. Berge, J. Castel and J. Martinez, *Lett.Pept.Sci*., 1997, **4**, 235.
317. C.E. Augelli-Szafran, T.S. Purchase, B.D. Roth, B. Tait, B.K. Trivedi, M. Wilson, N. Suman-Chauhan and L. Webdale, *Bioorg.Med.Chem.Lett*., 1997, **7**, 2009.
318. B. Bellier, I. McCort-Tranchepain, B. Ducos, S. Danascimento, H. Meudal, F. Noble, C. Garbay and B.P. Roques, *J.Med.Chem*., 1997, **40**, 3947.
319. A.G.S. Blommaert, H. Dhôtel, B. Ducos, C. Durieux, N. Goudreau, A. Bado, C. Garbay and B.P. Roques, *J.Med.Chem*., 1997, **40**, 647.
320. Y. Feng, J.P. Taulane and M. Goodman, *Macromolecules*, 1997, **30**, 2947.
321. J.-F. Hernandez, B. Bersch, Y. Pétillot, J. Gagnon and G.J. Arlaud, *J. Pept.Res*., 1997, **49**, 221.
322. S. Rothemund, E. Krause, M. Beyermann and M. Bienert, *J.Pept.Res*., 1997, **50**, 184.
323. A. Miranda, S.L. Lahrichi, J. Gulyas, S.C. Koerber, A.G. Craig, A. Corrigan, C. Rivier, W. Vale and J. Rivier, *J.Med.Chem*., 1997, **40**, 3651.
324. M. Cebrat, M. Lisowski, I.Z. Siemion, M. Zimecki and Z. Wieczorek, *J. Pept.Res*., 1997, **49**, 415.
325. D.E. Ward, R. Lazny and M.S.C. Pedras, *Tetrahedron Lett*., 1997, **38**, 339.
326. F. Cavelier, R. Jacquier, J.-L. Mercadier, J. Verducci, M. Traris and A. Vey, *J.Pept.Res*., 1997, **50**, 94.
327. J.M. Ramanjulu, M.M. Joullié and W.-R. Li, *Synth.Commun*., 1997, **27**, 3259.
328. J.S. Davies, J. Howe, J. Jayatilake and T. Riley, *Lett.Pept.Sci*., 1997, **4**, 441.
329. M.A. Castiglione Morelli, F. Bisaccia, S. Spisani, M. de Biasi, S. Traniello and A.M. Tamburro, *J.Pept.Res*., 1997, **49**, 492.
330. C. Flouzat, F. Marguerite, F. Croizet, M. Percebois, A. Monteil and M. Combourieu, *Tetrahedron Lett*., 1997, **38**, 1191.
331. T. Suzawa, K. Shibata, T. Tanaka, Y. Matsuda and M. Yamasaki, *Bioorg. Med.Chem.Lett*., 1997, **7**, 1715.
332. W.L. Cody, J.X. He, M.D. Reily, S.J. Haleen, D.M. Walker, E.L. Reyner, B.H. Stewart and A.M. Doherty, *J.Med.Chem*., 1997, **40**, 2228.
333. M. Husbyn, L. Örning, K.S. Sakariassen and P.M. Fischer, *J.Pept.Res*., 1997, **50**, 475.
334. A.D. King, N.-W. Huh, L.G. Pedersen and R.G. Hiskey, *J.Pept.Res*., 1997, **50**, 34.
335. M. Maeda, Y. Izuno, K. Kawasaki, Y. Kaneda, Y. Mu, Y. Tsutsumi, K.W. Lem and T. Mayumi, *Biochem.Biophys.Res.Commun*., 1997, **241**, 595.
336. J.C. Spetzler, M. Meldal, E. Meinjohanns, L. Steinaa, S. Mouritsen and K. Bock, *J.Pept.Sci*., 1997, **3**, 397.
337. A. Juréus, Ü. Langel and T. Bartfai, *J.Pept.Res*., 1997, **49**, 195.

338. N.S. Sturm, A.M. Hutzler, C.S. David, B.Y. Azizeh, D. Trivedi and V.J. Hruby, *J. Pept. Res.*, 1997, **49**, 293.
339. B.Y. Azizeh, J.-M. Ahn, R. Caspari, M.D. Shenderovich, D. Trivedi and V.J. Hruby, *J. Med. Chem.*, 1997, **40**, 2555.
340. X.-M. Lu, A.J. Fischmann, M. Kenneway, R.G. Tompkins and V.R. Young, *J. Labelled Compd. Radiopharm.*, 1997, **39**, 205.
341. I. Mezö, S. Lovas, I. Pályi, B. Vincze, A. Kálnay, G. Turi, Z. Vadász, J. Seprödi, M. Idei, G. Tóth, E. Gulyás, F. Ötvös, M. Mák, J.E. Horváth, I. Teplán and R.F. Murphy, *J. Med. Chem.*, 1997, **40**, 3353.
342. P.E. Maligres, I. Houpis, K. Rossen, A. Molina, J. Sager, V. Upadhyay, K.M. Wells, R.A. Reamer, J.E. Lynch, D. Askin, R.P. Volante, P.J. Reider and P. Houghton, *Tetrahedron*, 1997, **53**, 10983.
343. R.M. Campbell, E.P. Heimer, M. Ahmad, H.G. Eisenbeis, T.J. Lambros, Y. Lee, R.W. Miller, P.R. Stricker and A.M. Felix, *J. Pept. Res.*, 1997, **49**, 527.
344. J. Klodt, M. Kuhn, U.C. Marx, S. Martin, P. Rösch, W.G. Forssmann and K. Adermann, *J. Pept. Res.*, 1997, **50**, 222.
345. K. Nokihara, V. Wray, E. Ando, S. Naruse and H. Tetsuo, *Regul. Pept.*, 1997, **70**, 111.
346. I. Augeven-Bour, S. Rebuffat, C. Auvin, C. Goulard, Y. Prigent and B. Bodo, *J. Chem. Soc., Perkin Trans.1*, 1997, 1587.
347. E.J. Schwartz, J. Goldberg, C. Clayberger, A.M. Krensky and J.H. Griffin, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 37.
348. R.J. Bergeron, C. Ludin, R. Müller, R.E. Smith and O. Phanstiel, *J. Org. Chem.*, 1997, **62**, 3285.
349. B. Kundu, M. Shukla, S.K. Khare, P.P. Gupta, R. Singh, Amernath and G.K. Patnaik, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1191.
350. A. Plech, M. Rykaczewska-Czerwinska, H. Bartosz-Bechowski, D. Lombardska-Sliwinska, M. Malota, M. Szewczyk, R. Brus and D. Konopinska, *Pol. J. Pharmacol.*, 1997, **49**, 119.
351. K.G. Carson, C.F. Schwender, H.N. Shroff, N.A. Cochran, D.L. Gallant and M.J. Briskin, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 711.
352. J.W. Corbett, N.R. Graciani, S.A. Mousa and W.F. Degrado, *Bioorg. Med. Chem.-Lett.*, 1997, **7**, 1371.
353. D.Y. Jackson, C. Quan, D.R. Artis, T. Rawson, B. Blackburn, M. Struble, G. Fitzgerald, K. Chan, S. Mullins, J.P. Burnier, W.J. Fairbrother, K. Clark, M. Berisini, H. Chui, M. Renz, S. Jones and S. Fong, *J. Med. Chem.*, 1997, **40**, 3359.
354. M.R. Attwood, E.A. Conway, R.M. Dunsdon, J.R. Greening, B.K. Handa, P.S. Jones, S.C. Jordan, E. Keech and F.X. Wilson, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 429.
355. Z.-W. Miao, Y. Jiang, X.-Q. Li, X.-J. Xu and Y.-Q. Tang, *Gaodeng Xuexiao Huaxue Xuebao*, 1997, **18**, 734.
356. B. Kuhlman, J.A. Boice, W.-J. Wu, R. Fairman and D.P. Raleigh, *Biochemistry*, 1997, **36**, 4607.
357. M. Zhao, H.K. Kleinman and M. Mokotoff, *J. Pept. Res.*, 1997, **49**, 240.
358. N. Rubio. M. Pujol, M. Munoz, M.A. Alsina, I. Haro and F. Reig, *Supramol. Sci.*, 1997, **4**, 449.
359. M. Mokotoff, D.P. Swanson, S.S. Jonnalagadda, M.W. Epperly and M.L. Brown, *J. Pept. Res.*, 1997, **49**, 510.
360. T. Kaiser and W. Voelter, *Z. Naturforsch., B, Chem. Sci.*, 1997, **52**, 281.
361. T. Kaiser and W. Voelter, *J. Prakt. Chem./Chem.-Ztg.*, 1997, **339**, 371.

362. H. Hojo, Y. Akamatsu, K. Yamauchi, M. Kinoshita, S. Miki and Y. Nakamura, *Tetrahedron*, 1997, **53**, 14263.
363. M.F. Giblin, S.S. Jurisson and T.P. Quinn, *Bioconjugate Chem.*, 1997, **8**, 347.
364. C. Haskell-Luevano, K. Toth, L. Boteju, C. Job, A.M. de L. Castrucci, M.E. Hadley and V.J. Hruby, *J. Med. Chem.*, 1997, **40**, 2740.
365. C. Haskell-Luevano, S. Hendrata, C. North, T.K. Sawyer, M.E. Hadley, V.J. Hruby, C. Dickinson and I. Gantz, *J. Med. Chem.*, 1997, **40**, 2133.
366. R.-H. Mattern, S.P. Gunasekera and O.J. McConnell, *Tetrahedron Lett.*, 1997, **38**, 2197.
367. V. Caciagli, F. Cardinali, A. Hänsicke, G. Tuchalaski and P. Lombardi, *J. Pept. Sci.*, 1997, **3**, 224.
368. P. Singh, C.R. Hurrell, J.B.C. Findlay and C.W.G. Fishwick, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 715.
369. J.-L. Fauchère, N. Kucharczyk, E. Jacoby, M. Lonchampt, P. Robineau, C. Dacquet, D. Regoli and E. Canet, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 203.
370. T.B. Wyman, F. Nicol, O. Zelphati, P.V. Scaria, C. Plank and F.C. Szoka, *Biochemistry*, 1997, **36**, 3008.
371. J.A. Shin, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2367.
372. N. Sugimoto and S.-i. Nakano, *J. Chem. Soc., Chem. Commun.*, 1997, 2125.
373. T. Carell and J. Butenandt, *Angew. Chem., Int. Ed.*, 1997, **36**, 1461.
374. J. Hlaváček, B. Bennettová, T. Barth and R. Tykva, *J. Pept. Res.*, 1997, **50**, 153.
375. D. Pawlak, N.N. Chung, P.W. Schiller and J. Izdebski, *J. Pept. Sci.*, 1997, **3**, 277.
376. B. Drouillat, B. Kellam, C. Dekany, M.S. Starr and I. Toth, *Bioorg. Med. Chem.-Lett.*, 1997, **7**, 2247.
377. H. Lin, S. Shah, I.K. Reddy, B.C. Yoburn and W. Zito, *Drug Des. Discovery*, 1997, **15**, 83.
378. K. Kitagawa, N. Mizobuchi, T. Hama, T. Hibi, R. Konishi and S. Futaki, *Chem. Pharm. Bull.*, 1997, **45**, 1782.
379. S. Liao, J. Lin, M.D. Shenderovich, Y. Han, K. Hasohata, P. Davis, W. Qiu, F. Porreca, H.I. Yamamura and V.J. Hruby, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 3049.
380. A. Capasso, P. Amodeo, G. Balboni, R. Guerrini, L.H. Lazarus, P.A. Temussi and S. Salvadori, *FEBS Lett.*, 1997, **417**, 141.
381. R.C. Haaseth, T. Zalewska, P. Davis, H.J. Yamamura, F. Porreca and V.J. Hruby, *J. Pept. Res.*, 1997, **50**, 171.
382. J.L. Torres and P. Clapés, *J. Pept. Sci.*, 1997, **3**, 99.
383. H. Choi, T.F. Murray, G.E. DeLander, W.K. Schmidt and J.V. Aldrich, *J. Med. Chem.*, 1997, **40**, 2733.
384. S. Arttamangkul, J.E. Ishmael, T.F. Murray, D.K. Grandy, G.E. DeLander, B.L. Kieffer and J.V. Aldrich, *J. Med. Chem.*, 1997, **40**, 1211.
385. K. Soderstrom, H. Choi, F.W. Berman, J,V. Aldrich and T.F. Murray, *Eur. J. Pharmacol.*, 1997, **338**, 191.
386. T. Chiba and Y. Sasaki, *Ann. Rep. Tohoku Coll. Pharm.*, 1996, **43**, 117.
387. S.E. Schullery, T. Mohammedshah, H. Makhlouf, E.L. Marks, B.S. Wilenkin, S. Escobar, C. Mousigian and D.L. Heyl, *Bioorg. Med. Chem.*, 1997, **5**, 2221.
388. G. Tóth, Z. Darula, A. Péter, F. Fülöp, D. Tourwé, H. Jaspers, P. Verheyden, Z. Böcskey, Z. Tóth and A. Borsodi, *J. Med. Chem.*, 1997, **40**, 990.
389. A. Misicka, S. Cavagnero, R. Horvath, P. Davis, F. Porreca, H.I. Yamamura and V.J. Hruby, *J. Pept. Res.* 1997, **50**, 48.
390. Z. Darula, A. Péter and G. Tóth, *J. Labelled Compd. Radiopharm.*, 1997, **39**, 817.
391. R. Tomatis, M. Marastoni, G. Balboni, R. Guerrini, A. Capasso, L. Sorrentino,

V. Santagada, G. Caliendo, L.H. Lazarus and S. Salvadori, *J.Med.Chem.*, 1997, **40**, 2948.
392. A. Misicka, A. Lipkowski, R. Horvath, P. Davis, F. Porreca, H.I. Yamamura and V.J. Hruby, *Life Sci.*, 1997, **60**, 1263.
393. R. Raghubir, T. Naqvi, S. Varma, W. Haq, K.B. Mathur and G.K. Patnaik, *Med.Chem.Res.*, 1997, **7**, 16.
394. K.M. Sivanandaiah, V.V.S. Babu, S.C. Shankaramma and M. Lakshmana, *Indian J.Pharmacol.*, 1997, **29**, 92.
395. T. Terashima, A. Ambo and Y. Sasaki, *Ann.Rep.Tohoku Coll.Pharm.*, 1996, **43**, 109.
396. N.V. Sumbatyan, K. Groger, O.N. Chichenkov and G.A. Korshunova, *Lett. Pept.Sci.*, 1997, **4**, 477.
397. T.M. Hackeng, C.M. Mounier, C. Bon, P.E. Dawson, J.H. Griffin and S.B.H. Kent, *Proc.Natl.Acad.Sci., U.S.A.*, 1997, **94**, 7845.
398. J.-X. Xu and S. Jin, *Gaodeng Xuexiao Huaxue Xuebao*, 1997, **18**, 1098.
399. K. Nokihara, Y. Nagawa, S.-P. Hong and H. Nakanishi, *Lett.Pept.Sci.*, 1997, **4**, 141.
400. R.M. Black, J.M. Balkovec, K.M. Nollstadt, S. Dreikorn, K.F. Bartizal and G.K. Abruzzo, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 2879.
401. B. Lammek, M. Czaja, I. Derdowska, P. Rekowski, H.I. Trzeciak, P. Sikora, W. Szkróbka, R. Stojko and G. Kupryszewski, *J.Pept.Res.*, 1997, **49**, 261.
402. M. Moutiez, G. Lippens, C. Sergheraert and A. Tartar, *Bioorg.Med.Chem. Lett.*, 1997, **7**, 719.
403. Y. Yanai, K. Irie, H. Ohigashi and P.A. Wender, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 117.
404. J.D. Wade, F. Lin, G. Talbo, L. Otvös, Y.-Y. Tan and G.W. Tregear, *Biomed.Pept., Proteins Nucleic Acids,* 1997, **2**, 89.
405. M. Zhao, S. Peng, S. Dilinuer, Y. Wang, Q. Zhou and W. Zhao, *Yaoxue Xuebao*, 1997, **32**, 271.
406. S.I. Klein, M. Czekaj, B.F. Molino and V. Chu, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 1773.
407. T.-A. Tran, R.H. Mattern, Q. Zhu and M. Goodman, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 997.
408. A. Muller, F. Schumann, M. Koksch and N. Sewald, *Lett.Pept.Sci.*, 1997, **4**, 275.
409. M.A. Sow, A. Molla, F. Lamaty and R. Lazaro, *Lett.Pept.Sci.*, 1997, **4**, 455.
410. M. Maeda, Y. Izuno, K. Kawasaki, Y. Kaneda, Y. Mu, Y. Tsutsumi, S. Nakagawa and T. Mayumi, *Chem.Pharm.Bull.*, 1997, **45**, 1788.
411. D. Liu, J. Karanicolas, C. Yu, Z. Zhang and G.A. Woolley, *Bioorg.Med. Chem.Lett.*, 1997, **7**, 2677.
412. Y.L. Zhang, H.-F. Lu, J.M. Becker and F. Naider, *J.Pept.Res.*, 1997, **50**, 319.
413. P. Francescato, G. Lugaro, G.L. Gianfranceschi, L. De Angelis and F. Chillemi, *J.Pept.Sci.*, 1997, **3**, 54.
414. G. Ösapay, L. Prokai, H.-S. Kim, K.F. Medzihradsky, D.H. Coy, G. Liapakis, T. Reisine, G. Melacini, Q. Zhu, S.H.-H. Wang, R.-H. Mattern and M. Goodman, *J.Med.Chem.*, 1997, **40**, 2241.
415. P.M. Smith-Jones, B. Stolz, R. Albert, H. Knecht and C. Bruns, *Nucl.Med. Biol.*, 1997, **24**, 761.
416. A. Pyluck, R. Yuan, E. Galligan, P. Primakoff, D.G. Myles and N.S. Sampson, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 1053.
417. T. Niidome, M. Kimura, T. Chiba, N. Ohmori, H. Mihara and H. Aoyagi, *J.Pept.Res.*, 1997, **49**, 563.

418. U. Norinder, C. Rivera and A. Undén, *J.Pept.Res.*, 1997, **49**, 155.
419. I.E. Kasheverov, Y.N. Utkin, P. Franke and V.I. Tsetlin, *J.Pept.Res.*, 1997, **50**, 408.
420. G. Bitan, I. Sukhotinsky, Y. Mashriki, M. Hanani, Z. Selinger and C. Gilon, *J.Pept.Res.*, 1997, **49**, 421.
421. N.H. Guo, H.C. Krutzsch, J.K. Inman, C.S. Shannon and D.D. Roberts, *J. Pept.Res.*, 1997, **50**, 210.
422. J.I. DeGraw, R.G. Almquist, C.K. Hiebert, A.K. Judd, L. Dousman, R.L. Smith, W.R. Waud and I. Uchida, *J.Med.Chem.*, 1997, **40**, 2398.
423. J.I. DeGraw, R.G. Almquist, C.K. Hiebert, W.T. Colwell, J. Crase, T. Hayano, A.K. Judd, L. Dousman, R.L. Smith, W.R. Waud and I. Uchida, *J.Med.Chem.*, 1997, **40**, 2386.
424. R.L. Smith, L. Dousman, W.R. Waud and J.I. DeGraw, *J.Med.Chem.*, 1997, **40**, 2407.
425. P.J.A. Weber and A.G. Beck-Sickinger, *J.Pept.Res.*, 1997, **49**, 375.
426. M. Sukumar, E.M. Ross amd T. Higashijima, *Biochemistry*, 1997, **36**, 3632.
427. T. Niidome, R. Kawakami, K. Okamoto, N. Ohmori, H. Mihara and H. Aoyagi, *J.Pept.Res.*, 1997, **50**, 458.
428. R.J. Andersen, J.E. Coleman, E. Piers and D.J. Wallace, *Tetrahedron Lett.*, 1997, **38**, 317.
429. V. Calabro, J.M. Sabatier, E. Blanc, C. Lecomte, J. Van Rietschoten and H. Darbon, *J.Pept.Res.*, 1997, **50**, 39.
430. S. Liang, Z. Xia and J. Xie, *Sci.China Ser.C:Life Sci.*, 1997, **40**, 449.
431. V. Krishnakumari and R. Nagaraj, *J.Pept.Res.*, 1997, **50**, 88.
432. N. Ohta, T. Mochizuki, M. Hoshino, L. Jun, H. Kobayashi and N. Yanaihara, *J.Pept.Res.*, 1997, **50**, 178.
433. M. Dettin, R. Roncon, M. Simonetti, S. Tormene, L. Falcigno, L. Paolillo and C. di Bello, *J.Pept.Sci.*, 1997, **3**, 15.
434. K.A. Higgins, W. Bicknell, H.H. Keah and M.T.W. Hearn, *J.Pept.Res.*, 1997, **50**, 421.
435. H.L. Ball, A.W.E. Chan, W.A. Gibbons, A.R.M. Coates and P. Mascagni, *J.Pept.Sci.*, 1997, **3**, 168.
436. C.Z. Dong, H. de Rocquigny, E. Rémy, S. Mellac, M.C. Fournié-Zaluski and B.P. Roques, *J.Pept.Res.*, 1997, **50**, 269.
437. B. Dahmani, D. Krebs, S. El Antri, F. Troalen and S. Fermandjian, *J.Biomol.-Struct.Dyn.*, 1997, **14**, 429.
438. G.K. Walkup and B. Imperiali, *J.Amer.Chem.Soc.*, 1997, **119**, 3443.
439. T. Matsubara, H. Shinohara and M. Sisido, *Macromolecules*, 1997, **30**, 2651.
440. E.L. Altschuler, N.V. Hud, J.A. Mazrimas and B. Rupp, *J.Pept.Res.*, 1997, **50**, 73.
441. C. Uchida, K. Wakamatsu, Y. Iizuka, K. Oguma and M. Oya, *Bull.Chem.Soc. Jpn.*, 1997, **70**, 1709.
442. D. Ranganathan and S. Kurur, *Tetrahedron Lett.*, 1997, **38**, 1265.
443. K. Tsutsumiuchi, K. Aoi and M. Okada, *Macromolecules*, 1997, **30**, 4013.
444. W.N.E. Van Dijk-Wolthuis, L. Van de Water, P. Van de Wetering, M. Steenbergen, J.J. Kettenes-Van des Bonch, W. Schuyl and W.E. Hennink, *Macromol.Chem.Phys.*, 1997, **198**, 3893.
445. G. Mezo, J. Kajtar, I. Nagy, M. Szekerke and F. Hudecz, *Biopolymers*, 1997, **42**, 719.
446. Gy. Szokan, M. Almas, K. Krizsen, A.R. Khlafulla, E. Tyihak and B. Szende, *Biopolymers*, 1997, **42**, 305.
447. R. Maget-Dana, A. Brack and D. Lelievre, *Supramol.Sci.*, 1997, **4**, 365.

448. S. Pan, F. Shi, L. Huang, Q. Zhou, Z. Lin and W. Yi, *Shengwu Yixue Gongchengxue Zazhi*, 1997, **14**, 101.
449. G. Guichard and D. Seebach, *Chimia*, 1997, **51**, 315.
450. T.J. Deming, *Nature*, 1997, **390**, 386.
451. K.-Q. Tian, B.B. Brown, D.P. Mack, C.A. Hutton and P.A. Bartlett, *J. Org. Chem.*, 1997, **62**, 514.
452. J.F. Lynas and B. Walker, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1133.
453. J. Lynas and B. Walker, *Biochem. J.*, 1997, **325**, 609.
454. J.A. Fehrentz, M. Paris, A. Heitz, J. Velek, F. Winternitz and J. Martinez, *J. Org. Chem.*, 1997, **62**, 6792.
455. A. Escherich, L. Ditzel, H.-J. Musiol, M. Groll, R. Huber and L. Moroder, *Biol. Chem.*, 1997, **378**, 893.
456. S.Y. Tamura, E.A. Goldman, T.K. Brunck, W.C. Ripka and J.E. Semple, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 331.
457. H. Noguchi, T. Aoyama and T. Shioiri, *Tetrahedron Lett.*, 1997, **38**, 2883.
458. J.E. Semple, D.C. Rowley, T.K. Brunck and W.C. Ripka, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 315.
459. S.Y. Tamura, J.E. Semple, J.E. Reiner, E.A. Goldman, T.K. Brunck, M.S. Lim-Wilby, S.H. Carpenter, W.E. Rote, G.L. Oldeshulte, B.M. Richard, R.F. Nutt and W.C. Ripka, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1543.
460. J.E. Semple, N.K. Minami, S.Y. Tamura, T.K. Brunck, R.F. Nutt and W.C. Ripka, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2421.
461. T.A. Lyle, Z. Chen, S.D. Appleby, R.M. Freidinger, S.J. Gardell, S.D. Lewis, Y. Li, E.A. Lyle, J.J. Lynch, A.M. Mulichak, A.S. Ng, A.M. Naylor-Olsen and W.M. Sanders, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 67.
462. T.J. Tucker, W.C. Lumma, S.D. Lewis, S.J. Gardell, B.J. Lucas, E.P. Baskin, R. Woltmann, J.J. Lynch, E.A. Lyle, S.D. Appleby, I.W. Chen, K.B. Dancheck and J.P. Vacca, *J. Med. Chem.*, 1997, **40**, 1565.
463. T.J. Tucker, W.C. Lumma, S.D. Lewis, S.J. Gardell, B.J. Lucas, J.T. Sisko, J.J. Lynch, E.A. Lyle, E.P. Baskin, R.F. Woltmann, S.D. Appleby, I.W. Chen, K.B. Dancheck, A.M. Naylor-Olsen, J.A. Krueger, C.M. Cooper and J.P. Vacca, *J. Med. Chem.*, 1997, **40**, 3687.
464. D.-M. Feng, S.J. Gardell, S.D. Lewis, M.G. Bock, Z. Chen, R.M. Freidinger, A.M. Naylor-Olsen, H.G. Ramjit, R. Woltmann, E.P. Baskin, J.J. Lynch, R. Lucas, J.A. Shafer, K.B. Danchek, I.W. Chen, S.-S. Mao, J.A. Krueger, T.R. Hare, A.M. Mulichak and J.P. Vacca, *J. Med. Chem.*, 1997, **40**, 3726.
465. L. Guo, G. Liu and L. Yun, *Zhongguo Yaowu Huaxue Zazhi*, 1997, **7**, 43.
466. D. Panagiotopoulos, K. Alexopoulos, J.M. Matsoukas, T. Mavromoustakos, E. Theodoropoulou, E. Kalatzis, P. Fatseas, S. Mahmoud and M.D. Hollenberg, *Epitheor. Klin. Farmakol. Farmacokinet., Int. Ed.*, 1997, **11**, 128.
467. S.-L. Lee, R. Alexander, A. Smallwood, R. Trievel, L. Mersinger, P.C. Weber and C. Kettner, *Biochemistry*, 1997, **36**, 13180.
468. J. Cacciola, R.S. Alexander, J.M. Fevig and P.F.W. Stouten, *Tetrahedron Lett.*, 1997, **38**, 5741.
469. A. Salimbeni, F. Paleari, R. Canevotti, M. Criscuoli, A. Lippi, M. Angiolini, L. Belvisi, C. Scolastico and L. Colombo, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2205.
470. C. Dominguez, D.J. Carini, P.C. Weber, R.M. Knabb, R.S. Alexander, C.A. Kettner and R.R. Wexler, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 79.
471. S. Kim, S.Y. Hwang, Y.K. Kim, M. Yun and Y.S. Oh, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 769.

472. T. Akiyama, S. Tsutsumi, E. Hatsushiba, S. Ohuchi and T. Okonogi, *Bioorg. Med. Chem. Lett*., 1997, **7**, 533.
473. P. Préville, J.X. He, M. Tarazi, M.A. Siddiqui, W.L. Cody and A.M. Doherty, *Bioorg. Med. Chem. Lett*., 1997, **7**, 1563.
474. S. Elgendy, G. Patel, D. Green, C.A. Goodwin, M.F. Scully, W. Husman, E. Skordalakes, V.V. Kakkar and J.J. Deadman, *Tetrahedron Lett*., 1997, **38**, 3305.
475. K. Midura-Nowaczek, I. Bruzzo, W. Roszkowska-Jakimiec and K. Worowski, *Farmaco*, 1997, **52**, 35.
476. J. Rozycki, G. Kupryszewski, K. Rolka, U. Ragnarsson, T. Zbyryt, W. Watorek and T. Wilusz, *Pol. J. Chem*., 1997, **71**, 176.
477. C.A. Veale, P.R. Bernstein, C.M. Bohnert, F.J. Brown, C. Bryant, J.R. Damewood, R. Earley, S.W. Feeney, P.D. Edwards, B. Gomes, J.M. Hulsizer, B.J. Kosmider, R.D. Krell, G. Moore, T.W. Salcedo, A. Shaw, D.S. Silberstein, G.B. Steelman, M. Stein, A. Strimpler, R.M. Thomas, E.P. Vacek, J.C. Williams, D.J. Wolanin and S. Woolson, *J. Med. Chem*., 1997, **40**, 3173.
478. P.D. Edwards, D.W. Andisik, C.A. Bryant, B. Ewing, B. Gomes, J.J. Lewis, D. Rakiewicz, G. Steelman, A. Strimpler, D.A. Trainor, P.A. Tuthill, R.C. Mauger, C.A. Veale, R.A. Wildonger, J.C. Williams, D.J. Wolanin and M. Zottola, *J. Med. Chem*., 1997, **40**, 1876.
479. M. Iqbal, P.A. Messina, B. Freed, M. Das, S. Chatterjee, R. Tripathy, M. Tao, K.A. Josef, B. Dembofsky, D. Dunn, E. Griffith, R. Siman, S.E. Senadhi, W. Biazzo, D. Bozyczko-Coyne, S.L. Meyer, M.A. Ator and R. Bihovsky, *Bioorg. Med. Chem. Lett*., 1997, **7**, 539.
480. S. Chatterjee, M.A. Ator, D. Bozyczko-Coyne, K. Josef, G. Wells, R. Tripathy, M. Iqbal, R. Bihovsky, S.E. Senadhi, S. Mallya, T.M. O'Kane, B.A. McKenna, R. Siman and J.P. Mallamo, *J. Med. Chem*., 1997, **40**, 3820.
481. N. Schaschke, H.-J. Musiol, I. Assfalg-Machleidt, W. Machleidt and L. Moroder, *Bioorg. Med. Chem. Lett*., 1997, **7**, 2507.
482. B.J. Moon and S.K. Kim, *Bull. Korean Chem. Soc*., 1997, **18**, 778.
483. A. De, P. Basak and J. Iqbal, *Tetrahedron Lett*., 1997, **38**, 8383.
484. N. Schaschke, I. Assfalg-Machleidt, W. Machleidt, D. Turk and L. Moroder, *Bioorg. Med. Chem*., 1997, **5**, 1789.
485. Y.L. Kong and S. Jin, *Chin. Chem. Lett*., 1997, **8**, 779.
486. K.J. Lee and D.H. Kim, *Bull. Korean Chem. Soc*., 1997, **18**, 1100.
487. C.K. Esser, R.L. Bugianesi, C.G. Caldwell, K.T. Chapman, P.L. Durette, N.N. Girotra, I.E. Kopka, T.J. Lanza, D.A. Levorse, M. MacCoss, K.A. Owens, M.M. Ponpipom, J.P. Simeone, R.K. Harrison, L. Niedwiecki, J.W. Becker, A.I. Marcy, M.G. Axel, A.J. Christen, J. McDonnell, V.L. Moore, J.M. Olszewski, C. Saphos, D.M. Visco, F. Shen, A. Colletti, P.A. Krieter and W.K. Hagmann, *J. Med. Chem*., 1997, **40**, 1026.
488. C.K. Esser, N.J. Kevin, N.A. Yates and K.T. Chapman, *Bioorg. Med. Chem. Lett*., 1997, **7**, 2639.
489. A.D. Baxter, J. Bird, R. Bhogal, T. Massil, K.J. Minton. J. Montana and D.A. Owen, *Bioorg. Med. Chem. Lett*., 1997, **7**, 897.
490. A.D. Baxter, R. Bhogal, J.B. Bird, G.M. Buckley, D.S. Gregory, P.C. Hedger, D.T. Manallack, T. Massil, K.J. Minton, J.G. Montana, S. Neidle, D.A. Owen and R.J. Watson, *Bioorg. Med. Chem. Lett*., 1997, **7**, 2765.
491. A. Miller, M. Askew, R.P. Beckett, C.L. Bellamy, E.A. Bone, R.E. Coates, A.H. Davidson, A.H. Drummond, P. Huxley, F.M. Martin, L. Saroglou, A.J. Thompson, S.E. van Dijk and M. Whittaker, *Bioorg. Med. Chem. Lett*., 1997, **7**, 193.

492. R. Hirayama, M. Yamamoto, T. Tsukida, K. Matsuo, Y. Obata, F. Sakamoto and S. Ikeda, *Bioorg. Med. Chem.*, 1997, **5**, 765.
493. Q. Wang, B. Pfeiffer, G.C. Tucker, J. Royer and H.-P. Husson, *Bioorg. Med. Chem.-Lett.*, 1997, **7**, 2477.
494. C.P. Decicco, J.L. Seng, K.E. Kennedy, M.B. Covington, P.K. Welch, E.C. Arner, R.L. Magolda and D.J. Nelson, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2331.
495. C. Blackburn, A. Pingali, T. Kehoe, L.W. Herman, H. Wang and S.A. Kates, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 823.
496. R.M.P. Tandon, W. Haq, M. Dikshit, G.K. Patnaik, G.K.Patnaik and M.M. Dhar, *Indian J. Chem.*, 1997, **36B**, 386.
497. M. Bessodes, K. Antonakis, C. Rougeot, F. Capony, M. Garcia and H. Rochefort, *Protein Pept. Lett.*, 1997, **4**, 281.
498. M. Kratzel, R. Hiessbock and A. Bernkop-Schnurch, *Farm. Vestn.*, 1997, **48**, 380.
499. J. Sueiras-Diaz, D.M. Jones, M. Szelke, J. Deinum, L. Svensson, C. Westerlund and M. Sohtell, *J. Pept. Res.*, 1997, **50**, 248.
500. D.M. Jones, J. Sueiras-Diaz, M. Szelke, B.J. Leckie, S.R. Beattie, J. Morton, S. Neidle and R. Kuroda, *J. Pept. Res.*, 1997, **50**, 109.
501. R. Paruszewski, P. Jaworski, J. Tautt and J. Dudkiewicz, *Pharmazie*, 1997, **52**, 206.
502. T.T. Tran, N. Patino, R. Condom, T. Frogier and R. Guedj, *J. Fluorine Chem.*, 1997, **82**, 125.
503. P.L. Beaulieu, D. Wernic, A. Abraham, P.C. Anderson, T. Bogri, Y. Bousquet, G. Croteau, I. Guse, D. Lamarre, F. Liard, W. Paris, D. Thibeault, S. Pav and L. Tong, *J. Med. Chem.*, 1997, **40**, 2164.
504. M.D. Shultz and J. Chmielewski, *Tetrahedron: Asymmetry*, 1997, **8**, 3881.
505. A.D. Abell and G.J. Foulds, *J. Chem. Soc., Perkin Trans.1*, 1997, 2475.
506. J. Iqbal, M. Mukhopadhyay, B.C. Das, S. Rayesh, A. De, R. Sanghi, S. Jain and J.P. Nandy, *Indian J. Chem.,* 1997, **36A**, 498.
507. U.K. Saha and R. Roy, *Tetrahedron Lett.*, 1997, **38**, 7697.
508. D. Qasmi, E. De Rosny, L. Rene, B. Badet, I. Vergely, N. Boggetto and M. Reboud-Ravaux, *Bioorg. Med. Chem.*, 1997, **5**, 707.
509. N. Choy, H.-i. Choi, W.H. Jung, C.R. Kim, H. Yoon, S.C. Kim, T.G. Lee and J.S. Koh, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2635.
510. M. Marastoni, S. Salvadori, F. Bortolotti and R. Tomatis, *J. Pept. Res.*, 1997, **49**, 538.
511. R. Zutshi, J. Franciskovich, M. Shultz, B. Schweitzer, P. Bishop, M. Wilson and J. Chmielewski, *J. Amer. Chem. Soc.*, 1997, **119**, 4841.
512. Y. Konda, Y. Takahashi, H. Mita, K. Makeda and H. Harigaya, *Chem. Lett.*, 1997, 345.
513. X. Zhang, J. Rodrigues, L. Evans, B. Hinkle, L. Ballantyne and M. Peña, *J. Org. Chem.*, 1997, **62**, 6420.
514. Q.M. Wang, R.B. Johnson, J.D. Cohen, G.T. Voy, J.M. Richardson and L.N. Jungheim, *Antiviral Chem. Chemotherap.*, 1997, **8**, 303.
515. W. Ogilvie, M. Bailey, M.-A. Poupart, A. Abraham, A. Bhavsar, P. Bonneau, J. Bordeleau, Y. Bousquet, C. Chabot, J.-S. Duceppe, G. Fazal, S. Goulet, C. Grand-Maitre, I. Guse, T. Halmos, P. Lavallee, M. Leach, E. Malenfant. J. O'Meara, R. Plante, C. Plouffe, M. Poirier, F. Soucy, C. Yoakim and R. Deziel, *J. Med. Chem.*, 1997, **40**, 4113.
516. C. Noula, G. Kokotos, T. Barth and C. Tzougraki, *J. Pept. Res.*, 1997, **49**, 46.
517. K. Kánai, S. Erdö, E. Susán, M. Fehér, J. Sipos, B. Podányi, A. Szappanos and I. Hermecz, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1701.

518. J.M.C. Golec, M.D. Mullican, M.A. Murcko, K.P. Wilson, D.P. Kay, S.D. Jones, R. Murdoch, G.W. Bemis, S.A. Raybuck, Y.-P. Luong and D.J. Livingston, *Bioorg.Med.Chem.Lett*., 1997, **7**, 2181.
519. M. Taliani, E. Bianchi and A. Pessi, *Lett.Pept.Sci*., 1997, **4**, 101.
520. H.H. Wasserman and A.K. Petersen, *Tetrahedron Lett*., 1997, **38**, 953.
521. D.M. Leonard, K.R. Shuler, C.J. Poulter, S.R. Eaton, T.K. Sawyer, J.C. Hodges, T.-Z. Su, J.D. Scholten, R.C. Gowan, J.S. Sebolt-Leopold and A.M. Doherty, *J.Med.Chem*., 1997, **40**, 192.
522. D.J. McNamara, E. Dobrusin, D.M. Leonard, K.R. Shuler, J.S. Kaltenbronn, J. Quin, S. Bur, C.E. Thomas, A.M. Doherty, J.D. Scholten, K.K. Zimmerman, B.S. Gibbs, R.C. Gowan, M.P. Latash, W.R. Leopold, S.A. Przybranowski and J.S. Sebolt-Leopold, *J.Med.Chem*., 1997, **40**, 3319.
523. H. Reuveni, A. Gitler, E. Poradosu, C. Gilon and A. Levitzki, *Bioorg. Med.Chem*., 1997, **5**, 85.
524. P. Stoeber, M. Schelhaas, E. Naegele, P. Hagenbuch, J. Retey and H. Waldmann, *Bioorg.Med.Chem*., 1997, **5**, 75.
525. G. Byk, Y. Lelievre, M. Duchesne, F.F. Clerc, D. Scherman and J.D. Guitton, *Bioorg.Med.Chem*., 1997, **5**, 115.
526. A. Basak, B. Ernst, D. Brewer, N.G. Seidah, J.S. Munzer, C. Lazure and G.A. Lajoie, *J.Pept.Res*., 1997, **49**, 596.
527. T. Gariani and R.J. Leatherbarrow, *J.Pept.Res*., 1997, **49**, 467.
528. F. Cavelier, A.T. Russell, C.J. Schofield and J.E. Baldwin, *J.Chem.Res., Synop.*, 1997, 200.
529. R.B. Silverman, H. Huang, M.A. Marletta and P. Martasek, *J.Med.Chem*., 1997, **40**, 2813.
530. M.D. Andrews, K.A, O'Callaghan and J.C. Vederas, *Tetrahedron*, 1997, **53**, 8295.
531. G. Rosse, U. Sequin, H. Mett, P. Furet, P. Traxler and H. Fretz, *Helv. Chim.Acta*, 1997, **80**, 653.
532. S.R. Nagarajan, B. Devadas, M.E. Zupec, S.K. Freeman, D.L. Brown, H.-F. Lu, P.P. Mehta, N.S. Kishore, C.A. McWherter, D.P. Getman, J.I. Gordon and J.A. Sikorski, *J.Med.Chem*., 1997, **40**, 1422.
533. D.L. Brown, B. Devadas, H.-F. Lu, S. Nagarajan, M.E. Zupec, S.K. Freeman, C.A. McWherter, D.P. Getman and J.A. Sikorski, *Bioorg.Med.Chem. Lett*., 1997, **7**, 379.
534. B. Devadas, S.K. Freeman, M.E. Zupec, H.-F. Lu, S.R. Nagarajan, N.N. Kishore, J.K. Lodge, D.W. Kuneman, C.A. McWherter, D.V. Vinjamoori, D.P. Getman, J.I. Gordon and J.A. Sikorski, *J.Med.Chem*., 1997, **40**, 2609.
535. J.C. Phelan, N.J. Skelton, A.C. Braisted and R.S. McDowell, *J.Amer. Chem.Soc*., 1997, **119**, 455.
536. W.D. Kohn, C.M. Kay and R.S. Hodges, *J.Pept.Sci*., 1997, **3**, 209.
537. J.K. Young, F. Mar, M. Xu, R.E. Humphreys, N.M. Clemente, J.M. Stattel, D.J. Nelson, J. Gambino and G.E. Wright, *J.Pept.Res.*, 1997, **50**, 122.
538. S. Datta, N. Shamala, A. Banerjee, A. Pramanik, S. Bhattacharjya and P. Balaram, *J.Amer.Chem.Soc*., 1997, **119**, 9246.
539. S. Datta, R. Kaul, R.B. Rao, N. Shamala and P. Balaram, *J.Chem.Soc., Perkin Trans.2*, 1997, 1659.
540. S. Lee, T. Kiyota, T. Kunitake, E. Matsumoto, S. Yamashita, K. Anzai and G. Sugihara, *Biochemistry*, 1997, **36**, 3782.
541. K.S. Broo, L. Brive, P. Ahlberg and L. Baltzer, *J.Amer.Chem.Soc*., 1997, **119**, 11362.
542. L. Brive, G.T. Dolphin and L. Baltzer, *J.Amer.Chem.Soc*., 1997, **119**, 8598.

543. M. Kjellstrand, K. Broo, L. Andersson, C. Farre, Å. Nilsson and L. Baltzer, *J.Chem.Soc., Perkin Trans.2,* 1997, 2745.
544. B. Sacca, F. Formaggio, M. Crisma, C. Toniolo and R. Gennaro, *Gazz.Chim. Ital.*, 1997, **127**, 495.
545. H.S.M. Lu, M. Volk, Y. Kholodenko, E. Gooding, R.M. Hochstrasser and W.F. DeGrado, *J.Amer.Chem.Soc.*, 1997, **119**, 7173.
546. D.L. Holmes, E.M. Smith and J.S. Nowick, *J.Amer.Chem.Soc.*, 1997, **119**, 7665.
547. J.S. Nowick and S. Insaf, *J.Amer.Chem.Soc.*, 1997, **119**, 10903.
548. E.M. Smith, D.L. Holmes, A.J. Shaka and J.S. Nowick, *J.Org.Chem.*, 1997, **62**, 7906.
549. M. Gatos, F. Formaggio, M. Crisma, C. Toniolo, G.M. Bonora, Z. Benedetti, B. di Blasio, R. Iacovino, A. Santini, M. Saviano and J. Kamphuis, *J.Pept.Sci.*, 1997, **3**, 110.
550. A.J. Doig, *J.Chem.Soc., Chem.Commun.*, 1997, 2153.
551. M. Gatos, F. Formaggio, M. Crisma, G. Valle, C. Toniolo, G.M. Bonora, M. Saviano, R. Iacovino, V. Menchise, S. Galdiero, C. Pedone and E. Benedetti, *J.Pept.Sci.*, 1997, **3**, 367.
552. J.S. Nowick, M. Pairish, I.Q. Lee, D.L. Holmes and J.W. Ziller, *J.Amer. Chem.Soc.*, 1997, **119**, 5413.
553. I.G. Jones, W. Jones and M. North, *Synlett.*, 1997, 63.
554. J. Voigt, M. Noltemeyer and O. Reiser, *Synlett.*, 1997, 202.
555. P.W. Baures, W.H. Ojala, W.B. Gleason and R.L. Johnson, *J.Pept.Res.*, 1997, **50**, 1.
556. M. Pellegrini, M. Royo, M. Chorev and D.F. Mierke, *J.Pept.Res.*, 1997, **49**, 404.
557. T. Hayashi, T. Asai and H. Ogoshi, *Tetrahedron Lett.*, 1997, **38**, 3039.
558. A. Calcagni, D. Rossi, M.P. Paradisi, G. Lucente, G. Liusi, E. Gavuzzo, F. Mazza, G. Pochetti and M. Paci, *Biopolymers*, 1997, **41**, 555.
559. F. Fabiola. G.V. Pattabhi, E.B. Raju and S. Durani, *J.Pept.Res.*, 1997, **50**, 352.
560. P. Sharma and T.P. Singh, *Curr.Top.Biophys.*, 1996, **20**, 158.
561. A. Lombardi, G. D'Auria, M. Saviano, O. Maglio, F. Nastri, L. Quartara, C. Pedone and V. Pavona, *Biopolymers*, 1996, **40**, 505.
562. S. Hanessian and H. Yang, *Tetrahedron Lett.*, 1997, **38**, 3155.
563. A. Bianco, T. Bertolini, M. Crisma, G. Valle, C. Toniolo, M. Maggini, G. Scorrano and M. Prato, *J.Pept.Res.*, 1997, **50**, 159.
564. K. Kim and J.P. Germanas, *J.Org.Chem.*, 1997, **62**, 2847.
565. K. Kim and J.P. Germanas, *J.Org.Chem.*, 1997, **62**, 2853.
566. K. Brickmann, P. Somfai and J. Kihlberg, *Tetrahedron Lett.*, 1997, **38**, 3651.
567. K.G.I. Nilsson, G. Ljunger and P.M. Melin, *Biotechnol.Lett.*, 1997, **19**, 889.
568. G. Arsequell and G. Valencia, *Tetrahedron: Asymmetry*, 1997, **8**, 2839.
569. T. Xu and J.K. Coward, *Biochemistry*, 1997, **36**, 14683.
570. J.-Q. Fan and Y.C. Lee, *J.Biol.Chem.*, 1997, **272**, 27058.
571. U.K. Saha, L.S. Griffith, J. Rademann, A. Geyer and R.R. Schmidt, *Carbohydr.Res.*, 1997, **304**, 21.
572. S.J. Danishefsky, S. Hu, P.F. Cirillo, M. Eckhardt and P.H. Seeberger, *Chem.-Eur.J.*, 1997, **3**, 1617.
573. L. Lay, M. Meldal, F. Nicotra, L. Panza and G. Russo, *J.Chem.Soc., Chem. Commun.*, 1997, 1469.
574. L.-X. Wang, M. Tang, T. Suzuki, K. Kitajima, Y. Inoue, S. Inoue, J.-Q. Fan and Y.-C. Lee, *J.Amer.Chem.Soc.*, 1997, **119**, 11137.
575. S.D. Debenham, J.S. Debenham, M.J. Burk and E.J. Toone, *J.Amer.Chem. Soc.*, 1997, **119**, 9897.

576. Z.-W. Guo, Y. Nakahara, Y. Nakahara and T. Ogawa, *Angew. Chem., Int. Ed.*, 1997, **36**, 1464.
577. Z.-W. Guo, Y. Nakahara, Y. Nakahara and T. Ogawa, *Bioorg. Med. Chem.*, 1997, **5**, 1917.
578. K. Witte, P. Sears, R. Martin and C.-H. Wong, *J. Amer. Chem. Soc.*, 1997, **119**, 2114.
579. C. Unverzagt, *Tetrahedron Lett.*, 1997, **38**, 5627.
580. C. Vogel, U. Jeschke, S. Kramer and A.-J. Ott, *Liebig's Ann./Recl.*, 1997, 737.
581. T. Kanemitsu, Y. Ogihara and T. Takeda, *Chem. Pharm. Bull.*, 1997, **45**, 643.
582. E. Meinjohanns, M. Meldal, T. Jensen, O. Werdelin, L. Galli-Stampino, S. Mouritsen and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1997, 871.
583. G. Klich, H. Paulsen, B. Meyer, M. Meldal and K. Bock, *Carbohydr. Res.*, 1997, **299**, 33.
584. Y. Nakahara, Y. Nakahara, Y. Ito and T. Ogawa, *Tetrahedron Lett.*, 1997, **38**, 7211.
585. Y. Suhara, M. Izumi, M. Ichikawa, M.B. Penno and Y. Ichikawa, *Tetrahedron Lett.*, 1997, **38**, 7167.
586. H. Paulsen, A. Schleyer, N. Mathieux, M. Meldal and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1997, 281.
587. A. Schleyer, M. Meldal, R. Manat, H. Paulsen and K. Bock, *Angew. Chem., Int. Ed.*, 1997, **36**, 1976.
588. Z.-W. Guo, Y. Nakahara and T. Ogawa, *Tetrahedron Lett.*, 1997, **38**, 4799.
589. P. Arya, S. Dion and G.K.H. Shimizu, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1537.
590. P. Coutrot, C. Grison and M. Lecouvey, *Bull. Soc. Chim. Fr.*, 1997, **134**, 27.
591. Z.-W. Guo, Y. Nakahara, Y. Nakahara and T. Ogawa, *Carbohydr. Res.*, 1997, **303**, 373.
592. A.A. Kandil, N. Chan, M. Klein and P. Chong, *Glycoconjugate J.*, 1997, **14**, 13.
593. E.C. Rodriguez, K.A. Winans, D.S. King and C.R. Bertoggi, *J. Amer. Chem. Soc.*, 1997, **119**, 9905.
594. R.L. Polt, L. Szabo and J. Ramza, *Postepy Hig. Med. Dosw.*, 1996, **50**, 493.
595. D. Qiu and R.R. Koganty, *Tetrahedron Lett.*, 1997, **38**, 45.
596. B. Liebe and H. Kunz, *Angew. Chem., Int. Ed.*, 1997, **36**, 618.
597. O. Seitz and C.H. Wong, *J. Amer. Chem. Soc.*, 1997, **119**, 8766.
598. N. Mathieux, H. Paulsen, M. Meldal and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1997, 2359.
599. M. Leuck and H. Kunz, *J. Prakt. Chem./Chem.-Ztg.*, 1997, **339**, 322.
600. S. Mehta, M. Meldal, V. Ferro, J.Ø. Duus and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1997, 1365.
601. H. Vegad, C.J. Gray, P.J. Somers and A.S. Dutta, *J. Chem. Soc., Perkin Trans. 1*, 1997, 1429.
602. G. Thoma, J.L. Magnani, R. Öhrlein, B. Ernst, F. Schwarzenbach and R.O. Duthaler, *J. Amer. Chem. Soc.*, 1997, **119**, 7414.
603. A.E. Zemlyakov, V.O. Kur'yanov, V.V. Tsikalov, E.A. Aksenova and V.Ya. Chirva, *Chem. Natl. Compd.*, 1997, **33**, 61.
604. J. Gervay, P.S. Ramamoorthy and N.N. Mamuya, *Tetrahedron.*, 1997, **53**, 11039.
605. G. Auger, M. Crouvoisier, M. Caroff, J. van Heijenoort and D. Blanot, *Lett. Pept. Sci.*, 1997, **4**, 371.
606. Y. Suhara, M.Izumi, M. Ichikawa, M.B. Penno and Y. Ichikawa, *Tetrahedron Lett.*, 1997, **38**, 7167.
607. G. Shapiro, D. Buchler, C. Dalvit, P. Frey, M. Del Carmen Fernandez, B. Gomez-Lor, E. Pombo-Villar, U. Stauss, R. Swoboda and C. Waridel, *Bioorg. Med. Chem.*, 1997, **5**, 147.
608. H. Fretz, *Lett. Pept. Sci.*, 1997, **4**, 171.

609. A. Paquet, B. Blackwell, M. Johns and J. Nikiforuk, *J.Pept.Res.*, 1997, **50**, 262.
610. A. Tholey and R. Hoffmann, *J.Pept.Sci.*, 1997, **3**, 186.
611. A. Shahripour, K.S. Para, M.S. Plummer, E.A. Lunney, D.R. Holland, J.R. Rubin, C. Humblet, J.H. Fergus, J.S. Marks, A.R. Saltiel and T.K. Sawyer, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 1107.
612. M.S. Plummer, E.A. Lunney, K.S. Para, A. Shahripour, C.J. Stankovic, C. Humblet, J.H. Fergus, J.S. Marks, R. Herrera *et al.*, *Bioorg.Med.Chem.*, 1997, **5**, 41.
613. T.M. Johnson, J.W. Perich, J.D. Bjorge, D.J. Fujita and H.-C. Cheng, *J. Pept.Res.*, 1997, **50**, 365.
614. M.N. Qabar, J. Urban and M. Kahn, *Tetrahedron*, 1997, **53**, 11171.
615. H. Fretz, *Lett.Pept.Sci.*, 1997, **3**, 343.
616. A. Bernhardt, M. Drewello and M. Schutkowski, *J.Pept.Res.*, 1997, **50**, 143.
617. Q. Xu, E.A. Ottinger, N.A. Sole and G. Barany, *Lett.Pept.Sci.*, 1997, **3**, 333.
618. C. Garca-Echeverra, C. Stamm, R. Wille, D. Arz and B. Gay, *Lett.Pept. Sci.*, 1997, **4**, 49.
619. R. Hoffmann, N.F. Dawson, J.D. Wade and L. Otvös, *J.Pept.Res.*, 1997, **50**, 132.
620. T. Wakamiya, R. Togashi, T. Nishida, K. Saruta, J.I. Yasuoka, S. Kusumoto, S. Aimoto, Y.K. Kumagaye, K. Nakajima and K. Nagata, *Bioorg. Med.Chem.*, 1997, **5**, 135.
621. K.F. Medzihradszky, N.J. Phillips, L. Senderowicz, P. Wang and C.W. Turck, *Protein Sci.*, 1997, **6**, 1405.
622. R. Hoffman, T. Hoffmann, A. Tholey, A.C. Schulte and H.R. Kalbitzer, *J.Pept.Res.*, 1997, **49**, 163.
623. Q. Dai and R. Chen, *Heteroat.Chem.*, 1997, **8**, 279.
624. N.M. O'Brien-Simpson, N.J. Ede, L.E. Brown, J. Swan and D.C. Jackson, *J.Amer.Chem.Soc.*, 1997, **119**, 1183.
625. N.J.C.M. Beekman, W.M.M. Schaaper, G.I. Tesser, K. Dalsgaard, S. Kamstrup, J.P.M. Langeveld, R.S. Boshuizen and R.H. Meloen, *J.Pept.Res.*, 1997, **50**, 357.
626. S. Eifler, I. Leblond, E. Trifilieff and J.-P. Lepoittevin, *Lett.Pept. Sci.*, 1997, **4**, 467.
627. Y. Hamuro, M.C. Calama, H.S. Park and A.D. Hamilton, *Angew.Chem., Int.Ed.*, 1997, **36**, 2680.
628. Z. Wang, W.P. Carney and R.A. Laursen, *J.Pept.Res.*, 1997, **50**, 483.
629. F. Reichel, P.R. Ashton and G.-J. Boons, *J.Chem.Soc., Chem.Commun.*, 1997, 2087.
630. P. Ciapetti, F. Soccolini and M. Taddei, *Tetrahedron*, 1997, **53**, 1167.
631. S. Jordan, C. Schwemler, W. Kosch, A. Kretschmer, E. Schwenner, U. Stropp and B. Mielke, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 681.
632. S. Jordan, C. Schwemler, W. Kosch, A. Kretschmer, U. Stropp, E. Schwenner and B. Mielke, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 687.
633. C.G. Simmons, A.E. Pitts, L.D. Mayfield, J.W. Shay and D.R. Corey, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 3001.
634. S. Basu and E. Wickstrom, *Bioconjugate Chem.*, 1997, **8**, 481.
635. C. García-Echeverría, D. Husken, C.S. Chiesi and K.H. Altmann, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 1123.
636. P. Lagriffoule, P. Wittung, M. Eriksson, K.K. Jensen, B. Norden, O. Buchardt and P.E. Nielsen, *Chem.-Eur.J.*, 1997, **3**, 912.
637. E. Vivès and B. Lebleu, *Tetrahedron Lett.*, 1997, **38**, 1183.
638. G. Lowe and T. Vilaivan, *J.Chem.Soc., Perkin Trans. 1*, 1997, 539.
639. G. Lowe and T. Vilaivan, *J.Chem.Soc., Perkin Trans. 1*, 1997, 547.
640. G. Lowe and T. Vilaivan, *J.Chem.Soc., Perkin Trans. 1*, 1997, 555.

641. T. Koch, H.F. Hansen, P. Andersen, T. Larsen, H.G. Batz, K. Otteson and H. Ørum, *J.Pept.Res.*, 1997, **49**, 80.
642. T. Pehk and A. Uri, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 2159.
643. M. Fujii, K. Yamamoto, J. Hidaka and T. Ohtsu, *Tetrahedron Lett.*, 1997, **38**, 417.
644. M. Fujii, K. Yoshida, J. Hidaka and T. Ohtsu, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 637.
645. X.X. Li, Y.G. Wang, Y.Z. Chen, L.R. Zhang and L.H. Zhang, *Chin.Chem. Lett.*, 1997, **8**, 385.
646. C. Guibourdenche, D. Seebach and F. Natt, *Helv.Chim.Acta*, 1997, **80**, 1.
647. N.M. Howarth and L.P.G. Wakelin, *J.Org.Chem.*, 1997, **62**, 5441.
648. T. Yamazaki, K. Komatsu, H. Umemiya, Y. Hashimoto, K. Shudo and H. Kagechika, *Tetrahedron Lett.*, 1997, **38**, 8363.
649. M. Beltran, M. Maseda, J. Robles, E. Pedroso and A. Grandas, *Lett.Pept. Sci.*, 1997, **4**, 147.
650. M. Beltran, M. Maseda, Y. Perez, J. Robles, E. Pedroso and A. Grandas, *Nucleosides Nucleotides*, 1997, **16**, 1487.
651. J. Robles, M. Maseda, M. Beltran, M. Concernau, E. Pedroso and A. Grandas, *Bioconjugate Chem.*, 1997, **8**, 785.
652. U. Diederichsen, *Angew.Chem., Int.Ed.*, 1997, **36**, 1886.
653. S. Scarfi, A. Gasparini, G. Damonte and U. Benatti, *Biochem.Biophys.Res. Commun.*, 1997, **236**, 323.
654. A. Farese, S. Pairot, N. Patino, V. Ravily, R. Condom and A. Aumelas, *Nucleosides Nucleotides*, 1997, **16**, 1893.
655. I.E. Ichetovkin, N.V. Sumbatyan, G. Bagerzadekh and G.A. Korshunova, *Vestn. Mosk.Univ., Ser.2;Khim.*, 1997, **38**, 120.
656. G.A. Korshunova, I.A. Ilicheva, N.V. Sumbatyan and K.-c. Hyun, *Lett. Pept.Sci.*, 1997, **4**, 473.
657. P. Ciapetti, A. Mann, A. Schoenfelder, M. Taddei, E. Trifilieff, I. Canet and J.L. Canet, *Lett.Pept.Sci.*, 1997, **4**, 341.
658. E. Lesnick, F. Hassman, J. Barbeau, K. Teng, K. Weiler, R. Griffey and S. Freier, *Nucleosides Nucleotides,* 1997, **16**, 1775.
659. T. Koch, M.B. Borre, M. Naesby, H.G. Batz and H. Orum, *Nucleosides Nucleotides*, 1997, **16**, 1771.
660. A. Peyman, E. Uhlmann, K. Wagner, S. Augustin, C. Weiser, D.W. Will and G. Breipohl, *Angew.Chem., Int.Ed.*, 1997, **36**, 2809.
661. H. Waldmann and S. Gabold, *J.Chem.Soc., Chem.Commun.*, 1997, 1861.
662. V. Nagarajan, V. Pattabhi, A. Johnson, V. Bobde and S. Durani, *J.Pept. Res.*, 1997, **49**, 74.
663. S.N. Mitra, S. Dey, Karthikeyan and T.P. Singh, *Biopolymers*, 1997, **41**, 97.
664. E Godier-Marc, D.J. Aitken and H.-P. Husson, *Tetrahedron Lett.*, 1997, **38**, 4065.
665. D.E. Hibbs, M.B. Hursthouse, I.G. Jones, W. Jones, K.M.A. Malik and M. North, *Tetrahedron*, 1997, **53**, 17417.
666. A.R. McMath, D. Guillaume, D.J. Aitken and H.-P. Husson, *Bull.Soc.Chim. Fr.*, 1997, 134, 105.
667. A.I. Jiménez, R. Venderesse, M. Marraud, A. Aubry and C. Cativiela, *Tetrahedron Lett.*, 1997, **38**, 7559.
668. R. Balakrishnan, R. Parthasarathy and N. Ramasubbu, *J.Pept.Res.*, 1997, **49**, 371.
669. E. Mossel, F. Formaggio, M. Crisma, C. Toniolo, Q.B. Broxterman, W.H.T. Boesten, J. Kamphuis, P.J.L.M. Quaedflieg and P. Temussi, *Tetrahedron: Asymmetry*, 1997, **8**, 1305.

670. R.-H. Mattern, Y. Amino, E. Benedetti and M. Goodman, *J.Pept.Res.*, 1997, **50**, 286.
671. D. Ranganathan, S. Kurur, K.P. Madhusudanan and I.L. Karle, *Tetrahedron Lett.*, 1997, **38**, 4659.
672. P.E. Thompson and M.T.W. Hearn, *Tetrahedron Lett.*, 1997, **38**, 2907.
673. T. Hintermann and D. Seebach, *Synlett,* 1997, 437.
674. M. Bogenstätter and W. Steglich, *Tetrahedron*, 1997, **53**, 7267.
675. G.R. Geier and T. Sasaki, *Tetrahedron Lett.*, 1997, **38**, 3821.
676. A. Nicolaou, G. Kokotos, V. Constantinou-Kokotou, C. Charitos, C. Noula, R. Verger and W.A. Gibbons, *J.Pept.Sci.*, 1997, **3**, 291.
677. L. Zhang and J.P. Tam, *J.Amer.Chem.Soc.*, 1997, **119**, 2363.
678. U. Schmidt and J. Langner, *J.Pept.Res.*, 1997, **49**, 67.
679. S. Gangwar, G.M. Pauletti, T.J. Siahaan, V.J. Stella and R.T. Borchardt, *J.Org.Chem.*, 1997, **62**, 1356.
680. E.-I. Pécheur, D. Hoekstra, J. Sainte-Marie, L. Maurin, A. Bienvenüe and J.R. Philippot, *Biochemistry*, 1997, **36**, 3773.
681. I.S. Weitz, M. Pellegrini, D.F. Mierke and M. Chorev, *J.Org.Chem.*, 1997, **62**, 2527.
682. J. Lohse, P.E. Nielsen, N. Harrit and O. Dahl, *Bioconjugate Chem.*, 1997, **8**, 503.
683. F.A. Bennett, D.J. Barlow, A.N.O. Dodoo, R.C. Hider, A.B. Lansley, M.J. Lawrence, C. Marriott and S.S. Bansal, *Tetrahedron Lett.*, 1997, **38**, 7449.
684. S. Østergaard and A. Holm, *J.Pept.Sci.*, 1997, **3**, 123.
685. Y. Arano, H. Akizawa, T. Uezono, K. Akaji, M. Ono, S. Funakoshi, M. Koizumi, A. Yokoyama, Y. Kiso and H. Saji, *Bioconjugate Chem.*, 1997, **8**, 442.
686. N. Galéotti, E. Plagnes and P. Jouin, *Tetrahedron Lett.*, 1997, **38**, 2459.
687. F. Nastri, A. Lombardi, G. Morelli, O. Maglio, G. D'Auria, C. Pedone and V. Pavone, *Chem.-Eur.J.*, 1997, **3**, 340.
688. S. Sakamoto, S. Sakurai, A. Ueno and H. Mihara, *J.Chem.Soc., Chem. Commun.*, 1997, 1221.
689. R. Vanderesse, L. David, V. Grand, M. Marraud, J.P. Mangeot and A. Aubry, *Tetrahedron Lett.*, 1997, **38**, 2669.
690. F. André, A. Vicherat, G. Boussard, A. Aubry and M. Marraud, *J.Pept. Res.*, 1997, **50**, 372.
691. C. Frochot, R. Vanderesse, A. Driou, G. Linden, M. Marraud and T. Mahn, *Lett.Pept.Sci.*, 1997, **4**, 219.
692. R. Wilcock, P. Speers, G. Warhurst, M. Rowland and K.T. Douglas, *Amino Acids,* 1997, **12**, 185.
693. P. Wipf and T.C. Henninger, *J.Org. Chem.*, 1997, **62**, 1586.
694. M. Kira, T. Matsubara, H. Shinohara and M. Sisido, *Chem.Lett.*, 1997, 89.
695. D. Muller, I. Zeltser, G. Bitan and C. Gilon, *J.Org.Chem.*, 1997, **62**, 411.
696. R.V. Hoffman and J. Tao, *J.Org.Chem.*, 1997, **62**, 2292.
697. A. Tanaka, H. Sakai, T. Ishikawa, T. Aoki, Y. Motoyama and H. Takasugi, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 521.
698. M.C. Chandy and V.N.R. Pillai, *Indian J.Chem.*, 1997, **36B**, 303.
699. P. Furet, B. Gay, C. García-Echeverría, J. Rahuel, H. Fretz, J. Schoepfer and G. Caravatti, *J.Med.Chem.*, 1997, **40**, 3551.
700. B. Kellam, B.W. Bycroft and S.R. Chhabra, *Tetrahedron Lett.*, 1997, **38**, 4849.
701. V.J. Hruby, T.P. Davis, D.F. O'Brien, F. Porreca and H.I. Yamamura, *Protein Eng.*, 1997, **10**(Suppl), 76.
702. F.Kh. Iskhakova, O.V. Esipova and E.N. Zvonkova, *Bioorg.Khim.*, 1995, **21**, 517.
703. M. Scheunemann and B. Johannsen, *Forschungszent.Rossendorf FZR*, 1997, 18.

704. N. Galéotti, M. Giraud and P. Jouin, *Lett.Pept.Sci.*, 1997, **4**, 437.
705. P. Coutrot, C. Grison, S. Geneve, C. Didierjean, A. Aubry, A. Vicherat and M. Marraud, *Lett.Pept.Sci.*, 1997, **4**, 415.
706. T. Yamashita, E. Tsuru, E. Banjyo, M. Doe, K. Shibata, M. Yasuda and M. Gemba, *Chem.Pharm.Bull.*, 1997, **45**, 1940.
707. L. Chaloin, P. Vidal, J. Mery, G. Divita and F. Heitz, *Lett.Pept.Sci.*, 1997, **4**, 231.
708. Y. Kong, S. Jin, X. Jin and Q. Yang, *Hecheng Huaxue*, 1997, **5**, 357.
709. A.G. Shipov, E.P. Kramarova, N.A. Kalashnikova, E.A. Besova and Yu.I. Baukov, *Russ.J.Gen.Chem.*, 1997, **67**, 1736.
710. T.A. Gudasheva, N.I. Zaitseva, N.A. Bondarenko, I.E. Shcherbakova, L.S. Asmakova, G.G. Rosantsev, R.U. Ostrovskaya, T.A. Voronina and S.B. Seredenin, *Khim.-Farm.Zh.*, 1997, **31**, 10.
711. G.J. Moore, *Drug.Dev.Res.*, 1997, **42**, 157.
712. M. Liakopoulou-Kyriakides, C. Pachatouridis, L. Ekateriniadou and V.P. Papageorgiou, *Amino Acids*, 1997, **13**, 155.
713. F. André, M. Marraud, T. Tsouloufis, S.J. Tzartos and G. Boussard, *J.Pept.Sci.*, 1997, **3**, 429.
714. N. Voyer and B. Guérin, *J.Chem.Soc., Chem.Commun.*, 1997, 2329.
715. D. Kreile, D.E. Sile, E.Kh. Korchagova, J. Popelis, V.A. Slavinskaya, G. Cipens and E. Lukevics, *Latv.Kim.Z.*, 1997, 62.
716. P. Shi, X. Hao, Y. Chen, Y. Zheng and Q. Tao, *Yaoxue Xuebao*, 1997, **32**, 106.
717. G. Stavropoulos, V. Magafa, M. Liakopoulou-Kyriakides, Z. Sinakos and A. Aaberg, *Amino Acids*, 1997, **13**, 171.
718. Z. Cheng, Y. Li, Z. Yue, G. Yang and Y. Jiang, *Hecheng Huaxue*, 1997, **5**, 109.
719. A.S. Karnoup, V.M. Shiryaev, V.N. Medvedkin and Yu.V. Mitin, *J.Pept. Res.*, 1997, **49**, 232.
720. A.S. Karnoup, V.M. Shiryaev, V.N. Medvedkin and Yu.V. Mitin, *Bioorg. Khim.*, 1997, **23**, 56.
721. D.J. Skanchy, R. Wilson, T. Poh, G.-H. Xie, C.W. Demarest and J.F. Stobaugh, *Electrophoresis*, 1997, **18**, 985.
722. D. Waidelich, *LaborPraxis*, 1997, **21**, 14.
723. R. Hoffmann, M. Segal and L. Otvös, *Anal.Chim.Acta,* 1997, **352**, 327.

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

BY ANAND S. DUTTA

1 Introduction

The subject matter included this year is broadly similar to that included last year.[1] Most of the publications covered in this chapter were published in 1997. However, some of the 1996 publications (not covered last year) have been included. This is especially so in the case of peptides (e.g. melanocortins, phage library leads and some protein-protein interactions) not discussed last year due to space restrictions. No work published in patents or in unrefereed form (such as conference proceedings) has been included. Non-peptide ligands acting on the peptide receptors have again been included in sections on biologically active peptides. This is becoming an important aspect of peptide research because in the past 5-10 years many non-peptide compounds have been shown to act as antagonists of the endogenous ligand. More recently, in some cases (e.g. angiotensin II, bradykinin and CCK), the non-peptide ligands have also been shown to display agonist properties. As in the 1996 chapter, due to space limitations, the structure-activity studies on non-peptide series of compounds are not described in detail. Only the more potent compounds from each series are highlighted to give an idea about the structural types displaying the desired activity and pharmacokinetic profile. As last year, a small section dealing with the advances in formulation and delivery technology has been included. In addition, some new sections like phage library leads and protein-protein interaction inhibitors have been included. In the first two sections below (peptide backbone modifications and di-, tri-peptide mimetics and cyclic peptides), only examples from those peptides are included which are not covered in the remaining sections on the biologically active peptides. Throughout this chapter, amino acids are denoted by their three letter codes following standard nomenclature. For the naturally occurring L-amino acids, no stereochemistry is specified in the text.

2 Peptide Backbone Modifications and Di- and Tri-peptide Mimetics

Such changes have traditionally been introduced into peptides to improve potency, by altering the conformational preferences and optimising side chain

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interactions, and their pharmacokinetic profile when administered *in vivo*. A number of analogues of enkephalin, dynorphin(1-8), thymopentin and allatostatins containing various amide bond replacements have been reported. In addition, a number of heterocyclic ring systems have been described which can lead to conformationally restricted peptides with increased metabolic stability. Some of these residues, if suitably substituted, can act as di- or tri-peptide replacements. In other cases, the non-peptide moiety acts to induce the types of conformations (e.g. β- and γ-turns and β-sheets) which a flexible peptide can attain due to the presence of certain amino acid residues.

2.1 ψ[CH_2NH]-Aminomethylene Analogues – 2,4-Dimethoxybenzyl group was reported for secondary amine protection in the synthesis of Leu-enkephalin and dynorphin(1-8) analogues.[2] The protecting group was cleaved with mixtures of trifluoroacetic acid containing phenol, thioanisole or trifluoromethanesulfonic acids. Except Tyr-Gly-Gly-Pheψ[CH_2NH]Leu, which was somewhat more potent than Leu-enkephalin at the guinea pig ileum, all the other analogues {Tyrψ[CH_2NH]Gly-Gly-Phe-Leu, Tyr-Glyψ[CH_2NH]Gly-Phe-Leu and Tyr-Gly-Glyψ[CH_2NH]Phe-Leu} were much less potent. Aminomethylene pseudopeptide analogues of cockroach neuropeptides, allatostatins (inhibitors of juvenile hormone), that have a Tyr-Xaa-Phe-Gly-Leu-NH_2 as a C-terminal sequence have been reported.[3] In one of these neuropeptides, Asp-Arg-Leu-Tyr-Ser-Phe-Gly-Leu-NH_2, the Leu^3-Tyr^4 peptide bond was found to be susceptible to endopeptidases. In order to increase resistance to degradation, ψ[CH_2NH] peptide bond surrogate was introduced at this position. The pseudopeptide Asp-Arg-Leuψ[CH_2NH]Tyr-Ser-Phe-Gly-Leu-NH_2 was nearly equiactive to the parent peptide in an *in vitro* juvenile hormone inhibition assay.

2.2 ψ[CH=CH]-Isosteres – Solid-phase synthesis protocol for the synthesis of peptides containing (E)-alkene amide isostere linkages is reported.[4] In addition, a stereocontrolled synthesis of the (E)-alkene dipeptide isostere of Trp-Val has been reported. The stereospecific α-alkylation of the δ-amino-γ-mesyloxy-α,β-unsaturated ester *via* the organocyanocopper-Lewis acid mediated reaction, based on 1,3-transfer of chirality, was successfully applied for the key step in the synthetic sequence.[5]

2.3 ψ[$COCH_2$]-Ketomethylene Isosteres – Analogues of thymopentin (Arg^1-Lys^2-Asp^3-Val^4-Tyr^5) (amino acid residues 32-36 of a 49 amino acid peptide isolated from human thymus tissue) containing ketomethylene groups in place of the various amide bonds are reported.[3,6–8] The pentapeptide thymopentin is known for its activity as an immunomodulating agent. Most of the pentapeptides containing a ketomethylene group between residues 1 and 2, Argψ[CO-CH_2]Nle-Asp-Val-Phe, Argψ[CO-CH_2]Nle-Asp-Val-Phe-NH_2, Argψ[CO-CH_2]Nle-Asp-D-Val-Phe, and residues 3 and 4, Arg-Lys-Aspψ[CO-CH_2]Val-Phe, Arg-Nle-Aspψ[CO-CH_2]Val-Phe, Arg-Pro-Aspψ[CO-CH_2]Val-Phe, Arg-Pro-Aspψ[CO-CH_2]Val-Phe-NH_2, Arg-MeLys-Aspψ[CO-CH_2]Val-Phe, Arg-Lys-Aspψ[CO-CH_2]Ala-Phe and Arg-Lys-Aspψ[CO-CH_2]Val-Tyr, were about 2-5-fold less

potent in the binding assay.[6] Similarly the analogues modified between residues 4 and 5, Arg-Lys-Asp-Valψ[CO-CH_2]Phe, Arg-Pro-Asp-Val([CO-CH_2]Phe, Ac-Arg-Pro-Asp-Valψ[CO-CH_2]Phe, Arg-Nle-Asp-Valψ[CO-CH_2]Phe, Ac-Arg-Nle-Asp-Valψ[CO-CH_2]Phe, Arg-Leu-Asp-Valψ[CO-CH_2]Phe, Arg-Ala-Asp-Valψ[CO-CH_2]Phe, Arg-D-Lys-Asp-Valψ[CO-CH_2]Phe, Arg-Nle-Asp-Alaψ[CO-CH_2]Phe, Arg-Nle-Asp-Valψ[CO-CH_2]Val, Arg-MeNle-Asp-Valψ[CO-CH_2]Phe and Arg-MeNle-Asp-Valψ[CO-CH_2]Val, were less potent (up to 3-fold) than the parent peptide in binding to CEM cells.[7] Reduction of one of the analogues, Arg-Nle-Asp-Val(ψCO-CH_2]Phe, gave the corresponding CHOH analogue Arg-Nle-Asp-Valψ[CHOH-CH_2]Phe which was about 5-fold less potent than the keto-methylene analogue. Only a few thymopentin analogues, Arg-Lys-Aspψ[CO-CH_2]Val-Phe and Arg-Lys-Aspψ[CO-CH_2]Val-Tyr, were more strongly bound to CEM cells than thymopentin itself. However, the stability of the peptides in mouse and human plasma was increased. Two of the compounds, Arg-MeNle-Asp-Valψ[CO-CH_2]Phe and Arg-MeNle-Asp-Valψ[CO-CH_2]Val, were not degraded in mouse and human plasma.

The ketomethylene pseudopeptides of thymopentin were tested *in vivo* in the mouse type II collagen arthritis model and the rat adjuvant arthritis model (using assessment of inflammation and antibody production). The compounds were also tested for immune-potentiating activity *in vitro* using induction of the lymphocyte marker (Thy-1.2) in mouse spleen cells and stimulation of T-cell proliferation. A number of compounds which were stable to degradation in plasma, e.g. Arg-Nle-Asp-Valψ[CO-CH_2]Val, Arg-MeNle-Asp-Valψ[CO-CH_2]Val, Arg-MeNle-Asp-Valψ[CO-CH_2]Phe and Arg-MeLys-Aspψ[CO-CH_2]Val-Phe, were shown to be active in various models at a dose of 0.001-1 mg kg^{-}. The parent peptide Arg-Lys-Asp-Val-Tyr did not show significant activity at these dose levels.[8]

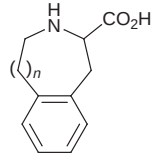
A ketomethylene analogue of allatostatins, Asp-Arg-Leuψ[COCH$_2$]Phe-Ser-Phe-Gly-Leu-NH_2, was nearly equiactive to the parent peptide in an *in vitro* juvenile hormone inhibition assay.[3]

2.4 Retro- and Retro-inverso Pseudopeptides – Peptides corresponding to the immunodominant loop located at residues 135-158 on caspid protein VP1 of foot-and-mouth disease virus generally elicit high levels of anti-peptide and virus neutralising antibodies. It had been shown previously that the antigenic activity of peptide 141-159 of VP1 of a variant of serotype A [Gly-Ser-Gly-Val-Arg-Gly-Asp-Phe-Gly-Ser-Leu-Ala-Pro-Arg-Val-Ala-Arg-Gln-Leu-Cys] can be mimicked by a retro-inverso (all D retro or retroenantio) peptide analogues. This retro-inverso analogue induced greater and longer-lasting antibody titers than did the corresponding L-peptide. A single inoculation of the retro-inverso analogue elicits a high level of neutralising antibodies that persist longer than those induced against the corresponding L-peptide and confer substantial protection in guinea pigs challenged with the cognate virus.[9]

2.5 Rigid Di- and Tri-peptide and Turn Mimetics - In addition to the peptide bond replacements mentioned above, a number of heterocyclic systems (e.g.

Freidinger lactams, diketopiperazine) have been described which could be inserted within the peptide chain to alter the conformations of the biologically active peptides. Some of these conformationally constricting residues, if suitably substituted, can act as di- or tri-peptide replacements. In other cases, the non-peptide moiety acts to induce the types of conformations (e.g. β- and γ-bends and α-helical turns) which a flexible peptide can attain due to the presence of certain amino acid residues.

Conformationally constrained phenylalanine analogue Tic (**1**, n = 0) has been incorporated in many peptides in the past. More recently, syntheses of additional conformationally constrained analogues of phenylalanine like Sic (**1**, n = -CH_2-), Hic (**1**, n = -CH_2-CH_2-) and Nic (**1**, n = -CH_2-CH_2-CH_2-) have been reported.[10] Stereoselective synthesis routes to Freidinger lactams (also incorporated previously in many peptides) of various ring sizes containing a spectrum of C-terminal amino acid residues (**2**) are reported. Using some of these lactams, angiotensin converting enzyme inhibitors were synthesised.[11] One of the more potent compounds was (**3**) which inhibited ACE with an IC_{50} of 8 nM. Synthetic routes to the diazepin-3-one derivatives like (**4**) and (**5**) (R = -CH_2Ph or -CH_3) have been reported.[12]



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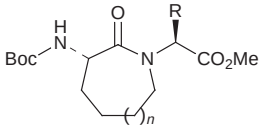

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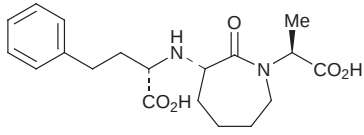

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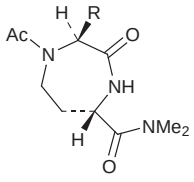

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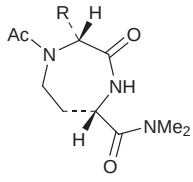
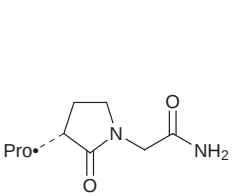


(5)

Diketopiperazine derivatives have been incorporated recently as 'skeletons' in various peptide and non-peptide structures. Incorporation of such structures into the Pro-Leu-Gly-NH_2-related structures like (**6**) and (**7**) (reported previously to be more potent than Pro-Leu-Gly-NH_2 in several pharmacological assay systems) led to compounds (**8-10**). These analogues were designed to explore the idea that the N-terminal "C5" conformation, which was found in the crystal structure of (**6**) and which was mimicked in (**8**) by the diketopiperazine function, was a factor in the high potency of these two agents.[13] Although compounds (**8-10**) had similar pharmacological profile to compounds (**6**) and (**7**), the potency seen was less than that seen for the starting compounds, suggesting that while the N-terminal "C5" conformation may play a role in the potency of the γ-lactam



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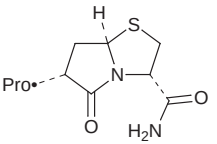

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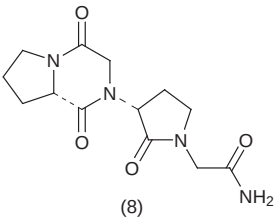

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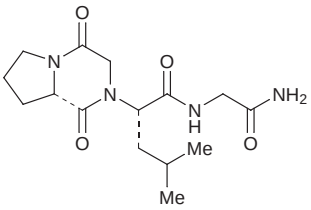

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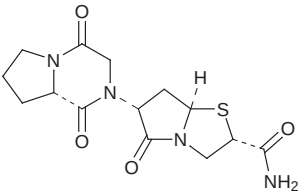
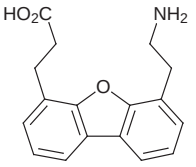


(10)

peptidomimetics of Pro-Leu-Gly, it does not appear to be the primary factor. In the 6-hydroxydopamine-lesioned animal model of Parkinson's disease, (**9**) altered apomorphine-induced rotational behaviour in a dose-dependent manner. The maximum effect occurred at a dose of 0.01 mg kg^{-1} i.p. and resulted in a 52 $\pm$ 14% increase in rotations compared to apomorphine administered alone.

Synthetic routes to a protected (Boc and Fmoc) dibenzofuran-based amino acid template (**11**) designed to generate a β-sheet folding structure, and an Fmoc protected phenothiazine derivative (**12**), suggested to be a possible surrogate for the α-carbon backbone of five residue turns are reported.[14,15]



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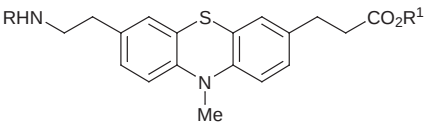

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3 Cyclic Peptides

Conformationally restricted cyclic peptide analogues of biologically active peptides are included in the sections dealing with individual peptides (Section 4). Sequences of the remaining cyclic peptides, their biological activities and some of the conformational studies using cyclic peptides are mentioned below.

In the recent past, backbone to backbone cyclic peptides have been reported in addition to the side-chain to side-chain and N- to C-terminal cyclic peptides. The synthetic routes to the backbone to backbone cyclic peptides require

N^{α}-substituted amino acid derivatives. Various methods for the synthesis of such derivatives have been reported.[16,17] In one synthetic route, preparation of a family of amino acids that contain ω-aminoalkyl groups [Boc-NH-$(CH_2)_n$-N(Fmoc)-CH(R)-COOH] and ω-carboxyalkyl groups [t-BuOCO-$(CH_2)_n$-N(Fmoc)-CH(R)-COOH] linked to the α-amino moiety was achieved by alkylation of suitably monoprotected alkylenediamines, Boc-$NH(CH_2)_nNHCH_2Ph$ (where n = 2-6), and protected ω-amino acids, $Me_3COCO(CH_2)_nNHCH_2Ph$ (where n = 1-5), with triflates of α-hydroxy acid esters, $TfOCHRCO_2CH_2Ph$ (where R = Me, Bzl). The alkylation proceeded with inversion of configuration yielding optically pure products. The N^{α}-(ω-aminoalkyl)amino acids and N^{α}-(ω-carboxyalkyl)amino acids were orthogonally protected to allow their incorporation into peptides by solid-phase synthesis methodology.[16]

Synthesis of a library of 60 backbone-bicyclic substance P analogues was prepared by the simultaneous multiple peptide synthesis method. The peptides, containing both a lactam and a disulfide ring, were synthesised and screened for NK_1 and NK_3 activity. The peptides were weak agonists at the tachykinin receptors.[18]

3.1 Naturally Occurring Cyclic Peptides, Synthetic Analogues and Conformational Studies – Several proline-rich peptides have been isolated from plant seeds, roots and fruits.[19–26] Cyclic nonapeptide, cyclolinopeptide B [c(Pro-Pro-Phe-Phe-Val-Ile-Met-Leu-Ile)], was isolated from the seeds of *Linum usitatissimum*. It showed potent immunosuppressive activity (inhibition of mitogen-induced response of human peripheral blood lymphocytes, IC_{50} 44 ng ml^{-1}) which was comparable with that of cyclosporin A.[19] Another similar immunosuppressive cyclic nonapeptide, cyclolinopeptide A [c(Val-Pro-Pro-Phe-Phe-Leu-Ile-Ile-Leu)], inhibited calcium-dependent activation of T lymphocytes comparably to the actions of cyclosporin A and FK506. The concentration required for complete inhibition, however, was 10 times higher than that of cyclosporin A.[20] Calcineurin, a phosphatase which plays an important role in T lymphocyte signalling, was inhibited *in vitro* by cyclolinopeptide A by a mechanism dependent on the peptidyl-prolyl *cis-trans* isomerase cyclophilin A but not FKBP12.

Proline-rich cyclic decapeptides, cycloleonuripeptide A [c(Gly-Pro-Pro-Pro-Tyr-Pro-Pro-Met-Ile)], B [c(Gly-Pro-Pro-Pro-Tyr-Pro-Pro-Met(O)-Ile)], C [c(Gly-Pro-Pro-Pro-Tyr-Pro-Pro-Met(O)-Ile)] and D [c(Ser-Pro-Pro-Pro-Tyr-Phe-Gln-Thr-Pro-Ile)], were isolated from the fruits of *Leonurus heterophyllus*.[21,22] Distance geometry calculations and restrained energy minimisation data from NMR studies indicated that the backbone structures of cycloleonuripeptides A, B and C consist of two turns, a (βVI turn at Pro^3-Pro^4 and a β I turn at Pro^7-Met^8. X-Ray diffraction studies indicated that the decapeptide backbone of cycloleonuripeptide D contained two β-turns, one type I β-turn at Pro-Ile and one type III β-turn at Pro-Tyr.

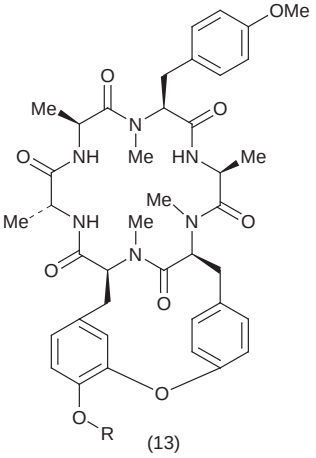
Solid and solution state conformations of a cyclic heptapeptide, c(Gly-Tyr-Gly-Gly-Pro-Phe-Pro), isolated from the roots of *Stellaria yunnanensis* were studied.[23] The main conformational feature of the peptide was a type II and a type II′ β-turn.

Three-dimensional structures in DMSO-d_6 of two cyclic heptapeptides and pentapeptides, segetalin D [c(Gly-Leu-Ser-Phe-Ala-Phe-Pro)], E [c(Gly-Tyr-Val-Pro-Leu-Trp-Pro)], G [c(Gly-Val-Lys-Tyr-Ala)] and H [c(Gly-Tyr-Arg-Phe-Ser)], isolated from seeds of *Vaccaria segatalis* were elucidated by computational and NMR methods.[24,25] The backbone structure of segetalin D had a β II turn at Pro^7-Gly^1 and a β I turn at Phe^4-Ala^5 and that of segetalin E a β II turn at Pro^7-Gly^1 and a β VI turn at Val^3-Pro^4. In addition, each had three intramolecular hydrogen bonds, which constructed a classical P-bulge conformation, as suggested by calculations and NMR studies. The backbone structure of segetalin G contains one β II-like turn at Tyr^4-Ala^5, and that of segetalin H a β II′ turn at Gly^1-Tyr^2 and one γ turn at Arg^3-Phe^4-Ser^5 sequence.

Thionation of oestrogen-like active cyclic peptides, segetalins A and B, with Lawesson's reagent each provided two thiosegetalins; thiosegetalin Al [Gly^1-ψ(CS-NH)-Val^2, Trp^5-ψ(CS-NH)-Ala^6]segetalin A, thiosegetalin A2 [Gly^1-ψ(CS-NH)-Val^2, Ala^6-ψ(CS-NH)-Gly-1]segetalin A, thiosegetalin B1 [Gly^1-ψ(CS-NH)-Val^2, Ala^3-ψ(CS-NH)-Trp^4]segetalin B, and thiosegetalin B2 [Gly^1-ψ(CS-NH)-Val^2, Trp^4-ψ(CS-NH)-Ala-1]segetalin B. Thiosegetalin A2 only showed oestrogen-like activity against ovariectomized rats.[26] On the basis of their conformations (analysed by NMR experiments), the backbone conformation was considered to play an important role in oestrogen-like activity for segetalins.

Anabaenopeptins C and D, two new ureido bond-containing peptides, were isolated from the cultured cyanobacterium *Oscillatoria agardhii* (NIES-204). The gross structures of anabaenopeptins C and D were elucidated by extensive 2D NMR techniques and chemical degradation The absolute stereochemistry of usual amino acids in anabaenopeptins C and D was determined by GC and HPLC analyses.[27]

Analogues of RA-VII (**13**), tuberactinomycins [**14**; tuberactinomycin A, R=OH, R^1=OH, R^2=$CONH_2$; tuberactinomycin B (viomycin), R = H, R^1 = OH, R^2 = $CONH_2$; tuberactinomycin N, R = OH, R^1 = H, R^2 = $CONH_2$; tuberactinomycin O, R = H, R^1 = H, R^2 = $CONH_2$] and capreomycin [**15**; capreomycin IA, R^1 = OH, R^2 = -$CONH_2$; capreomycin IB, R^1 = H, R^2 = -$CONH_2$; capreomycin IIA, R^1 = OH, R^2 = -$CONH_2$ (des-β-lysine); capreomycin IIB, R^1 = H, R^2 = -$CONH_2$ (des-β-lysine)] have been synthesised.[28–31] In order to increase water solubility, a number of analogues of RA-VII (R = Me) were synthesised by incorporating basic groups in the molecule.[28] Two of the compounds [**13**; R = -CH_2-CH_2-NMe_2 or -CH_2-CH_2-N(i-Pr)$_2$] showed antitumour activity against P388 leukaemia in mice. Some other analogues [**13**; R = -CH_2-CH_2-NEt_2, -CH_2-CH_2-N(Ph)Et or -CH_2-CH_2-N(CH_2-Ph)$_2$] were inactive. One of the more potent analogues [**13**; R = -CH_2-CH_2-N(i-Pr)$_2$] was also active against colon cells in mice at a dose of 1.6-3.13 mg kg^{-1} day^{-1}. Analogues of tuberactinomycin B (viomycin) and capreomycin were obtained by replacing the R^1 and/or R^2 groups by various substituted aromatic groups and evaluated against various micro-organisms [*pasteurella spp*., methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci].[29–31] Although most of the analogues showed antibacterial activity, the overall biological profiles of various analogues were different. Modification and replacement of the β-lysine side chain



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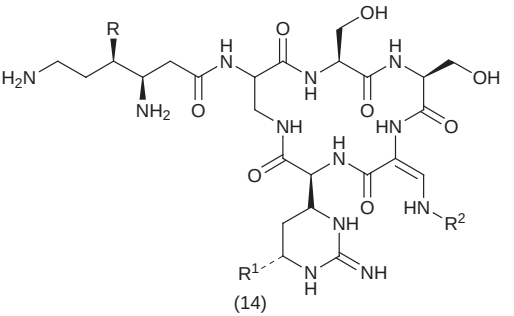

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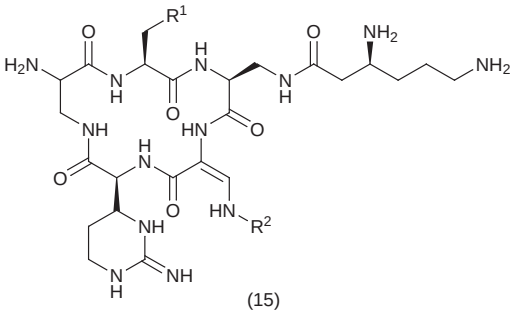
of capreomycin and tuberactinomycin cyclic pentapeptides also resulted in compounds with good antibacterial potency against multidrug-resistant pathogens.

Tris-bridged cyclic peptides consisting of three units of piperazin-2-one derivatives, a serine, a histidine and a carboxylic acid were synthesised as an active site mimic of lipase.[32] The catalytic activity of the synthetic peptides [**16**; **a**: R = -$CONH_2$, **b**: R = -COOH, **c**: R = -CH_2-COOH, **d**: R = -$CONHCH_2COOH$, **e**: R = -$CONHCH_2CH_2COOH$, **f**: R = -H] for the transesterification of 4-nitrophenyl acetate in dimethylsulfoxide was examined. Compound (**16f**), in which the carboxylic acid part was replaced by methyl piperizin-2-one (bridged alanylglycine), exhibited less activity than the other five compounds. Compounds (**16b-16e**), which bear a carboxylic acid function, were found to accelerate the transesterification of 4-nitrophenyl acetate.



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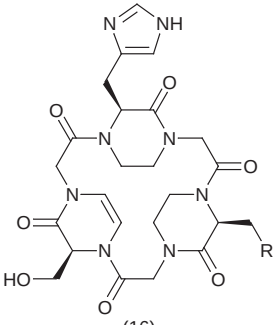

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4 Biologically Active Peptides

4.1 Peptides Involved in Alzheimer's Disease – Recent developments in the pathophysiology and pharmacotherapy of Alzheimer's disease have been reviewed.[33–36] The brains of individuals with Alzheimer's disease are characterised by extracellular deposition of β-amyloid protein (Aβ), intracellular neurofibrillary tangles, and loss of neurones. *In vivo*, amyloids are composed of two broad categories of constituents. The first is the disease, or process specific protein, and the second a set of common structural components the majority of which are the building blocks of basement membranes (e.g. heparan sulfate proteoglycan, laminin, type IV collagen, serum amyloid P). The involvement of these components, apolipoproteins and neurotrophin receptors in Alzheimer's disease has been the subject matter of a number of publications.[37–50]

Molecular genetic studies of families suffering from genetic forms of early onset Alzheimer's disease have identified three genes and their protein products (all integral membrane proteins) as being crucially involved in the etiology of Alzheimer's disease.[37] One of them is β-amyloid precursor protein (the precursor of β-amyloid), and the other two (presenilins S182 and STM2), are homologous in amino acid sequence to one another but are unrelated to β-amyloid precursor protein. Immunofluorescence experiments have indicated that portions of the presenilins are expressed at the cell surface.[38] Using mice expressing wild-type and mutant presenilin genes, it has been shown that overexpression of mutant, but not wild-type, presenilin 1 selectively increases brain Aβ42(43) peptide.[39] The carboxy-terminal 105 amino acid fragment of the amyloid precursor protein was shown to increase the gene expression of proinflammatory cytokines IL-1β and IL-6 in cultured rat cortical glial cells.[40] Highest levels of both IL-1(βand IL-6 transcripts were detected in the culture exposed to the carboxy terminal 105 aggregates for 4 days. This exacerbation of cytokine expression may be in part responsible for chronic inflammation linked to slowly progressive neurodegeneration characteristic to Alzheimer's disease.

The β-amyloid peptide, which forms extracellular cerebral deposits in Alzheimer's disease, is derived from a large membrane-spanning glycoprotein referred to as the β-amyloid precursor protein. The protein is normally cleaved within the β-amyloid region by a putative proteinase (α-secretase) to generate large soluble amino-terminal derivatives, and this event prevents the β-amyloid peptide formation. Studies using mice devoid of gelatinase A (generated by gene targeting) indicated that gelatinase A does not play an essential role in the generation and release of soluble derivatives of β-amyloid peptide under physiological conditions.[41] The Aβ peptide was shown to bind selectively to the membranes containing gangliosides (binding affinity ranging from 10^{-6} to 10^{-7} M). The addition of ganglioside-containing vesicles to the peptide solution accelerated the rate of fibril formation as compared with that of the peptide alone, thus indicating that the membrane-bound form of the peptide may act as a specific "template" (seed) that catalyses the fibrillogenesis process *in vivo*.[42] The heparin binding domains of the amyloid precursor protein have been narrowed down to the 316-346 and 416-447 amino acid regions.[43]

The data on the role of apolipoproteins in the stimulation or inhibition of formation of neurofibrillary tangles in the brain has been controversial.[44–47] For example, some studies have indicated that accumulation of Aβ in neurones is facilitated by the presence of ApoE, which may provide a suitable lipidic environment to stabilise the hydrophobic Aβ. Stabilisation of Aβ inside the neurones above a threshold level may trigger cell death.[44] The death of neurones would result in the release of Aβ, possibly in a complex with ApoE, which over time evolves into neuritic plaques. Other studies have indicated that apolipoprotein E inhibits the β amyloid fibril formation by forming a complex with β amyloid(1-42), thus eliminating free β amyloid(1-42) from the reaction mixture. *In vivo*, such complexes may be removed by receptor-dependent or receptor-independent processes.[45,46] Apolipoprotein J has also been implicated in complex formation with Aβ and resulting in the receptor-mediated clearance of Aβ(1-40)-apolipoprotein J complex.[47]

The neurotrophin receptor $p75^{NTR}$ has been implicated in an alternative/additional mechanism in the pathophysiology of Alzheimer's disease.[48–50] Using rat cortical neurones and NIH3T3 cell line engineered to stably express $p75^{NTR}$, β-amyloid peptide was shown to bind specifically to $p75^{NTR}$. Furthermore, 3T3 cells expressing $p75^{NTR}$, but not wild-type control cells lacking the receptor, were shown to undergo apoptosis in the presence of aggregated β-amyloid. Normal neural crest-derived melanocytes that express physiologic levels of $p75^{NTR}$ undergo apoptosis in the presence of aggregated β-amyloid, but not in the presence of control peptide synthesised in reverse.[48] A fragment of $p75^{NTR}$ from the carboxyl terminus of the receptor [human NTR(368-381), Ac-Ala-Thr-Leu-Asp-Ala-Leu-Leu-Ala-Ala-Leu-Arg-Arg-Ile-Gln-NH_2], and a variant form of this peptide, [Ac-Ala-Thr-Leu-Asp-Ala-*Lys*-Leu-Ala-Ala-Leu-Arg-Arg-Ile-Gln-NH_2], were studied *via* NMR techniques and *in vitro* assays for apoptotic activity.[49] The wild-type peptide induced apoptosis and adopted a helical conformation oriented parallel to the surface of lipid micelles, whereas the variant form adopted a non-helical conformation in the presence of lipid and showed no activity.

4.2 Antimicrobial Peptides – Work on peptide antibacterials has been reviewed.[51] A number of naturally occurring antibacterial and antifungal peptides [e.g. brevinins, esculentin (both isolated from skin secretions of the European frog *Rana esculenta*), tracheal antimicrobial peptide, misgurin (isolated from mudfish), protegrin (isolated from porcine leukocytes), cathelicidins and cecropin] have been shown to display potent antibacterial (against Gram negative and Gram positive bacteria) and antifungal properties. Most of these peptides are polycationic in nature and some contain Cys-Cys disulfide bridges. Isolation of new antimicrobial peptides, SAR studies on the older peptides and biological evaluations of the isolated peptides have been reported. In general, most of these peptides have been associated with haemolytic activity in addition to the antibacterial and antifungal properties. Attempts have been made to synthesise analogues of these peptides which do not display haemolytic activity in mamma-

lian cells. In addition, enzymes like peptide deformylase have been isolated from micro-organisms.[52]

4.2.1 Antibacterial Peptides. – A novel antimicrobial peptide (misgurin) was isolated from the leach, *Misgurnus anguillicaudatus*.[53] The 21-amino-acid peptide (Arg-Gln-Arg-Val-Glu-Glu-Leu-Ser-Lys-Phe-Ser-Lys-Lys-Gly-Ala-Ala-Ala-Arg-Arg-Arg-Lys) is a strongly basic peptide (5 arginine and 4 lysine residues). Misgurin showed a strong antimicrobial activity *in vitro* against a broad spectrum of micro-organisms without significant haemolytic activity and was about 6 times more potent than magainin 2. Scanning electron microscopy confirmed that the peptide caused damage to the cell membrane by a pore-forming mechanism similar to that of magainin 2. A cysteine-rich, 18-residue β-sheet peptide protegrin (Arg-Gly-Gly-Arg-Leu-Cys-Tyr-Cys-Arg-Arg-Arg-Phe-Cys-Val-Cys-Val-Gly-Arg-NH_2), isolated from porcine leukocytes, showed antimicrobial activity (MICs 0.12 to 2 μg ml^{-1}) against a broad range of micro-organisms.[54] Immunocompetent mice inoculated with *P. aeruginosa* or *S. aureus* exhibited 93 to 100% mortality in the vehicle control group compared with 0 to 27% mortality in animals that received a single intraperitoneal injection of protegrin-1 (0.5 mg kg^{-1}). Mice inoculated with *S. aureus* and dosed 0 to 60 min later with a single intravenous injection of protegrin-1 (5 mg kg^{-1}) had a mortality of 7 to 33%, compared to a mortality of 73 to 93% in the vehicle controls. In leukopenic mice inoculated with vancomycin-resistant *Enterococcus faecium*, mortality was 87% in the vehicle control group and 33% in animals injected with protegrin-1 (2.5 mg kg^{-1}). Two novel phenylalanine-rich antimicrobial peptides, styelin A [Gly-X-Phe-Gly-HOLys-Ala-Phe-X-Ser-Val-Ser-Asn-Phe-Ala-HOLys-hydroxyLys-His-HOLys-Thr-Ala] and styelin B [Gly-X-Phe-Gly-Pro-Ala-Phe-His-Ser-Val-Ser-Asn-Phe-Ala-HOLys-HOLys-His-HOLys-Thr-Ala], purified from the haemocytes of *Styela clava* were effective against a panel of Gram negative and Gram positive bacterial pathogens of humans (MICs < 1.5 μg ml^{-1}).[55]

Molecular modelling studies of protein sequences identified two positively charged, highly amphipathic amino acid segments in the cytoplasmic tail of the transmembrane envelope protein of human HIV-1. These peptide sequences (amino acids 828-856 and 768-788 of HIV-1 strain HXB2R Env) resemble magainins and cecropins in overall secondary structural properties and high hydrophobic moment.[56] Several peptides [e.g. Arg-Val-Ile-Glu-Val-Val-Gln-Gly-Ala-Cys-Arg-Ala-Ile-Arg-His-Ile-Pro-Arg-Arg-Ile-Arg-Gln-Gly-Leu-Glu-Arg-Ile-Leu, Asp-Leu-Trp-Glu-Thr-Leu-Arg-Arg-Gly-Gly-Arg-Trp-Ile-Leu-Ala-Ile-Pro-Arg-Arg-Ile-Arg-Gln-Gly-Leu-Glu-Leu-Thr-Leu, Arg-Ile-Ala-Gly-Tyr-Gly-Leu-Arg-Gly-Leu-Ala-Val-Ile-Ile-Arg-Ile-Cys-Ile-Arg-Gly-Leu-Asn-Leu-Ile-Phe-Glu-Ile-Ile-Arg, Tyr-His-Arg-Leu-Arg-Asp-Leu-Leu-Leu-Ile-Val-Thr-Arg-Ile-Val-Glu-Leu-Leu-Gly-Arg-Arg, Phe-Leu-Ile-Arg-Gln-Leu-Ile-Arg-Leu-Leu-Thr-Trp-Leu-Phe-Ser-Asn-Cys-Arg-Thr-Leu-Leu-Ser-Arg-Val-Tyr and Leu-Leu-Ser-Arg-Val-Tyr-Gln-Ile-Leu-Gln-Pro-Ile-Leu-Gln-Arg-Leu-Ser-Ala-Thr-Leu-Gln-Arg-Ile-Arg-Glu-Val-Leu-Arg] were potent and selective antibacterial agents and killed bacteria at 50-100-fold lower concentration than that required to lyse

erythrocytes. Novel antimicrobial peptides were also isolated from European bumblebee, *Bombus pascuorum* and rabbit leukocytes.[57,58]

Several cysteine-rich β-defensin peptides have been reported previously as broad-spectrum bactericidal agents. The human β-defensin-1 [Asp-His-Tyr-Asn-Cys-Val-Ser-Ser-Gly-Gly-Gln-Cys-Leu-Tyr-Ser-Ala-Cys-Pro-Ile-Phe-Thr-Lys-Ile-Gln-Gly-Thr-Cys-Tyr-Arg-Gly-Lys-Glu-Lys-Cys-Cys-Lys] gene maps adjacent to the human α-defensin cluster and is expressed in the respiratory, gastrointestinal and genitourinary tracts. A mouse β-defensin gene, expressed at high levels in the mouse kidney and at lower levels in brain, heart, lung, uterus, spleen, skeletal muscle, stomach, and small intestine, maps to mouse chromosome 8 at or near the location of the mouse α-defensin genes.[59] The mouse β-defensin sequence [Asp-Gln-Tyr-Lys-Cys-Leu-Gln-His-Gly-Gly-Phe-Cys-Leu-Arg-Ser-Ser-Cys-Pro-Ser-Asn-Thr-Lys-Leu-Gln-Gly-Thr-Cys-Lys-Pro-Asp-Lys-Pro-Asn-Cys-Cys-Lys] is similar to the human peptide.

In addition to the cationic peptides mentioned above, some anionic peptides have been reported recently as antibacterial agents. These peptides, based on the inactive precursor proteins (or zymogens) that contain homopolymeric regions of Asp [e.g. ovine trypsinogen activation peptide and frog (*Xenopus laevis*) PYL activation peptide] at the amino terminus were synthesised and tested against previously described surfactant-associated anionic peptide.[60] Peptides like Asp-Asp-Asp-Asp-Lys, Gly-Asp-Asp-Asp-Lys, Ala-Asp-Asp-Asp-Asp-Asp-Asp, Gly-Ala-Asp-Asp-Asp-Asp-Asp, Ala-Ala-Asp-Asp-Asp-Asp-Asp and Gly-Asp-Asp-Asp-Asp-Asp-Asp inhibited the growth of both Gram negative (MIC 0.08-3.0 mM) and Gram positive (MIC 0.94-2.67 mM) bacteria.

As mentioned above, most of the antimicrobial peptides have been associated with haemolytic activity in addition to the antibacterial and antifungal properties. Attempts have been made to synthesise analogues of these peptides which do not display haemolytic activity in mammalian cells. Conformational studies have provided evidence that the peptides exist in the form of amphipathic helices. Based on this model, various SAR studies have been carried out in an attempt to reduce cytotoxicity of these peptides.[61–64] For example, peptides like Gly-Ile-Leu-Ser-Lys-Leu-Gly-Lys-Ala-Leu-Lys-Lys-Ala-Ala-Lys-His-Ala-Ala-Lys-Ala, Gly-Arg-Phe-Arg-Arg-Leu-Gly-Arg-Lys-Phe-Lys-Lys-Leu-Phe-Lys-Lys-Tyr-Gly, Gly-Leu-Leu-Arg-Arg-Leu-Arg-Asp-Phe-Leu-Lys-Lys-Ile-Gly-Glu-Lys-Phe-Lys-Lys-Ile-Gly-Tyr and Gly-Leu-Leu-Arg-Arg-Leu-Arg-Lys-Lys-Ile-Gly-Glu-Ile-Phe-Lys-Lys-Tyr-Gly, predicted to assume a highly amphipathic α-helical conformation, displayed a potent antibacterial activity against selected Gram positive and Gram negative bacteria (MICs 1-8 μM), including some antibiotic resistant strains. However, the cytotoxic activity of the peptides, determined on several normal and transformed cell lines, was somewhat reduced (20-50% haemolysis of various cell types at 8-100 μM concentration). In the case of magainin analogues, peptides like [Leu^2, Arg^{11}, Ala^{20}]-, [Ile^6, Leu^{15}]- and [Ile^6, Ala^8, Leu^{15}, Ile^{17}]-magainin-NH_2 were shown to adopt an α-helical conformation when bound to phospholipid vesicles.[62] However, the antibacterial and haemolytic activity of the peptides increased with enhanced hydrophobicity. The two analogues, [Ile^6, Leu^{15}]- and [Ile^6, Ala^8, Leu^{15}, Ile^{17}]-magainin-NH_2, which were somewhat more

potent than magainin as antibacterial agents, also showed increased haemolytic activity. A strong correlation was found between the haemolytic effect and the bilayer-permeabilizing activity against phosphatidylcholine-rich vesicles. The antibacterial and haemolytic activities of several other magainin analogues like [Ile^6, Arg^{11}, Arg^{14}, Trp^{16}]-magainin-NH_2 were similar to that of magainin but one of the more hydrophobic analogues, [Ile^6, Val^9, Trp^{12}, Thr^{15}, Ile^{17}]-magainin-NH_2, was more potent than magainin in both assays.[63] Two novel cationic peptide analogues of the proline-rich peptide indolicidin (Ile-Leu-Pro-Trp-Lys-Trp-Pro-Trp-Trp-Pro-Trp-Arg-Arg), designed to increase the number of positively charged residues, CP-11 (Ile-Leu-Lys-Lys-Trp-Pro-Trp-Trp-Pro-Trp-Arg-Arg-Lys) and CP-11C (Ile-Leu-Lys-Lys-Trp-Pro-Trp-Trp-Pro-Trp-Arg-Arg-Lys-OMe), showed improved antibacterial and antifungal activity and a reduced ability to lyse erythrocytes.[64]

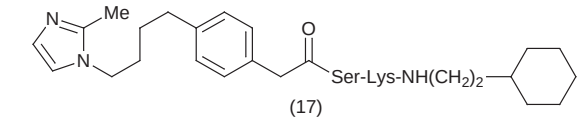
Attempts have been made to define the mechanism of the antimicrobial and cytotoxic effects of various peptides mentioned above.[65–67] For example, a comparison of the chemotherapeutic activity of three synthetic antibacterial peptides showed that Lys-Leu-Lys-Leu-Leu-Leu-Leu-Leu-Lys-Leu-Lys-NH_2 and its D-enantiomer showed significant chemotherapeutic activity in methicillin-resistant *Staphylococcus aureus*-infected mice, whereas Lys-Leu-Lys-Leu-Leu-Leu-Lys-Leu-Lys-NH_2, which showed the highest antibacterial activity among them *in vitro*, was found to be inactive. The active peptides were shown to activate human neutrophils to produce superoxide, thus indicating that prevention of the infection by these peptides was not simply due to their direct bactericidal activity but also due to augmentation of the systemic defence mechanism mediated by neutrophils.[65] Studies using some polymeric synthetic peptides like $(Lys\text{-}Leu\text{-}Ala\text{-}Lys\text{-}Leu\text{-}Ala\text{-}Lys)_3$, $(Lys\text{-}Leu\text{-}Ala\text{-}Lys\text{-}Lys\text{-}Leu\text{-}Ala)_n$ (n = 3, 4), $(Lys\text{-}Leu\text{-}Gly\text{-}Lys\text{-}Lys\text{-}Leu\text{-}Gly)_3$, $(Lys\text{-}Phe\text{-}Ala\text{-}Lys\text{-}Phe\text{-}Ala\text{-}Lys)_3$, $(Lys\text{-}Phe\text{-}Ala\text{-}Lys\text{-}Lys\text{-}Phe\text{-}Ala)_n$ (n = 3, 4) and $(Lys\text{-}Leu\text{-}Ala\text{-}Lys\text{-}Lys\text{-}Leu\text{-}Ala)_3$ indicated that aggregation of the peptides may be responsible for some of the cytotoxicity of these peptides.[67]

4.2.2 Antifungal Peptides. – In addition to the SAR studies on the naturally occurring antifungal peptides like histatins, cecropin A and lipopeptides like pneumocandins,[68–71] attempts have been made to design antifungal compounds by inhibiting one of the fungal enzymes, myristoyl-CoA-protein N-myristoyltransferase.[72–76] In comparison to histatin-5 (cationic, antifungal peptide present in human saliva, ED_{50} 8 μM), two of its analogues obtained by replacing Phe^{14}-His^{15} or His^{18}-His^{19} sequences by Ala-Ala were much less potent against *C. albicans* (ED_{50} 67-149 μM).[68–71] Assessment of the candidacidal activity of histatin-5 with the well-characterised azole-resistant strains suggested that the mode of action of histatins against *Candida* is distinct from that of azole-based antifungal agents because histatin-5 kills both azole-sensitive and azole-resistant strains equally well. Cecropin A (a pore-forming 37-amino acid peptide) was shown to achieve complete lethality for germinating *Aspergillus* spp. at 99 μg ml^{-1} and for germinating and non-germinating *Fusarium* spp. at 6 μg ml^{-1}.[69] In an attempt to reduce haemolytic activity associated with cecropin A, hybrid

peptides of cecropin A and melittin were synthesised. One of the analogues, Lys-Trp-Lys-Leu-Phe-Lys-Lys-Ile-Gly-Ile-Gly-Ala-Leu-Leu-Lys-Ala-Ala-Lys-Lys-Gly-NH_2, had similar antifungal activity but the haemolytic activity was significantly reduced (0.8% at 200 μg ml^{-1}) in comparison to the parent hybrid peptide (14.1% at 200 μg ml^{-1}). Two other analogues, Lys-Trp-Lys-Leu-Phe-Lys-Lys-Ile-Gly-Ile-Gly-Lys-Val-Leu-Lys-Val-Leu-Lys-Lys-Gly-NH_2 and Lys-Trp-Lys-Leu-Phe-Lys-Lys-Ile-Gly-Ile-Gly-Ala-Leu-Leu-Lys-Ala-Ala-Lys-Val-Gly-NH_2, were less potent in the antifungal and haemolytic assays.[70]

Myristoyl-CoA-protein N-myristoyltransferase (a cytosolic monomeric enzyme) (NMT) covalently attaches the 14-carbon saturated fatty acid myristate, *via* an amide bond, to the N-terminal glycine residues of a variety of cellular proteins. Genetic studies have shown the enzyme to be essential for the viability of fungal pathogens which cause systemic infection in immunosuppressed humans and hence is a target for development of fungicidal drugs. The discovery of NMT inhibitors is based on the identification of the octapeptide substrate Gly-Leu-Tyr-Ala-Ser-Lys-Leu-Ser-NH_2 derived from the N-terminal fragment of ADP ribosylation factor 2 (a protein myristoylated by the enzyme).[72] The substitution of alanine for glycine provided a competitive inhibitor of the fungal and human enzymes. Incorporation of Arg, Nle, His or Orn in place of the lysine residue gave significantly weaker inhibitors. The N-terminal tetrapeptide from a substrate-based inhibitor, Ala-Leu-Tyr-Ala-Ser-Lys-Leu-NH_2, was replaced with an ω-aminoalkanoyl moiety having an optimal 11-carbon chain for inhibition [11-aminoundecanoyl-Ser-Lys-Leu-NH_2 (IC_{50} 1.2 μM)]. Compounds with $NH_2(CH_2)_8CO$-, $NH_2(CH_2)_9CO$- and $NH_2(CH_2)_{11}CO$- groups at the N-terminal were comparable in potency but the $NH_2(CH_2)_6CO$-analogue was inactive up to a concentration of 100 μM. Replacement of the C-terminal Leu residue in $NH_2(CH_2)_{10}CO$-Ser-Lys-Leu-NH_2 by Gly, Ala, Val, Ile and Phe residues led to less potent compounds. Only the cyclohexylalanine (IC_{50} 0.36 μM) and cyclohexylglycine containing compounds were 2-3-fold more potent than the parent peptide. Removal of the carboxamide moiety in $NH_2(CH_2)_{10}CO$-Ser-Lys-Cha-NH_2 led to a metabolically stable dipeptide inhibitor $NH_2(CH_2)_{10}CO$-Ser-Lys-NH-CH_2-cycloC_6H_{11} (IC_{50} 0.11). Introduction of the larger 2-cyclooctylethyl group decreased the inhibitory activity (IC_{50} 0.3 μM). Partial rigidification of the flexible aminoundecanoyl chain produced the dipeptide derivatives like **17** (*C. albicans* NMT IC_{50} 0.056 μM, human NMT IC_{50} 14.1 μM), **18** (*C. albicans* NMT IC_{50} 0.75 μM, human NMT IC_{50} 62 μM), **19** (*C. albicans* NMT IC_{50} 0.019 μM, human NMT IC_{50} 8.2 μM) and **20** (*C. albicans* NMT IC_{50} 0.38 μM, human NMT IC_{50} 840 μM; about 2200-fold less potent against the human enzyme).[72–75] Compound **20** displayed fungistatic activity against *C. albicans*. Replacement of the lysine residue gave compounds like **21** which retained significant antifungal activity, but many other analogues containing heterocyclic lysine mimetics exhibited much weaker activity against the fungal enzyme.[76]

4.3 ACTH/CRF Peptides – Some of the work has been reviewed.[77] Three regions of corticotrophin receptors ($CRFR_1$ and $CRFR_2$) were shown to be important for optimal binding of rat or human corticotrophin releasing factor



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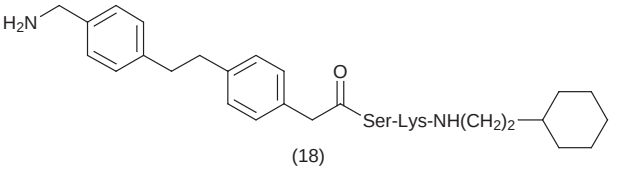

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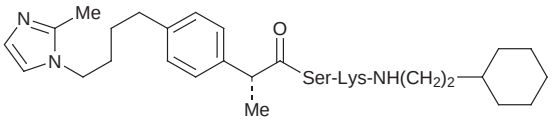

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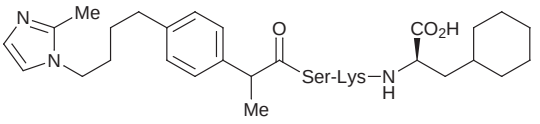

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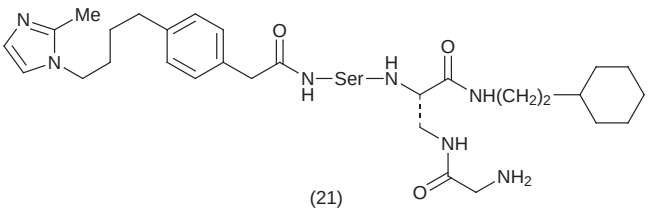
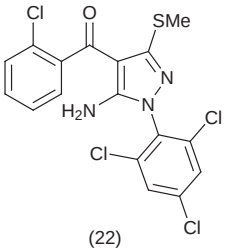


(21)

and/or receptor activation by using chimeric receptor constructs of the two human receptor subtypes, followed by generating point mutations of the receptor.[78] The first region was mapped to the junction of the third extracellular domain and the fifth transmembrane domain; substitution of three amino acids of $CRFR_1$ in this region (Val^{266}, Tyr^{267}, and Thr^{268}) by the corresponding $CRFR_2$ amino acids (Asp^{266}, Leu^{267}, and Val^{268}) increased the EC_{50} value for corticotrophin binding by approximately 10-fold. The other two regions were localised to the second extracellular domain of the $CRFR_1$ involving amino acids 175-178 and His^{189} residue. Substitutions in these two regions each increased the EC_{50} value for r/hCRF by approximately 7- to 8-fold only in the presence of the amino acid 266-268 mutation involving the first region, suggesting that their roles in peptide ligand binding might be secondary. A fourth region in the third extracellular domain, Asp^{254}, was identified to be important for sauvagine but not corticotrophin or urocortin binding; thus, the three peptide ligands not only

interact with a different set of regions on $CRFR_1$ and $CRFR_2$ but also differentially interact with some of the same regions. Two amino acid residues, His^{199} in the third transmembrane domain and Met^{276} in the fifth transmembrane domain, were shown to be important for binding the non-peptide high-affinity $CRFR_1$ antagonist NBI 27914. Mutations of His^{199} and Met^{276} to the corresponding amino acids in $CRFR_2$ each decreased the binding affinity of NBI 27914 for $CRFR_1$ by 40- and 200-fold, respectively, thus indicating that the transmembrane regions are critically important in forming the binding pocket for the nonpeptide antagonist.[79]

Non-peptide antagonists of corticotrophin-releasing factor have been reported.[80] The initial weak lead (**22**) was identified by a high throughput receptor binding screen. Structural modifications led to orally active pyrrolo[3,4-d]pyrimidine series of compounds. Compound (**23**) (Ki 2.7 nM at CRF_1 receptor) demonstrated activity in anxiotytic and antidepressant models after intraperitoneal and oral administration. The influence of central injection of a peptide corticotrophin-releasing factor receptor antagonist, astressin, {c(30-33)[D-Phe^{12}, $Nle^{21,38}$, Glu^{30}, Lys^{33}]r/hCRF12-41)}, on exogenous and endogenous corticotrophin-releasing factor-induced gastric ileus and stimulation of bowel discharges was investigated in conscious rats.[81] The peptide reduced corticotrophin-releasing factor- and stress-related alterations of gastrointestinal motor functions, without an intrinsic effect in these *in vivo* systems when injected into the CSF at doses of 1-10 μg.



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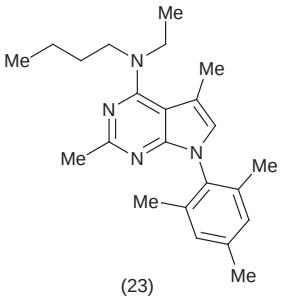

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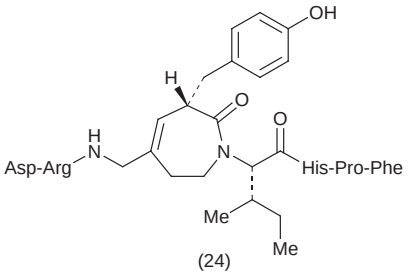
4.4 Angiotensin II Analogues and Non-peptide Angiotensin II Receptor Ligands

Various aspects of angiotensin II research (including angiotensinogen) have been reviewed.[82–85] Hydroxyethylene analogues of angiotensin (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) and its N- and C-terminal fragments [Arg-Val-Tyr-Ile-His-Pro-Phe and Val-Tyr-Ile-His-Pro-Phe] were synthesised and evaluated in a binding assay. The three analogues Aspψ[$CHOHCH_2NH$]Arg-Val-Tyr-Ile-His-Pro-Phe, Argψ[$CHOHCH_2NH$]Val-Tyr-Ile-His-Pro-Phe and Valψ-[$CHOHCH_2NH$]Tyr-Ile-His-Pro-Phe were much less potent than the parent peptides.[86] However, the pseudopeptides were more stable than the parent peptides in kidney homogenates. Lipidated angiotensin II agonists and antagonists were evaluated for their biological activities.[87] N-Acetyl-Ser^1 angiotensin

palmitoylated on the serine hydroxyl function was inactive. A Ser palmitoylated analogue of an angiotensin antagonist [N-Ac-Ser^1, β-D-Nal^8]angiotensin was also inactive. In contrast to the Ser palmitoylated analogues, [Sar^1, Tyr(palmitoyl)^4]angiotensin obtained by palmitoylating [Sar^1]angiotensin on the phenolic hydroxyl of Tyr^4 was comparable in potency to [Sar^1]angiotensin, and exhibited prolonged activity. Similarly, the palmitoylated analogue of [Sar^1, D-Nal^8]angiotensin, [Sar^1, Tyr(palmitoyl)^4, D-Nal^8]angiotensin, retained the antagonist activity. All the above palmitoyl derivatives were easily converted into the nonlipidated active form by lipolysis or saponification. [Sar^1, Tyr^4(O-octadecyl)]angiotensin, an analogue to Tyr-palmitoylated [Sar^1]angiotensin with an octadecyl phenyl ether in position 4, was inactive.[87]

Conformational analysis studies using theoretical calculations and H^1-NMR spectroscopy on tripeptide model compounds indicated that the cyclic moieties of some of the angiotensin cyclic peptide analogues reported earlier {e.g. c[Hcy^{3,5}]-angiotensin II (pD_2 8.4 in isolated rabbit aortic rings and IC_{50} 2.1 nM for binding in a rat uterine membrane assay), c[Cys^{3,5}]-angiotensin II, and c[Pen^{3,5}]-angiotensin II (IC_{50} values 43 and 2.6 nM, respectively)} may assume an inverse γ-turn conformation.[88] On the basis of these results, the amino acid residues 3-5 in angiotensin II were substituted with two different γ-turn mimetics giving four diastereomeric angiotensin II analogues. The analogues like (**24**) and (**25**) (some retained binding affinity) were either inactive or much less potent than angiotensin II in inducing contractile responses at the rabbit aortic strips.



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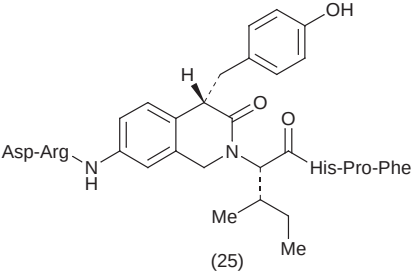

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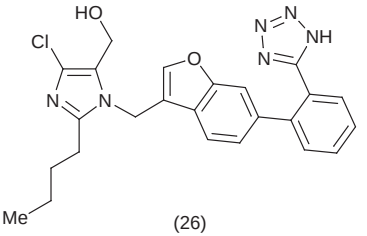
As in the last few years, a number of publications have appeared on the chemistry and biology of non-peptide antagonists of angiotensin.[89–101] Structures of some of the compounds (**26-32**) are shown below. Benzofuran derivatives like (**26**) were weak antagonists of angiotensin (pIC_{50} values 3.36-6.15).[90] In contrast, several other compounds like (**27-29**) were potent antagonists of angiotensin at the AT_1 receptors and showed activity in several *in vivo* antihypertensive tests.[89,91,96] For example compound (**27**) (UR-7280) decreased blood pressure at a dose of 0.3 mg kg^{-1} when administered orally and compound (**29**) (EMD90423) after oral administration (3 mg kg^{-1}) to cynomolgus monkeys demonstrated good efficacy and a long duration of action as an antihypertensive agent.

Chemical modifications and receptor mutations were shown to have significant effects on the agonist and antagonist properties of some non-peptide compounds



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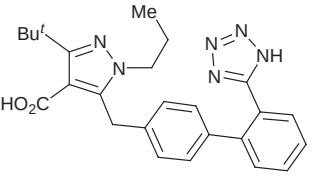
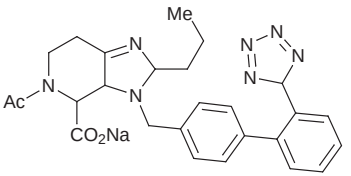


(27) UR 7280



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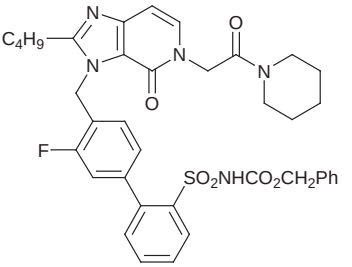
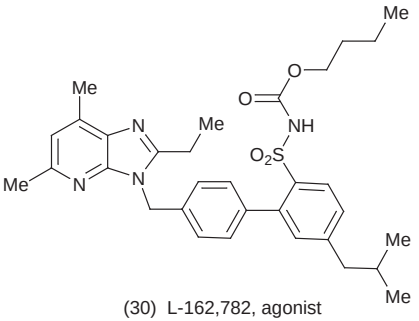


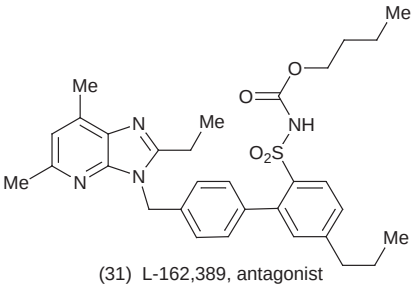
(29) EMD90423

like (**30**-**32**). Compound (**32**, L-162,313) has been reported previously as angiotensin agonist. The nonpeptide ligands that differ chemically by only a single methyl group but have agonistic (**30**, L-162,782) and antagonistic (**31**, L-162,389) properties *in vivo* were characterised on the cloned angiotensin AT_1 receptor. Both compounds bound with high affinity (K_i = 8 and 28 nM, respectively) to the AT_1 receptor expressed transiently in COS-7 cells. A series of point mutations in the transmembrane segments of the AT_1 receptor had only a minor effect on the binding affinity of the nonpeptide compounds, with the exception of $Ala^{104}Val$ at the top of transmembrane segment III, which selectively impaired the binding of (**29**) and (**30**). Substitutions in the middle of transmembrane segments III, VI, or VII, which did not affect the binding affinity of the compounds, impaired or eliminated the agonistic efficacy of the non-peptides but with only minor or no effect on the angiotensin potency or efficacy.[92]

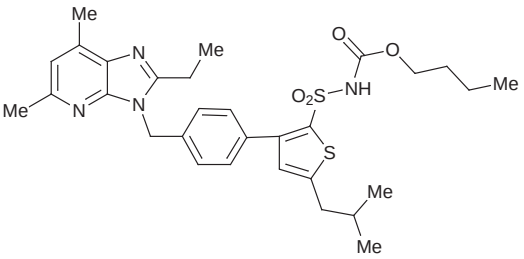
4.5 Bombesin/Neuromedin Analogues – The role of bombesin/gastrin releasing peptide in various form of cancer, especially small cell lung cancer, has been discussed in the past. Some of the more recent work has indicated that although many human melanoma cell lines express GRP-preferring bombesin receptor mRNA, only one of these cell lines (A375-6) expressed functional receptors in sufficient numbers to allow detection of bombesin binding. Growth of A375-6 cells could be neither stimulated by exogenous bombesin nor inhibited by blocking the receptors, therefore excluding a role for bombesin/GRP in autocrine stimulation.[102] However, in H-128 human SCLC line xenografted nude mice, administration of a bombesin receptor antagonist [D-Tpi^6, $Leu^{13}\psi(CH_2NH)$-Leu^{14}]bombesin(6-14) (RC-3095) (20 μg day^{-1} $animal^{-1}$ for 4 weeks) caused



(30) L-162,782, agonist



(31) L-162,389, antagonist



(32) L-162,313, agonist

70% reduction in tumour weight and volume. The numbers of receptors both for bombesin and epidermal growth factor were also reduced.[103] Similarly, in the oestrogen-dependent mouse mammary tumours, the concentration of epidermal growth factor receptors was reduced by RC-3095 (about 60%) 6 hours after injection and returned to original levels after 24 hours. Levels of mRNA also fell in parallel with the receptor numbers and were nearly normal after 24 hours. In the hormone-independent mouse mammary cancers, the number of epidermal growth factor receptors decreased progressively (undetectable after 6 hours) and returned to normal levels after 24 hours, but mRNA levels remained lower for 48 hours.[104]

Recent discoveries of various bombesin/GRP receptor subtypes, (gastrin-releasing peptide receptor, neuromedin B receptor, bombesin receptor subtype-3 and bombesin receptor subtype 4; all G-protein-coupled receptors), and the involvement of human bombesin receptor subtype 3 (BRS-3) in obesity has created a new interest in the field.[105] Function of this receptor has been studied using BRS-3-deficient mice. BRS-3-deficient mice were shown to gain more weight and the rate of weight loss was less when the food supply was restricted.

Unlike the remaining three receptors [GRP-R, NMB-R, and bb4] which bind bombesin with dissociation constants in the nanomolar range, the BRS-3 receptor has low affinity for bombesin ($K_d > 1$ μM) and its natural ligand has not yet been identified.[106,107] Amino acid substitutions in mouse GRP and human BRS-3 receptors have been carried out to identify the residues involved in interaction with various ligands. Four substitutions ($Arg^{288}His$, $Gln^{121}Arg$, $Pro^{199}Ser$ and

Ala[308]Ser) in mouse GRP-R resulted in a significant decrease in bombesin affinity.[107] The analogous mutations in BRS-3 (Arg[127]Gln, His[294]Arg, Ser[205]Pro and Ser[315]Ala) resulted in a receptor with a 100-fold increase in bombesin and GRP affinities relative to wild-type BRS-3.

Attempts are being made to identify selective ligands of BRS-3 receptor subtype by various techniques.[108,109] A number of bombesin-like peptides (agonists and antagonists) were tested for calcium mobilisation in BR-2 cells (Balb/3T3 fibroblasts transfected with BRS-3 cDNA). Neuromedin B, litorin and ranatensin containing a C-terminal Gly-His-Phe-Met-NH_2 tetrapeptide promoted calcium mobilisation at 1 μM. In contrast, GRP, bombesin and alytesin which have Gly-His-Leu-Met-NH_2 tetrapeptide at the C-terminus were active at 10 μM. Testing of a series of NMB analogues truncated at the amino terminus and carboxyl terminus indicated that the minimal size of NMB required for retention of full activity was Ac-NMB(3-10) (Ac-Leu-Trp-Ala-Thr-Gly-His-Phe-Met-NH_2). Systematically replacing each residue with alanine, or changing its chirality, demonstrated that the carboxyl-terminal residues His[8], Phe[9], and Met[10] of NMB are important for optimal activity.[108] Two of the most potent agonist peptides at the BRS-3 receptor were [D-Phe[6]]-BN(6-13) propyl amide (EC_{50} 84 nM) and [D-Phe[6]-Phe[13]]-BN(6-13) propyl amide (EC_{50} 5 nM). Conversion of the most potent BRS-3 agonist peptide, [D-Phe[6]-Phe[13]]-BN(6-13) propyl amide, into a more NMB-like peptide resulted in a much less potent analogue, [D-Phe[6]-Leu[7], Thr[10], Phe[13]]-BN(6-13) propyl amide. A number of other analogues reported to be agonists or antagonists of bombesin, 4-pyridylcarbonyl-[His[7], D-Ala[11], Lys(CO-CH_2-CH_2-Ph)[12]]-BN(7-13)-OMe, [D-Phe[6]]-BN(6-13)-OMe, [D-Phe(F_5)[6], D-Ala[11]]-BN(6-13)-OMe, [Leu[13]ψ(CH_2NH)Leu[14]]-BN and [desaminoPhe[6], His[7], D-Ala[11], D-Pro[13]ψ(CH_2NH)Phe[14]]-BN(6-14), did not show agonist or antagonist activity up to a concentration of 10 μM.

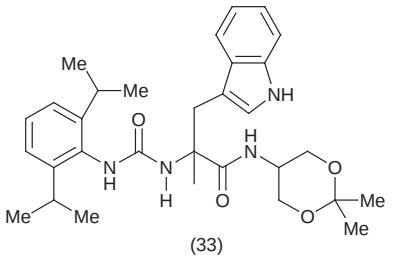
In another study, two cell lines stably expressing transfected hBRS-3 receptors (NCI-H1299 and Balb 3T3) were used to study the binding of various analogues of bombesin.[109] Radiolabelled NMB-R or GRP-receptor ligands like [[125]I-D-Tyr[0]]NMB, [[125]I-Tyr[4]]BN, [125]I-GRP, [[125]I-D-Tyr[6]]BN(6-13)-OMe] did not demonstrate increased saturable binding to these clones compared with the results in the native non-transfected cells. One of the bombesin analogues, [D-Tyr[6], β-Ala[11], Phe[13], Nle[14]]BN(6-14) and the iodinated derivative [[125]I-D-Tyr[6], β-Ala[11], Phe[13], Nle[14]]BN(6-14) demonstrated no saturable binding to the non-transfected Balb 3T3 cell lines, a low but significant level of binding to non-transfected NCI-H1299 cells that have low levels of hBRS-3 receptors and a significant binding to each of ten different H1299 clones and four Balb 3T3 clones that had been stably transfected with hBRS-3 receptors. [D-Phe[6], β-Ala[11], Phe[13], Nle[14]]BN(6-14) inhibited the binding of [[125]I-D-Tyr[6], β-Ala[11], Phe[13], Nle[14]]BN(6-14) to hBRS transfected NSCLC-1299 and Balb 3T3 cells (K_i 4.2 and 8.9 nM, respectively). [D-Phe[6], β-Ala[11], Phe[13], Nle[14]]BN(6-14) stimulated >5-fold increase in [[3]H]inositol phosphate at concentrations below 100 nM (data not shown). A number of other peptides [bombesin, GRP, NMB, ranatensin, litorin, alytesin, [Leu[8]]phyllolitorin, phyllolitorin, neuromedin C, NMB and [Phe[13]]bombesin] showed no affinity (>2 μM) for the two transfected cell lines.[109]

Peptides inactive in the hBRS-3 receptor transfected cell lines include: [D-Phe^{12}]BN, [Tyr^4, D-Phe^{12}]BN, [D-Phe^6, D-Phe^{12}, Leu^{14}]BN, [Leu$^{13}\psi$(CH_2NH)-Leu^{14}]BN, [D-Phe^6, Leu$^{13}\psi$(CH_2NH)Phe(p-Cl)^{14}]BN(6-14), [D-Phe^6, Leu^{13}(ψCH_2NH)D-Phe^{14}]BN(6-14), [3-Ph-Pr^6, His^7, D-Ala^{11}, D-Pro$^{13}\psi$(CH_2NH)Pro^{14}]BN(6-14)-NH_2, [3-Ph-Pr^6, Pro^7, β-Ala^{11}, D-Pro$^{13}\psi$(CH_2NH)Phe^{14}]BN(6-14), [D-Phe^6]BN(1-13)-NH_2, [D-Phe^6]BN(6-13)-NH_2, [D-Phe^6]BN(6-13)-OMe, [D-Phe^6]BN(6-13)-NHPr, [D-Phe^6]BN(6-13)-NH-hexyl, [D-Tyr^6, D-Ala^{11}]BN(6-13)butylamide, [D-Phe^6]BN(6-13)-$NHNMe_2$, [D-Phe^6]BN(6-13)-$NHNH_2$, [D-Phe^1, β-Leu^8, des-Met^9]litorin, [D-Arg^1, D-Trp^{7,9}, Leu^{11}]SP, [D-Pro^4, D-Trp^{7,9,10}]SP(4-11), [D-Phe^6]BN(6-14), [D-Phe^6, D-Ala^{11}, Leu^{14}]BN(6-14), [Phe(p-Cl)^1, D-Ala^6, Leu^8, Nle^9]litorin and [D-Phe^1, Nle^9]litorin.

The C-terminal Leu^{13}-Leu^{14} modified analogues showed both agonist and antagonist properties.[110] All the agonist analogues were less potent than bombesin (pancreatic acini binding K_i 1.8 nM, amylase release EC_{50} 0.07 nM) both in the receptor binding and amylase release assays. The most potent agonist analogues were D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-βhPhe-NH_2 (pancreatic acini K_i 9.7 nM, amylase release EC_{50} 2.1 nM) and D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-βhLeu-NH_2 (pancreatic acini K_i 5.8 nM, amylase release EC_{50} 0.3 nM). The rest of the agonist analogues, D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-Aic-NH_2, D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-Tic-NH_2, D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-D-Tic-NH_2, D-Phe-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH_2CH_2]D-Leu-NH_2, D-Phe-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH_2CH_2]Leu-NH_2 and D-Phe-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH_2]Leu-NH_2, were much less potent. Amongst the antagonist peptides, D-Phe-Gln-Trp-Ala-Val-Gly-His-Leuψ[-CH_2]Leu-NH_2 (pancreatic acini K_i 4.3 nM, amylase release Ki 7.7 nM) was the most potent antagonist analogue in inhibiting bombesin-stimulated amylase secretion. The rest of the antagonist analogues, D-Phe-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH_2NH]Leu-NH_2, D-Phe-Gln-Trp-Ala-Val-Gly-His-βhLeu-D-Nle-NH_2, D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-βh-D-Phe-NH_2 and D-Phe-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH_2O]Nle-NH_2 were less potent.

Non-peptide antagonists of neuromedin B were synthesised using a rational approach. After defining the role of each amino acid side chain by alanine scanning in the minimum active fragment which indicated that Trp^8, Val^{10} and Leu^{13} were more important for the binding affinity to the bombesin receptors, a search within the company compound collection for various templates containing Trp, Val/Leu types of side chains led to a moderately active lead (**33**) (K_i 95 nM at neuromedin B receptors and >10,000 nM at GRP receptors). Replacing the three backbone NH groups by N-methyl groups to introduce conformational constraint led to less potent compounds (K_i values 120 to 490 nM). However, changes at the C-terminal end led to more potent (S) α-methyl-Trp derivative (**34**, PD 165929).

Analogues of bombesin(6-14) and bombesin(7-14) have been used as carriers for cytotoxic agents like doxorubicin and 2-pyrrolinodoxorubicin to create hybrid cytotoxic analogues. All the peptides contained a ψ[CH_2NH] peptide bond replacement between positions 13 and 14. The K_i value for one of the more potent compounds (**35**) was 1.6 nM on Swiss 3T3 cells. The cytotoxic bombesin



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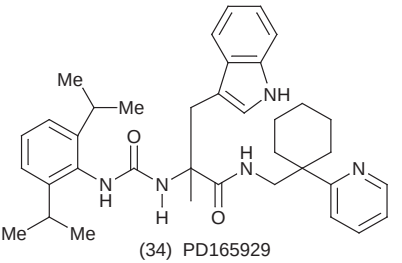

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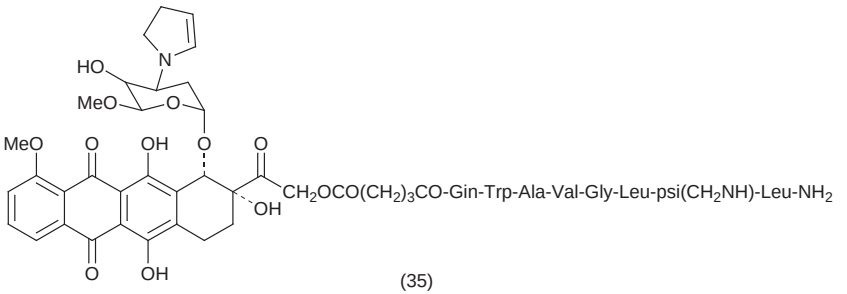

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analogues and their corresponding cytotoxic radicals exerted similar inhibitory effects on the *in vitro* growth of human pancreatic cancer, human lung cancer, human prostate cancer and human gastric cancer cell lines that have receptors for bombesin/GRP. *In vivo* experiments on nitrosamine-induced pancreatic cancers in golden hamsters indicated that both cytotoxic bombesin analogue and cytotoxic radical have significant antitumour activity.[112]

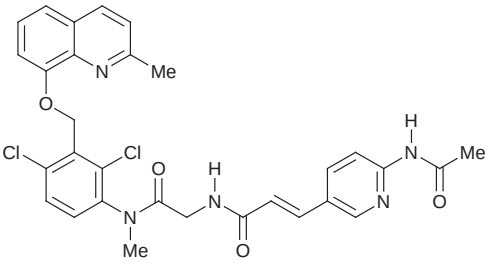
4.6 Bradykinin Analogues – Conformational properties of a bradykinin analogue, [Thr^6]bradykinin, were reported.[113,114] Bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), several metabolites like [des-Arg^9]bradykinin, [HyPro^3]bradykinin and partially processed N- and C-terminally extended intermediates [Leu-Leu-Pro-Ile-Val-Gly-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Ile-Ala, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Ile-Ala-Pro-Ala-Ser-Thr-Leu and Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Ile-Ala-Pro-Ala-Ser-Ile-Leu] were isolated from an extract of *Rana temporaria* skin.[115] Sequences of new bradykinin potentiating peptides have been disclosed.[116] A cDNA clone was isolated from a *Bothrops jararaca* venom gland cDNA library that encodes a 256 amino acid precursor for bradykinin potentiating peptides (ACE inhibitors) and a C-type natriuretic peptide. The seven bradykinin potentiating peptides [Gln-Ser-Trp-Pro-Gly-Pro-Asn-Ile-Pro-Pro, Gln-Gly-Gly-Trp-Pro-Arg-Pro-Gly-Pro-Glu-Ile-Pro-Pro, Gln-Asn-Trp-Pro-His-Pro-Gln-Ile-Pro-Pro, Gln-Gly-Arg-Ala-Pro-Gly-Pro-Pro-Ile-Pro-Pro, Gln-Gly-Arg-Ala-Pro-His-Pro-Pro-Ile-Pro-Pro and two repeating sequences of Gln-Lys-Trp-Ala-Pro] were present after the hydrophobic signal peptide sequence, followed by a putative

intervening sequence and a C-type natriuretic peptide [Gly-Ala-Ala-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-Gly-Thr-Met-Ser-Gly-Leu-Gly-Cys] at the C-terminus.[116]

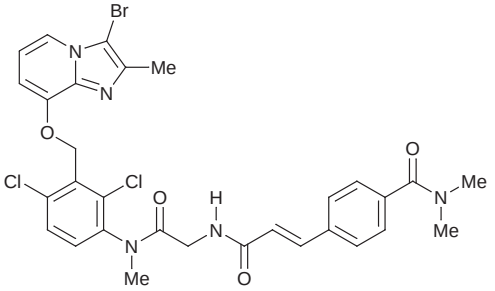
The tetrapeptide in the central portion of bradykinin and [des-Arg^{10}]kallidin was replaced by alkyl spacers of various lengths [-NH-(CH_2)n-CO-, n = 5-11]. When tested as agonists at the kinin receptors, the pseudopeptides showed significant activity only at the B_1 receptors.[117] In the rat isolated ileum longitudinal smooth muscle preparation (B_1 receptor), Arg-NH$(CH_2)_{10}$CO-Ser-Pro-Phe-Arg and Arg-NH$(CH_2)_{11}$CO-Ser-Pro-Phe-Arg showed weak agonist activity, but in the guinea pig ileum longitudinal smooth muscle preparation (B_2 receptor), Arg-NH$(CH_2)_5$CO-Ser-Pro-Phe-Arg, Arg-NH$(CH_2)_7$CO-Ser-Pro-Phe-Arg, Arg-NH$(CH_2)_{10}$CO-Ser-Pro-Phe-Arg and Arg-NH$(CH_2)_{11}$CO-Ser-Pro-Phe-Arg did not show any agonist or antagonist activity up to a concentration of 10 μM. The same modifications in the selective B_1 receptor agonist, [des-Arg^{10}]kallidin, also led to less potent compounds. Lys-Arg-NH$(CH_2)_5$CO-Ser-Pro-Phe was inactive and three other compounds [Lys-Arg-NH$(CH_2)_7$CO-Ser-Pro-Phe, Lys-Arg-NH$(CH_2)_{10}$CO-Ser-Pro-Phe and Lys-Arg-NH$(CH_2)_{11}$CO-Ser-Pro-Phe] were >10-fold less potent than the parent peptide in the B_1 receptor preparation.[117]

Non-peptide antagonist (**36**-**39**) and agonist (**40**) analogues of bradykinin have been disclosed.[118–125] A quinoline derivative (**36**, FR173657) displaced [^{3}H]bradykinin binding to B_2 receptors present in guinea-pig ileum membranes with an IC_{50} of 0.56 nM and in rat uterus with an IC_{50} of 1.5 nM. In human lung fibroblast (IMR-90) cells, FR173657 displaced [^{3}H]bradykinin binding to B_2 receptors (IC_{50} of 2.9 nM, K_i of 0.36 nM), but did not reduce [^{3}H]-des-Arg^{10}-kallidin binding to B_1 receptors. In guinea-pig isolated preparations, FR173657 antagonised bradykinin-induced contractions (pA_2 9.2). Compound (**37**) was about 5-10-fold less potent than (**37**).[122] *In vivo*, oral administration of (**36**) in anaesthetised guinea-pigs inhibited bradykinin-induced bronchoconstriction in a dose dependent manner (ED_{50} 0.075 mg kg^{-1}), but did not inhibit histamine-mediated bronchoconstriction even at 1 mg kg^{-1}. Compound (**36**) also inhibited carrageenin-induced paw oedema (ED_{50} 6.8 mg kg^{-1}) 2 h after the carrageenin injection in rats.[118] Plasma exudation induced by intrapleural injection of bradykinin into male rats were significantly inhibited by oral administration of (**36**) (3-30 mg kg^{-1}, 1h before bradykinin) in a dose-dependent manner. The inhibitory effect of 30 mg kg^{-1} dose persisted for more than 4 hours. In another test (carrageenin-induced-exudation), oral administration of (**36**) (30 mg kg^{-1}) also blunted (50-77%) the plasma exudation response 1, 3, 5, and 7 h after carrageenin, causing a significant parallel reduction (42-57%) in the volume of exudates.[119] Compound (**36**) also showed activity in a number of other test systems.[120–122] A proposed overlap between a nonpeptide bradykinin antagonist (**38**) (WIN64338) and a cyclic hexapeptide bradykinin antagonist (**39**) indicated that the naphthalene ring of (**38**) lies over the tetrahydroisoquinoline moiety of (**39**). This then places one of the cyclohexyl rings near the octahydroisoquinoline and the positively charged guanidine over the Arg^5 of the cyclic peptide.[123]

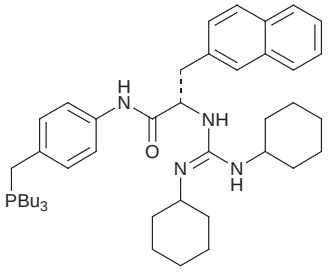
The nonpeptide bradykinin receptor agonist (**40**) (FR190997) stimulated phosphatidylinositol hydrolysis in Chinese hamster ovary cells permanently



(36) FR173657



(37) FR167344



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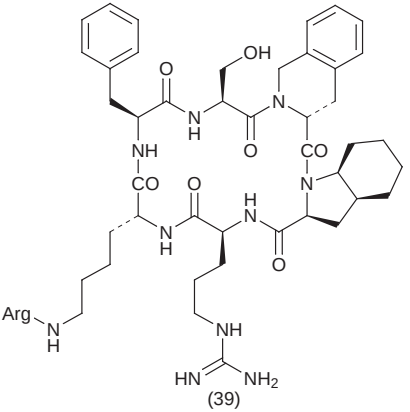
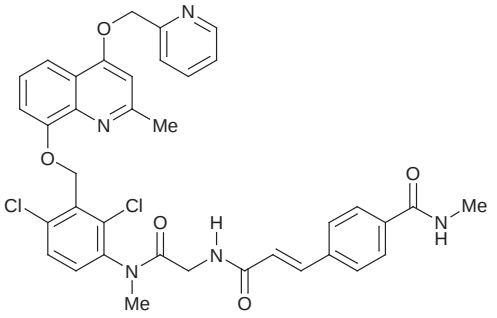


(39)

expressing the human bradykinin B_2 receptor at concentrations between 1 nM and 1μM. The response of phosphatidylinositol hydrolysis was antagonised by the B_2 receptor selective antagonist Hoe 140, [D-Arg, HyPro^3, β-thienyl-Ala^4, D-Tic^7, Oic^8]-bradykinin. At 100 nM concentration, Hoe140 inhibited the bradykinin-stimulated and (**40**)-stimulated (both at 100 nM) phosphatidylinositol hydrolysis by 60 and 63%, respectively. In competitive binding experiments (CHO cell membranes expressing the human bradykinin receptor subtypes),

FR190997 showed a high affinity binding to the B_2 receptor (IC_{50} 5.3 nM) and no binding affinity for the B_1 receptor. Intravenous administration of bradykinin or (**40**) (both at 10 μg kg^{-1}) caused a fall in blood pressure. However, the duration of the hypotensive response to (**40**) (12.4 min.) was significantly longer than the response to bradykinin (1.9 min.).[124]



(40) FR190997

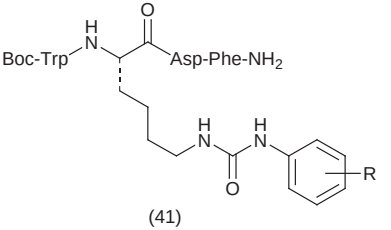
4.7 Cholecystokinin Analogues – As in previous years, peptide and non-peptide analogues of CCK have been reported. The peptide analogues have been based on the C-terminal tetra- and hexapeptide derivatives. Although some new structural subtypes have been reported, the non-peptide ligands have been primarily based on the benzodiazepine-type skeleton reported in the past. In addition, some dual CCK/histamine antagonists have been reported. CCK-4 (Trp-Met-Asp-Phe-NH_2) pseudopeptide analogues incorporating the (*R*) or (*S*) ψ[CH(CN)NH] peptide bond surrogate at the Nle^{31}-Asp^{32} or Trp^{30}-Nle^{31} bonds have been described. Z-Trpψ[(*S*)CH(CN)NH]Nle-Asp-Phe-NH_2 retained the high CCK_B receptor affinity [IC_{50} CCK_A 1714 nM, CCK_B 14.9 nM] of Boc-[Nle^{31}]-CCK-4 [IC_{50} CCK_A 4000 nM, CCK_B 65 nM], and was a potent and selective CCK_B antagonist in the isolated guinea pig ileum.[126] In comparison to the (*S*) analogue, the corresponding (*R*) analogue was less potent at the CCK_B receptor (IC_{50} 45.3 nM) but somewhat more potent at the CCK_A receptor (IC_{50} 483 nM). Some of the truncated analogues, Z-Trpψ[(*S*)CH(CN)NH]Asp-Phe-NH_2, Z-Trpψ[(*R*)CH(CN)NH]Asp-Phe-NH_2, Z-Trpψ[(*S*)CH(CN)NH]Asp-Phe(NH_2)-OH and Z-Trpψ[(*R*)CH(CN)NH]Asp-Phe(NH_2)-OH, retained activity at the CCK_A receptor (IC_{50} 272-719 nM) but were very poor ligands at the CCK_B receptor (IC_{50} >8630 nM). Further truncation led to much less potent analogues, Z-Trpψ[(*S*)CH(CN)NH]Phe-NH_2, Z-Trpψ[(*R*)CH(CN)NH]Phe-NH_2, at both the receptor subtypes. The two more potent tetrapeptide analogues, Z-Trpψ[(*S*)CH(CN)NH]Nle-Asp-Phe-NH_2 and Z-Trpψ[(*R*)CH(CN)NH]Nle-Asp-Phe-NH_2, inhibited the CCK-4 induced contractions in the isolated guinea pig ileum preparation with pA_2 values of 8.0 and 7.1, respectively.

In contrast to Boc-CCK-4, which is 70-fold selective for the CCK_B receptor, modified lysine containing tetrapeptides possessing the general structure Boc-

Trp-Lys[N^{ε}-CO-NH-Ph(R)]-Asp-Phe-NH_2 (**41**) have been reported in the past to be potent and selective full agonists at the CCK_A receptor. Further SAR studies involving modifications of the substituted phenylurea moiety appended off the lysine revealed that relatively minor structural modification within the tetrapeptide, resulted in loss of CCK_A receptor selectivity and development of a trend toward CCK_B selectivity.[127] Lysine derivatives (**41**, R = 2-Me, 3-Me, 4-Me, 2-MeO, 3-MeO, 4-MeO, 2-COOMe, 3-COOMe, 4-COOMe, 2-NO_2, 3-NO_2, 4-NO_2, 3-Ac, 4-Ac) showed varying levels of selectivity for the CCK_A receptors. The most selective agonist (R = 2-Me) (CCK_A IC_{50} 3.8 nM and CCK_B IC_{50} 1400 nM) was about 350-fold more selective for the CCK_A receptors. A few of the compounds (R = 4-NO_2 or 4-Ac) lost selectivity and were marginally more potent agonists at the CCK_B receptors. These tetrapeptides, e.g. Boc-Trp-Lys[N^{ε}-CO-NH-(4-NO_2-Ph)]-Asp-Phe-NH_2 and Boc-Trp-Lys[N^{ε}-CO-NH-(4-Ac-Ph)]-Asp-Phe-NH_2, were full agonists relative to CCK-8 in stimulating intracellular calcium mobilisation in a cell line that expresses the, CCK_B receptor.

Constrained cyclic pseudopeptide CCK_B agonists have been synthesised by replacing the Trp residue of Boc-Trp-MeNle-Asp-Phe-NH_2 (K_i = 0.8 nM, CCK_A/CCK_B > 6000) by α-Me-D-Trp and the MeNle residue by 2-aminononane-1,9-dicarboxylate or similar amino acids followed by cyclisation from the N-terminal Trp amino group and 2-aminononane-1,9-dicarboxylate side chain.[128] Several compounds like (**42-44**) demonstrated activity in both CCK_B (K_i 15-62 nM) and CCK_A (K_i 271-2880 nM) receptor binding assays. The most potent compound, (**42**) [(S) at the α-carbon of the aminononane derivative] had a K_i value of 15 nM for guinea pig cortex membranes (CCK_A/CCK_B = 147) and behaved as a potent and full agonist in a functional assay [stimulation of inositol phosphate accumulation in CHO cells transfected with the rat CCK_B receptor (EC_{50} = 7 nM)]. The cyclic peptide (**42**) also stimulated gastric acid secretion in anaesthetised rats after intravenous injection (ED_{50} value 7.6 nmol/kg/h) (CCK-8 ED_{50} 4 nmol kg^{-1} h^{-1}).[128]

The C-terminal octapeptide of CCK, Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (Ki CCK_A 0.09 nM and CCK_B 0.41 nM) has been modified to improve selectivity for the CCK_A receptor subtype.[129] A close analogue of the octapeptide,



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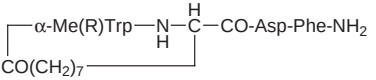
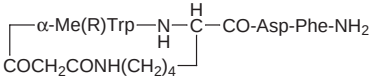


(42) RB360-I and RB360-II



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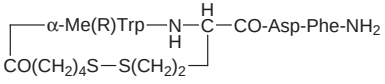
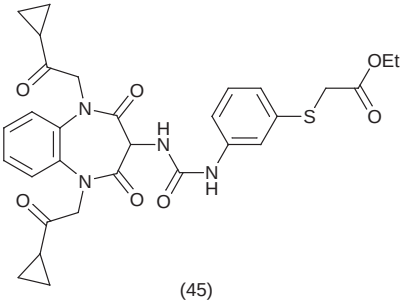


(44) RB380

4-hydroxyphenylacetyl-Met-Gly-Trp-Met-Asp-MePhe-NH_2, was about 20-fold more selective than the octapeptide. Replacing the Asp residue by a D-Asp residue led to a 100-fold more selective analogue, 4-hydroxyphenylacetyl-Met-Gly-Trp-Met-D-Asp-MePhe-NH_2 [K_i CCK_A 0.98 nM and CCK_B 84 nM]. The MePhe residue in this peptide was important for the potency and selectivity. The corresponding Phe analogue was similar in selectivity to CCK-8 but was much less potent [K_i CCK_A 19 nM and CCK_B 150 nM]. Additional modifications like replacement of the Met residues by Nle or Ile and the Asp residue by MeAsp or D-MeAsp resulted in further enhancement in CCK_A selectivity. 4-Hydroxyphenylacetyl-Nle-Gly-Trp-Nle-D-Asp-MePhe-NH_2, 4-hydroxyphenylacetyl-Nle-Gly-Trp-Nle-MeAsp-Phe-NH_2, 4-hydroxyphenylacetyl-Nle-Gly-Trp-Ile-MeAsp-MePhe-NH_2, 4-hydroxyphenylacetyl-Ile-Gly-Trp-Ile-MeAsp-Phe-NH_2 and 4-hydroxyphenylacetyl-Nle-Gly-Trp-Nle-D-MeAsp-Phe-NH_2 showed high affinity at the CCK_A receptors (K_i 0.03-0.5 nM) and poor affinity at the CCK_B receptors (2000-48000-fold selectivity). Most of the analogues inhibited feeding in 24-hour fasted rats when administered intraperitoneally. The most potent and selective analogue, 4-hydroxyphenylacetyl-Nle-Gly-Trp-Nle-D-MeAsp-Phe-NH_2 [K_i CCK_A 0.03 nM and CCK_B 1600 nM] inhibited feeding in rats [ED_{50} values 0.06 and 0.21 μg kg^{-1} at 30 min. and 3 hour period, respectively]. One of the less selective compounds, 4-hydroxyphenylacetyl-Nle-Gly-Trp-Nle-MeAsp-Phe-NH_2, [K_i CCK_A 0.03 nM and CCK_B 224 nM] was similar in potency to the more selective analogue 4-hydroxyphenylacetyl-Nle-Gly-Trp-Nle-D-MeAsp-Phe-NH_2 in rats, but when administered intranasally to dogs it was about 4-fold more potent [ED_{50} values 5.0 and 20 μg kg^{-1}, respectively].[129]

As reported in previous years, a number of benzodiazepine derivatives (**45**-**48**) have been described as CCK_B/gastrin antagonists.[130–134] In addition, CCK_A agonists based on the benzodiazepine skeleton have been reported. Compound (**45**) inhibited gastric acid secretion induced by pentagastrin in anaesthetised rats (ED_{50} 0.06 mg kg^{-1}).[130] In a pentagastrin-induced acid secretion model (Heidenhain pouch dogs), YF476 (**46**) and the corresponding NMe_2 analogue were potent inhibitors of acid secretion (ED_{50} 0.01 mg kg^{-1}). When administered orally, YF476 was about 90-fold more potent than YM022 (reported earlier) and displayed a long duration of action (>6 h at a dose of 100 nmol kg^{-1}).[132] Many compounds like (**47**) were synthesised to improve aqueous solubility. The compounds showed high affinity for the CCK_B receptor (CCK_B IC_{50} < 2.5 nM, CCK_A/CCK_B > 2000), and a significant improvement *in vivo* half-life was observed for a selection of compounds. Compound (**47**) inhibited the pentagastrin-induced excitation of single neurones in a slice preparation of the rat ventromedial hypothalamic neurones with a K_b <1 nM.[134] In addition to the above CCK_B/gastrin receptor antagonists, benzodiazepine-based CCK_A/CCK_B antagonists have also been synthesised. Compound (**48**) had high affinity at both CCK_A (IC_{50} 9.2 nM) and CCK_B receptors (IC_{50} 0.38 nM). Analogues containing a -S-CH_3 or -$COCH_3$ group in place of the tetrazole were similar in potency to (**48**), whereas the -COOH analogue was about 2-fold less potent than (**48**) at the CCK_B receptor and about 16-fold less potent at the CCK_A receptors.[135]

Analogues of CCK_A agonist (**49**) (GW5823) [ED_{50} in a functional assay 109



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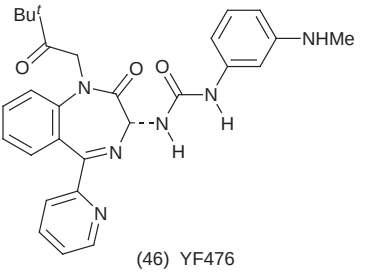
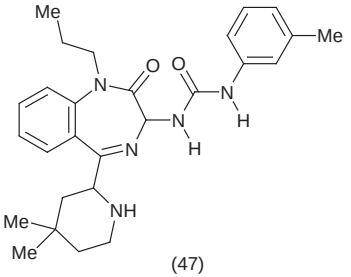


(46) YF476



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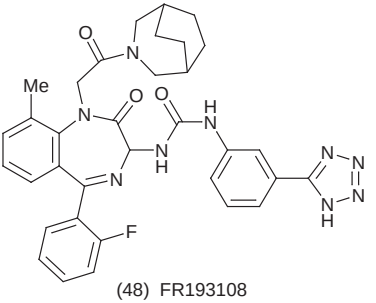
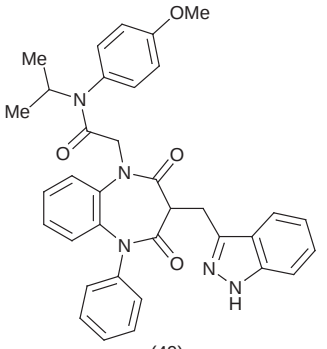


(48) FR193108

nM, CCK-8 ED_{50} 2 nM] which demonstrated oral efficacy in a rat feeding model have been synthesised.[136] In comparison to the indazole analogue (**49**), the corresponding benzofuran, indole, benzothiophene, benzisoxazole and benzisothiazole analogues did not give a full agonist response (30-80% CCK-8). Evaluation of several analogues in an *in vivo* mouse gall-bladder emptying assay revealed compound (**49**) to be the most potent and efficacious of all the analogues tested. It reduced food intake to 40% of vehicle controls when given orally at a dose of 10 μmol kg^{-1}.

In addition to the benzodiazepine-based antagonists, some other chemically distinct CCK antagonists (**50-54**) have also been reported.[137–141] Compound (**51**) (S-0509) which may be considered as a ring opened benzodiazepine structure showed high affinity for the CCK_B/gastrin receptor [IC_{50} values for gastrin, CCK_B and CCK_A receptors 1.52, 23.5 and 2813 nM, respectively]. Administration (id) of (**51**) led to inhibition of gastric acid secretion induced by pentagastrin in anaesthetised rats with an ED_{50} value of 0.014 mg kg^{-1}.[138] The 2-Adoc-α-Me-D-Trp-hydroxyproline derivatives of the general formula (**52**) [R = O-(1-naphthyl), -OPh(*p*-Cl), -OPh(*p*-F), -OPh(*p*-I), -OPh(*p*-NO_2), -OPh(*o*,*p*-Cl_2), -OPh(*o*,*p*-F_2), -OCH_2Ph, and -OCH_2Ph(*m*-Cl)] were less potent than 2-Adoc-α-Me-D-Trp-N(CH_2COOH)-CH_2CH_2Ph (RB210) reported earlier.[139] Conformationally constrained series of CCK_B/gastrin receptor ligands were synthesised by replacing the Phe residue in compound (**52**) by analogues of Tic containing 6-9-membered rings.[10,141] The 9-membered ring analogue (**53**) was one of the more potent CCK_B/gastrin antagonist (rat stomach pK_B 9.08, mouse cortex pIC_{50} 8.3).



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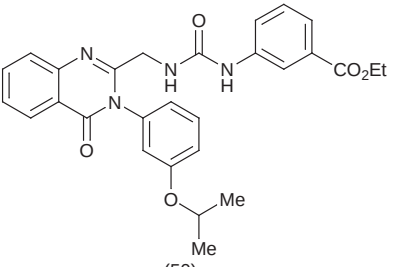

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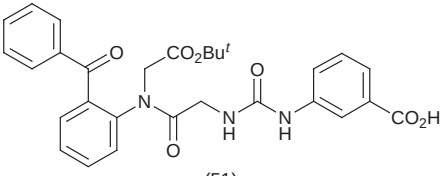

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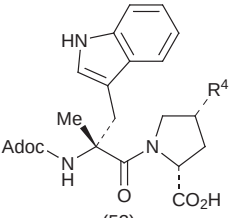

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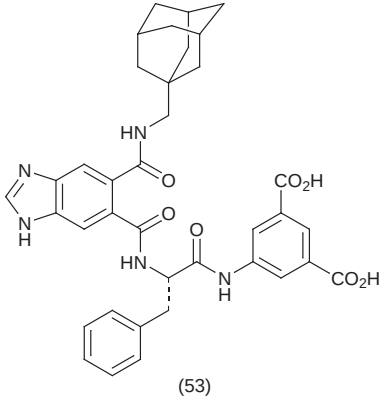

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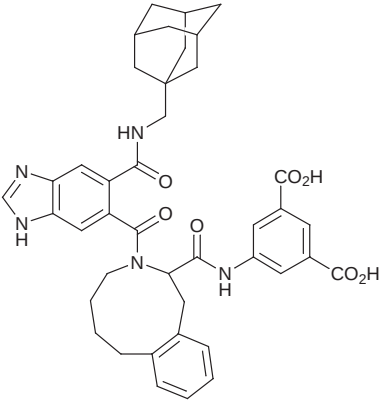
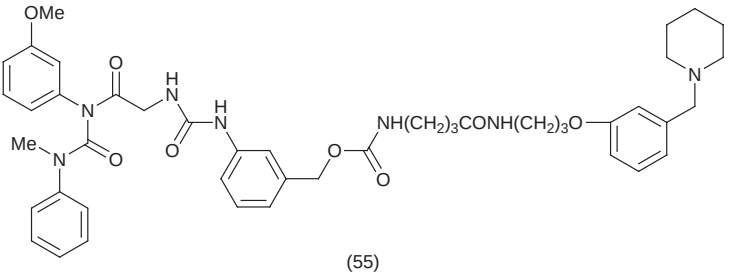


(54)

In comparison, the 6-, 7- and 8-membered ring containing analogues were poor CCK_B/gastrin receptor antagonists (rat stomach pK_B values 7.6-7.9, mouse cortex pIC_{50} 6.63-6.75).

Further SAR studies on the previously reported dual histamine H_2 and gastrin receptor antagonists were attempted to improve their low oral absorbability by decreasing the high hydrophobicity of the parent hybrid compounds.[142–144] Many such compounds containing a benzazepine, benzoxazepine, benzothiazepine or

benzodiazepine skeleton and various linking groups like -Gly-β-Ala-γ-Abu-, -γ-Abu-γ-Abu- led to compounds with CCK and gastrin antagonist activity. Compounds like (**55**) [IC_{50} values for gastrin, CCK_B and CCK_A receptors 21, 380 and 1800 nM, respectively; histamine H_2 pA_2 7.6] showed some improvement in oral bioavailability.



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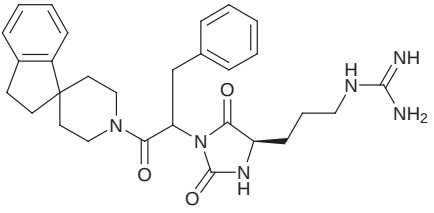
4.8 Complement-related Peptide/Non-peptide Analogues – Peptide and non-peptide agonists and antagonists of C3a and C5a are reported. In a human neutrophil cDNA library, an orphan G-protein-coupled receptor (HNFAG09) with 37% nucleotide identity to the C5a receptor (C5a-R, CD88) was identified. Mammalian cells stably expressing HNFAG09 specifically bound [^{125}I]-C3a and responded to a synthetic C3a carboxyl-terminal analogue and to human C3a but not to rC5a with a robust calcium mobilisation response.[145] Casoxin C (Tyr-Ile-Pro-Ile-Gln-Tyr-Val-Leu-Ser-Arg) isolated from the tryptic digest of bovine κ-casein as an anti-opioid peptide in longitudinal strips of guinea pig ileum had a contractile profile on ileal strips similar to that of human complement C3a. It also had affinity for C3a receptors (IC_{50} 40 μM) in a radioreceptor assay. In comparison, the IC_{50} values for C3a, C3a(70-77) and another C3a agonist (Trp-Trp-Gly-Lys-Lys-Tyr-Arg-Ala-Ser-Lys-Leu-Gly-Leu-Ala-Arg) were 0.089, 57 and 0.22 μM, respectively. Casoxin C also showed phagocyte-stimulating activities.[146]

Peptides synthesised with sequence homology to the C-terminal region of C5a [C5a 65-74 (Tyr^{65}, Phe^{67}) Tyr^{65}-Ser^{66}-Phe^{67}-Lys^{68}-Asp^{69}-Met^{70}-Gln^{71}-Leu^{72}-Gly^{73}-Arg^{74})] have been shown to behave as full agonists relative to the natural factor but with much reduced potencies.[147] For example, the decapeptide C5a 65-74 (Tyr-Ser-Phe-Lys-Asp-Met-Gln-Leu-Gly-Arg) retains 0.1% potency of C5a (decapeptide EC_{50} 9.6 μM, C5a EC_{50} 20 nM in inducing smooth muscle contraction of human foetal artery). Two other peptides, Tyr-Ser-Phe-Lys-Asp-Met-Pro-Leu-D-Ala-Arg and Tyr-Ser-Phe-Lys-Pro-Met-Pro-Leu-D-Ala-Arg, were somewhat more potent (2-8% C5a). Based on these findings, the C-terminal region of C5a was regarded as the biological effector region of the polypeptide. Some of the N-terminally extended analogues of the above decapeptide, e.g. Ac-Tyr-Ser-Phe-Lys-Pro-Met-Pro-Leu-D-Ala-Arg, Ahx-Tyr-Ser-Phe-Lys-Pro-Met-Pro-Leu-D-Ala-Arg, Lys-Trp-Ahx-Tyr-Ser-Phe-Lys-Pro-Met-Pro-Leu-D-Ala-

Arg, Lys-Tyr-Ser-Phe-Lys-Pro-Met-Pro-Leu-D-Ala-Arg, Lys-Gly-Lys-Ahx-Tyr-Ser-Phe-Lys-Pro-Met-Pro-Leu-D-Ala-Arg, Lys-Gly-Lys-Gly-Lys-Ahx-Tyr-Ser-Phe-Lys-Pro-Met-Pro-Leu-D-Ala-Arg, Lys-Gly-Lys-Gly-Lys-Gly-Gly-Gly-Tyr-Ser-Phe-Lys-Pro-Met-Pro-Leu-D-Ala-Arg, $C5a_{37-46}$-AhX-Tyr-Ser-Phe-Lys-Pro-Met-Pro-Leu-D-Ala-Arg and $C5a_{12-20}$-AhX-Tyr-Ser-Phe-Lys-Pro-Met-Pro-Leu-D-Ala-Arg were somewhat more potent than the decapeptide (10-24% C5a) in inducing contractile responses in the human foetal artery. All the analogues were much less potent than C5a in the polymorphonuclear leukocytes (neutrophils) β-glucuronidase release assay (<2%) and in a receptor binding assay (<0.04% C5a). Although the N-terminally $C5a_{12-20}$- (Lys-Tyr-Lys-His-Ser-Val-Val-Lys-Lys) extended analogue, $C5a_{12-20}$-AhX-Tyr-Ser-Phe-Lys-Pro-Met-Pro-Leu-D-Ala-Arg, was the most potent compound in each of the three assay systems (13%, 5.2% and 1.04% C5a, respectively, in the human foetal artery, β-glucuronidase release and the receptor binding assays), the potency in the receptor binding assay was not always related to the activities in the other assays.[147]

Non-peptide agonist and antagonists of C5a have been identified by random screening.[148,149] A C5a receptor ligand lead (**56**), a hydantoin derivative incorporating an arginine and a phenylalanine residue, was identified by random screening [^{125}I-C5a displacement from a human neutrophil preparation]. The structural optimisation for binding affinity resulted up to a 100-fold improvement in binding affinity (compounds **57**, **58**, IC_{50} 0.3 μM). Replacement of the Phe residue by cyclohexylalanine gave the most potent analogues. A number of other compounds containing a Nal(1), Nal(2), hPhe, Trp, Val or Met side chain in place of the Phe side chain were much less potent (IC_{50} values 5-30 μM). Replacement of the Arg side chain by Lys or hArg side chain or by a $-(-CH_2)_3-NH-CO-NH_2$ or $-CH_2Ph-4-NH_2$ group also resulted in less potent compounds. Both compounds (**57**) and (**58**) acted as functional agonists [stimulated myeloperoxidase release from neutrophils (eliciting 86-90% of the increase in myeloperoxidase release at 30 μM that is produced by a peptide agonist Tyr-Phe-Lys-Ala-Cha-Gly-Leu-D-Phe-Arg at 1 μM)].[148] SAR studies on a random screening lead identified using a C5a binding assay (human monocyte cell line U937) led to an antagonist (**59**) of C5a (IC_{50} 0.8 μM).[149] Compound (**59**) was also tested in a functional assay (respiratory burst) based on the release of hydrogen peroxide from neutrophils on activation. At a concentration of 30 μM, (**59**) blocked the respiratory burst induced by 100 nM of C5a.

4.9 Endothelin Analogues – An ET_B receptor subtype has been identified in canine spleen membranes using ET_B-selective agonists endothelin-3, IRL-1620, sarafotoxin 6c as well as ET_A-selective antagonists BQ123 and related cyclic pentapeptides. The receptor binds both ET_A and ET_B selective ligands.[150] Receptor binding and antagonist properties of endothelin-1 analogues, [Thr^{18}, γ-methylleucine^{19}]endothelin-1, [Thr^{18}, Leu^{19}]endothelin-1 and [Thr^{18}, Cha^{19}]endothelin-1, were investigated using cloned human ET_A and ET_B receptors expressed in CHO cells. All three analogues inhibited the binding of [^{125}I]endothelin-1 from ET_A and ET_B receptor expressing cells (K_d values 160-370 and 62-190 pM, respectively) and were devoid of any agonist activity (stimulation of



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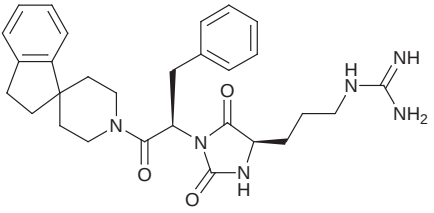

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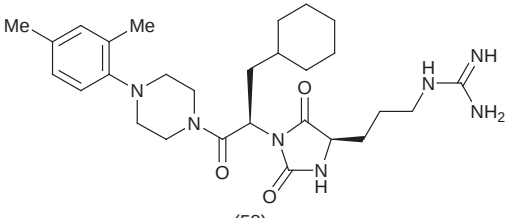

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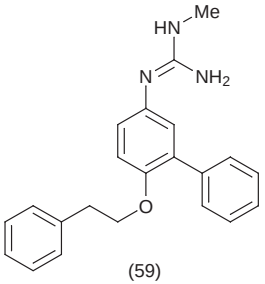


(59)

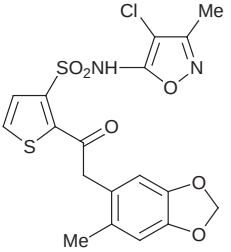
arachidonic acid release) in endothelin ET_A receptor-expressing cells. In endothelin ET_B receptor-expressing cells, [Thr[18], γ-methylleucine[19]]endothelin-1 and [Thr[18], Cha[19]]endothelin-1 did not stimulate arachidonic acid release up to a concentration of 1 μM, but [Thr[18], Leu[19]]endothelin-1 did stimulate arachidonic acid release (pD_2 value 8.9). However, the maximal release was only 40% of the maximal release induced by endothelin-1. In another agonist assay (inositol phosphate accumulation), both [Thr[18], γ-methylleucine[19]]endothelin-1 and [Thr[18], Cha[19]]endothelin-1 had no effect on inositol phosphate accumulation up to a concentration of 1 μM, but [Thr[18], Leu[19]]endothelin-1 stimulated accumulation of inositol phosphate in a concentration dependent manner (pD_2 value 9.1). However, the maximal release was only 10% of the maximal release induced by endothelin-1. Thus [Thr[18], Leu[19]]endothelin-1 was a partial agonist at the CCK_B receptor in both the assays.[151]

RES-701-1 [c(Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp)-Trp-Phe-Phe-Asn-Tyr-Tyr-Trp], a cyclic 16 amino acid peptide in which the N-terminal glycine amino group and β-carboxyl of Asp[9] were linked by an amide bond, was reported

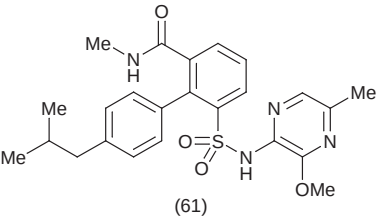
previously as an ET_B-selective antagonist.[152] N-Terminal cyclic peptide of RES-701-1 was chemically combined to C-terminal peptide of various endothelin and sarafotoxin family of peptides. Several of the hybrid peptides, RES-701-1(1-10)/[Ala^{15}]ET-1(12-21) [c(Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp)Trp-Val-Tyr-Phe-Ala-His-Leu-Asp-Ile-Ile-Trp], RES-701-1(1-10)/[Ala^{15}]ET-3(12-21) [c(Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp)Trp-Val-Tyr-Tyr-Ala-His-Leu-Asp-Ile-Ile-Trp] and RES-701-1(1-10)/[Ala^{15}]SRXT6b(12-21) [c(Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp)Trp-Leu-Tyr-Phe-Ala-His-Gln-Asp-Val-Ile-Trp] were found to be selective ET_B antagonists. The three hybrid peptides were more potent (ET_B IC_{50} 0.04-3.8 nM, ET_A IC_{50} >100 nM) than RES-701-1 (ET_B IC_{50} 10 nM, ET_A IC_{50} >5000 nM). Fragments of endothelins and sarafotoxins, e.g. ET-1(12-21), [Ser^{15}]ET-1(12-21), ET-3(12-21) and SRXT6b(12-21), were poor antagonists of endothelin (ET_B IC_{50} 1000-3000 nM).[152]

Combined ET_A/ET_B receptor antagonists based on the C-terminal hexapeptide of endothelin (His^{16}-Leu^{17}-Asp^{18}-Ile^{19}-Ile^{20}-Trp^{21}), such as Ac-D-Dip^{16}-Leu-Asp-Ile-Ile-Trp^{21} [PD 142893, ET_A IC_{50} = 58 nM, ET_B IC_{50} = 130 nM] and Ac-D-Bhg^{16}-Leu-Asp-Ile-Ile-Trp^{21} [PD 145065, ET_A IC_{50} = 4 nM, ET_B IC_{50} = 15 nM], have been reported previously. However, these compounds are readily cleaved by carboxypeptidases. By performing a reduced amide bond and N-methyl amino acid scan, it was discovered that N-methylation of Ile^{20} resulted in a compound (Ac-D-Bhg^{16}-Leu-Asp-Ile-MeIle-Trp^{21}, PD 156252) that retained full receptor affinity at both endothelin receptor subtypes (ET_A IC_{50} = 1 nM, ET_B IC_{50} 40 nM) along with enhanced proteolytic stability ($t_{1/2}$ 538 min.) and cellular permeability. Interestingly, N-methylation of this bond allows the cis configuration to be readily accessible which greatly alters the preferred structure of the entire molecule and may be responsible for the observed enhanced metabolic stability.[153] Other N-Me amino acid containing analogues, Ac-N-Me-D-Dip^{16}-Leu-Asp-Ile-Ile-Trp^{21}, Ac-D-Dip^{16}-MeLeu-Asp-Ile-Ile-Trp^{21}, Ac-D-Dip^{16}-Leu-MeAsp-Ile-Ile-Trp^{21}, Ac-D-Dip^{16}-Leu-Asp-MeIle-Ile-Trp^{21}, Ac-D-Dip^{16}-Leu-Asp-Ile-MeIle-Trp^{21}, Ac-D-Dip^{16}-Leu-Asp-Ile-Ile-MeTrp^{21}, were somewhat less potent (ET_A IC_{50}s = 30 nM - 4.5 μM, ET_B IC_{50} 80 nM - 11 μM). Similarly, the aminomethylene analogues, Ac-D-Dip^{16}ψ[CH_2NH]Leu-Asp-Ile-Ile-Trp^{21}, Ac-D-Dip^{16}-Leuψ[CH_2NH]Asp-Ile-Ile-Trp^{21}, Ac-D-Dip^{16}-Leu-Aspψ[CH_2NH]Ile-Ile-Trp^{21}, Ac-D-Dip^{16}-Leu-Asp-Ileψ[CH_2NH]Ile-Trp^{21}, Ac-D-Dip^{16}-Leu-Asp-Ile-Ileψ[CH_2NH]Trp^{21} were also less potent (ET_A IC_{50} = 250 nM - 2.7 μM, ET_B IC_{50} 20 nM - 1.3 μM).[153]

SAR studies have continued on previously reported non-peptide antagonists of endothelin with the aim of improving the pharmacokinetic profiles. Thiophenesulfonamide series of compounds yielded several potent ET_A selective antagonists.[154–158] One of these (**60**, TBC11251) bound competitively to human ET_A receptors with a K_i of 0.43 nM and an IC_{50} of 1.4 nM (IC_{50} for ET_B = 9800 nM) and inhibited ET-1-induced stimulation of phosphoinositide turnover [K_i 0.686 nM, pA_2 of 8.0]. The compound has a serum half-life in the rat and the dog of 6-7 h and 60-100% oral bioavailability.[157] Another series of arylsulfonylamide derivatives displaying ET_A selective antagonist activity included compounds like (**61**) (ET_A pIC_{50} 9.3) which inhibited big endothelin-1-induced pressor response

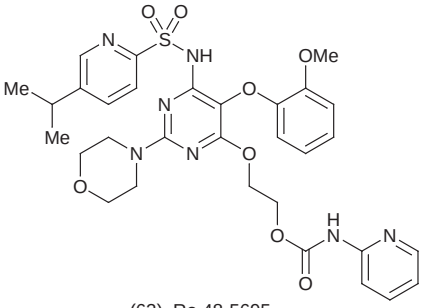


(60) TBC11251

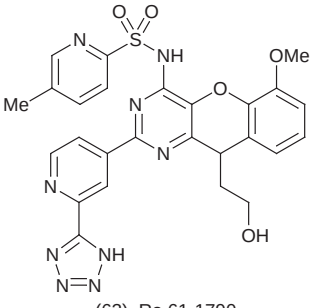


(61)

when administered orally. Compound (**61**) had a duration of action in excess of 4 hours at a dose of 2.5 mg kg^{-1} in the conscious rat.[159,160] Examples of sulfonamide derivatives acting as endothelin antagonists on both the ET_A and ET_B receptor subtypes include compounds like (**62**, Ro48-5695) which display potent binding (IC_{50} values at ET_A and ET_B receptors 0.3 and 5 nM, respectively) and high functional antagonistic potency *in vitro*. As a sodium salt, (**60**) showed 40-60% oral bioavailability in dogs and rats.[161–163] *In vivo*, Ro 61-1790 (**63**) inhibited the pressor effect of big ET-1 in pithed rats with an ID_{50} value of 0.05 mg kg^{-1}. Intravenous bolus dose of (**63**) induced a long-lasting antihypertensive effect in deoxycorticosterone acetate salt rats. Other structurally different endothelin antagonists, obtained primarily by SAR around random screening leads, include compounds like PD156707 (**64**) (IC_{50}s = 0.37 and 480 nM at ET_A and ET_B receptors, respectively), (**65**) (IC_{50} 260 and 200 nM for ET_A and ET_B receptors, respectively) and (**66**) (IC_{50} hET_A 0.52 nM, pET_B 0.85 nM).[164–169] The ET_A/ET_B antagonist (**66**) was obtained by chemical modifications of ABT-627, an ET_A selective antagonist reported earlier.

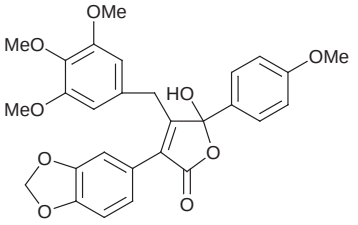


(62) Ro 48-5695

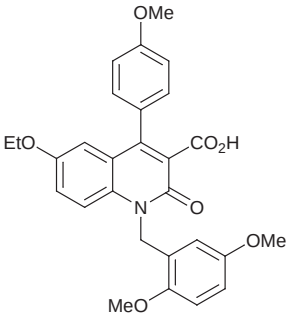


(63) Ro 61-1790

4.10 Growth Hormone-releasing Peptide and Non-peptide Analogues – Potent peptides which release growth hormone *in vitro* and *in vivo* have been known for the past ten years. Examples of such peptides include His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 (GHRP-6), Ala-His-D-Nal(2)-Ala-Trp-D-Phe-Lys-NH_2, D-Ala-D-Nal(2)-Ala-Trp-D-Phe-Lys-NH_2 (GRP-2) and His-D-2-methyl-Trp-Ala-Trp-D-



(64) PD156707



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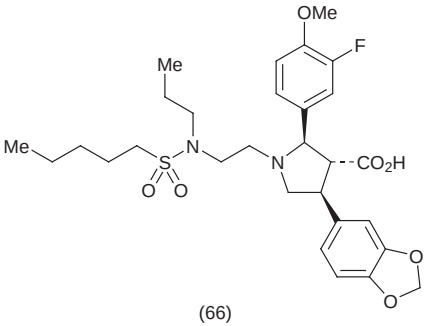
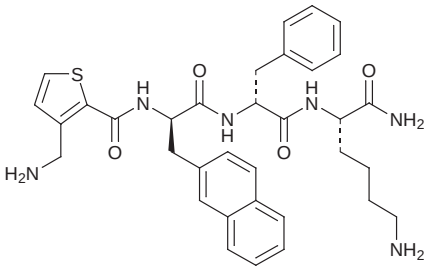


(66)

Phe-Lys-NH_2 (hexarelin). These peptides have no structural homology with growth hormone or growth hormone-releasing hormone. Both GRP-2 and GRP-6 act synergistically with growth hormone-releasing factor to release growth hormone. Co-administration of GHRP-2 and GHRP-6 at maximal concentrations had no further effect on growth hormone release than either one alone. Both the peptides were able to desensitise cells to each other but not to growth hormone-releasing factor. The effect of GHRP-2 was inhibited by peptide antagonist, but was not affected by growth hormone-releasing factor antagonist, [Ac-Tyr^1, D-Arg^2]-GRF. Thus the two peptides appear to stimulate growth hormone release from rat pituitary cells via the same receptor and mechanism, but different from growth hormone-releasing factor.[170,171] One of the above peptides, [His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH_2], when administered to β-thalassemia patients was shown to raise serum growth hormone levels by both intravenous (1 μg kg^{-1}) and oral (20 mg per patient) routes of administration. Growth hormone releasing hormone (1 μg kg^{-1}, i.v.) did not have significant effect on growth hormone levels.[172] Chemical modifications of a random screening pentapeptide lead, Ala-His-D-Nal-D-Phe-Lys-NH_2 (EC_{50} 6 nM), by replacement of the N-terminal Ala-His dipeptide by other dipeptide mimetics like 3-aminomethylbenzoic acid (EC_{50} 7 nM), 2-aminomethylbenzoic acid (EC_{50} 85 nM), 2,3-substituted thiophene (**67**, EC_{50} 27 nM), 2,4-substituted thiophene and 2,5-substituted thiophene, resulted in less potent compounds.[173]



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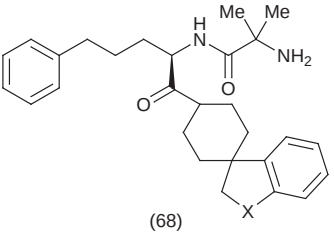
Antagonists of growth hormone-releasing hormone were synthesised based on the N-terminal 1-29 amino acids of the releasing hormone.[174] In comparison to the earlier reported antagonist [Ac-Tyr^1, D-Arg^2]hGH-RH(1-29)-NH_2, many of the new antagonists, Ibu-[D-Arg^2, Phe(*p*-Cl)^{6,10}, Abu^{15}, Nle^{27}]hGH-RH(1-28)-$NH(CH_2)_4$-NH-C(NH)-NH_2, Ph-CH_2CO-[D-Arg^2, Phe(*p*-Cl)^6, Abu^{15}, Nle^{27}]hGH-RH(1-28)-$NH(CH_2)_4$-NH-C(NH)-NH_2, Ph-CH_2CO-[D-Arg^2, Phe(*p*-Cl)^6, Abu^8, Ala^{15}, Nle^{27}]hGH-RH(1-29)-NH_2 and Ph-CH_2CO-[D-Arg^2, Phe(*p*-Cl)^6, Abu^{8,28}, Ala^{15}, Nle^{27}]hGH-RH(1-29)-NH_2, were more potent. The most potent antagonist, Ph-CH_2CO-[D-Arg^2, Phe(*p*-Cl)^6, Abu^{15}, Nle^{27}]hGH-RH(1-28)-$NH(CH_2)_4$-NH-C(NH)-NH_2, inhibited growth hormone-releasing hormone-induced growth hormone release (55-94% inhibition at 30 nM) in a superfused rat pituitary assay up to a period of 2 hours. It also inhibited serum growth hormone levels after intravenous (80 μg kg^{-1}), intraperitoneal (4 mg kg^{-1}) or intramuscular (2.2 mg kg^{-1}) administration but not after subcutaneous (4 mg kg^{-1}) administration.[175] Ph-CH_2CO-[D-Arg^2, Phe(*p*-Cl)^6, Abu^{15}, Nle^{27}]hGH-RH(1-28)-$NH(CH_2)_4$-NH-C(NH)-NH_2 also inhibited the growth of various insulin-like growth factors-I and/or -II dependent tumours including human lung cancers, ostoesarcomas, prostatic and colorectal cancers in nude mice and murine mammary tumours. Another potent antagonist, isobutyryl-[D-Arg^2, Phe(*p*-Cl)^6, Abu^{15}, Nle^{27}]hGH-RH(1-28)Agm, inhibited the growth of Caki-I cells and was also effective in reducing the tumour weight in nude mice bearing xenografts of human Caki-I renal cell carcinoma.[176]

SAR studies on non-peptide growth hormone secretagogues have been reported. In the spiroindane series of compounds, modification of the benzylic position of the spiroindane resulted in increased potency and oral bioavailability. In both structures [(**68**) and the corresponding compound containing Trp in place of Phe], polar substituents (X = CO or CHOH) led to more potent compounds than the corresponding (X = CH_2) analogues when given orally. For example, compound (**68**, X = CH_2) gave a 4-fold increase in serum growth hormone levels in the beagle dog model at a dose of 1 mg kg^{-1}, po, but the corresponding (X = CO) analogue gave a similar response at a dose of 0.25 mg kg^{-1}, po.[177] Compounds with a readily metabolised ester into the spiroindane benzylic position (**68**, X = CH-COOEt, L-163,833) resulted in short-acting compounds. The compound was rapidly converted into the corresponding acid *in vitro* (rat, dog, human plasma and liver microsomes) as well as *in vivo* (rat and dog) and the

resulting acid was rapidly cleared.[178] In the benzoazepinone series of compounds, replacement of the tetrazole group by various 5-membered ring systems [e.g. oxadiazole (**69**) (EC_{50} 30 nM) and triazole (EC_{50} 90 nM)] resulted in slight enhancement in affinity.[179] In comparison to the naphthoazepinone analogue (**70**) (ED_{50} 4 nM), the corresponding benzoazepinone analogue was much less potent (ED_{50} 30 nM).[180]



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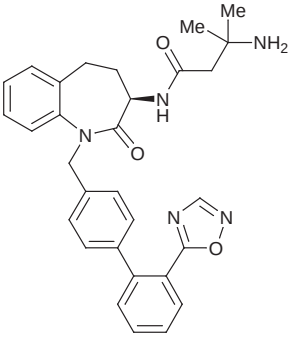

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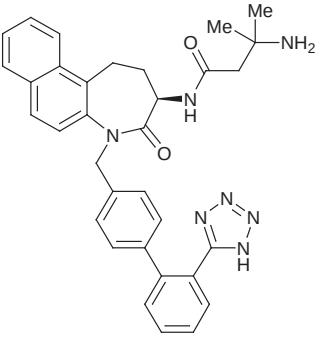

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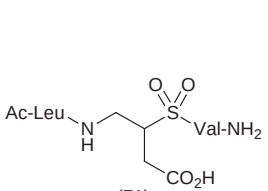
4.11 Integrin-related Peptide and Non-peptide Analogues – Many proteins like fibronectin, von Willebrand factor, thrombospondin, laminin, vitronectin and VCAM-1 are involved in cell-cell and cell-matrix interactions. A number of these processes are known to be involved in various diseases like thrombosis, bone disorders and cancer. Various aspects of integrin research have been described in recent reviews.[181–189] As in the previous year, most of the work has centred around glycoprotein IIb/IIIa and $\alpha_v\beta_3$ integrins. A region of type IV collagen, α(IV)531-543 [Gly-Glu-Phe-Tyr-Phe-Asp-Leu-Arg-Leu-Lys-Gly-Asp-Lys], has been reported to bind $\alpha_3\beta_1$ integrin and promote integrin-mediated tumour cell adhesion. An all-D enantiomeric peptide model of α1(IV)531-543 containing a D-Tyr at the C-terminal end [Gly-D-Glu-D-Phe-D-Tyr-D-Phe-D-Asp-D-Leu-D-Arg-D-Leu-D-Lys-Gly-D-Asp-D-Lys-D-Tyr] and the corresponding all-L analogue were found to support melanoma and breast carcinoma cell adhesion, spreading, and motility in a dose-dependent fashion. The adhesions of melanoma and breast carcinoma cells to both type IV collagen and fibronectin were effectively inhibited by both the peptides.[190]

A series of cyclic peptides (based on the Leu-Asp-Val sequence of CS1) like Cys-Trp-Leu-Asp-Val-Cys (disulfide bridge between Cys[1,6]) inhibited $\alpha_4\beta_1$-dependent binding of lymphocytes to VCAM-1 and CS1 with IC_{50} values of 1-3 μM.[191,192] The peptide also inhibited $\alpha_4\beta_7$-dependent lymphocyte binding to the ligands MAdCAM-1, VCAM-1 and CS-1. The cyclic peptide inhibited $\alpha_4\beta_1$-dependent, but not $\alpha_5\beta_1$-dependent, binding of cells to intact fibronectin. Other active compounds in the series included cyclic disulfide bridge containing peptides Cys-Glu-Trp-Leu-Asp-Val-Cys, Cys-Ile-Leu-Asp-Val-Cys, Cys-Trp-Leu-Asp-Ala-Cys and Cys-Leu-Asp-Val-Cys. Inactive cyclic peptides included Cys-Asp-Leu-Val-Trp-Cys, Cys-Trp-Leu-Asp-Cys, c(Glu-Trp-Leu-Asp-Val-Asp) and c(Glu-Trp-Leu-Asp-Val-Pro-Glu-Trp-Leu-Asp-Val) (IC_{50} values >1000 μM). In addition to Leu-Asp-Val containing peptides, Lys-Leu-Asp-Ala-Pro-Thr has been reported as a novel fibronectin ligand for activated α4 ($\alpha_4\beta_1$ and $\alpha_4\beta_7$) integrins.[193] Ac-Leu-Asp-Thr-NH_2 (IC_{50} 276 μM), Ac-Leu-Asp-Val-NH_2 (IC_{50} 126 μM) and the sulfonamide derivative **71** were active in the $\alpha_4\beta_7$ and its endothelial ligand (MAdCAM-1, mucosal addressin cell adhesion molecule-1) adhesion assay.[194] The sulfonamide derivative of **71** containing a Thr residue in place of Val did not show activity in the adhesion assay at a concentration of 500 μM. Sequences (e.g. Gly-Arg-Gly-Asp-Asn-Pro) involved in $\alpha_5\beta_1$ integrin recognition have been reported.[195,196]

Various peptide and non-peptide antagonists of $\alpha_v\beta_3$ integrin have been reported.[197–203] Based on the potent and selective inhibitor for the $\alpha_v\beta_3$ integrin, c(Arg-Gly-Asp-D-Phe-Val), several cyclic retro-inverso and related peptides [c(Arg-Gly-Asp-Phe-Val), c(Arg-Gly-Asp-Phe-D-Val), c(Arg-Gly-Asp-D-Phe-Val), c(Arg-Gly-D-Asp-Phe-Val), c(D-Arg-Gly-Asp-Phe-Val), c(D-Arg-Gly-D-Asp-D-Phe-Val), c(D-Arg-Gly-D-Asp-Phe-D-Val), c(D-Arg-Gly-Asp-D-Phe-D-Val), c(Arg-Gly-D-Asp-D-Phe-D-Val), c(D-Val-Phe-Asp-Gly-Arg), c(Val-D-Phe-Asp-Gly-Arg), c(Val-Phe-D-Asp-Gly-Arg), c(Val-Phe-Asp-Gly-D-Arg), c(Val-D-Phe-D-Asp-Gly-D-Arg), c(D-Val-Phe-D-Asp-Gly-D-Arg), c(D-Val-D-Phe-Asp-Gly-D-Arg), c(D-Val-D-Phe-D-Asp-Gly-Arg) and c(D-Val-D-Phe-D-Asp-Gly-D-Arg)] were tested on the isolated integrin $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$ receptors. One of the retro-inverso peptides [c(D-Val-D-Phe-D-Asp-Gly-Arg)] and one of the inverso [c(D-Arg-Gly-Asp-D-Phe-D-Val)] peptides inhibited vitronectin binding to $\alpha_v\beta_3$ receptor. Only c(Arg-Gly-Asp-Phe-D-Val) and c(Arg-Gly-Asp-D-Phe-Val) inhibited $\alpha_{IIb}\beta_3$. All the others were inactive at a concentration of 10 μM.[198] In comparison to the hexapeptide Gly-Arg-Gly-Asp-Ser-Pro ($\alpha_V\beta_3$ IC_{50} 5 μM, $\alpha_V\beta_5$ IC_{50} 0.58 μM, fibronectin IC_{50} 0.02 μM and IIb/IIIa IC_{50} 0.8 μM), an Arg-Gly-Asp cyclic peptide derivative containing a β-turn peptidomimetic (**72**) was a selective antagonist of $\alpha_v\beta_3$ receptor [$\alpha_v\beta_3$ IC_{50} 0.05 μM, $\alpha_v\beta_5$ IC_{50} 0.4 μM, fibronectin and IIb/IIIa IC_{50} >10 μM].[199]

Non-peptide antagonists of $\alpha_v\beta_3$ receptor have been based on the Arg-Gly-Asp derivatives reported previously as IIb/IIIa antagonists. Examples of compounds more selective against $\alpha_v\beta_3$ receptor include compounds like (**73-75**). The diaminopropionic acid derivative (**73**) was one of the more potent and selective compounds against $\alpha_v\beta_3$ integrin (IC_{50} 1.1 nM) and poorly active against $\alpha_v\beta_5$, $\alpha_5\beta_1$ and GPIIb/IIIa integrins (>500-fold less potent).[201] Compound (**74**)



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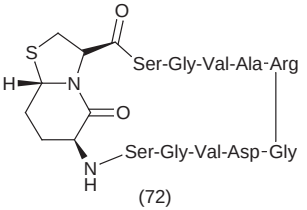

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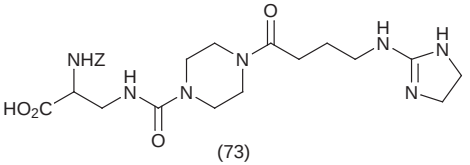
(SC56631) prevents osteoclast-mediated bone particle degradation with a potency similar to that of the disulfide bridge containing cyclic peptide Gly-Pen-Gly-Arg-Gly-Asp-Ser-Pro-Cys-Ala.[202] Intravenous infusion (0.5 mg kg^{-1} min^{-1}, $t_{1/2}$ <20 min) of the mimetic prevents the 55% loss of trabecular bone sustained by rats within 6 weeks of oophorectomy. Compound (**75**) was an inhibitor of both human osteoclast-mediated bone resorption and vitronectin-induced hepatotoxis of human endothelial cells.[203]



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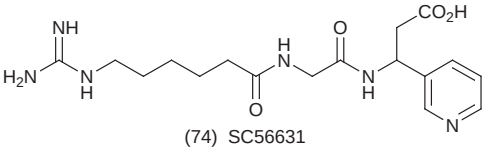
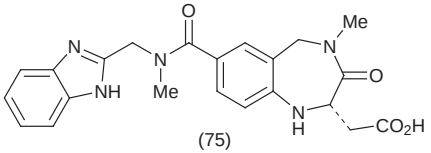


(74) SC56631



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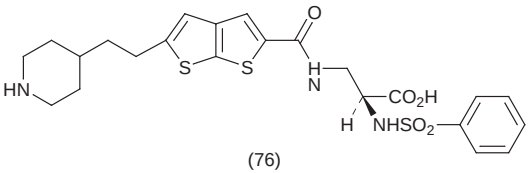
Design of newer IIb/IIIa antagonists has been based primarily around the compounds reported earlier. The main emphasis has been on the oral activity and duration of action. This work has resulted in compounds which inhibit platelet aggregation in animal models up to a period of 24 hours after oral administration.[204–210] For example, in L-2,3-diaminopropionic acid series of compounds, the more potent compounds (**76-78**) inhibited platelet aggregation (8-24 hours) in several animal species at a dose of 0.25-2 mg kg^{-1} when given orally. Similarly, compounds containing an ornithine or an ornithine analogue (**79**) were also potent inhibitors of platelet aggregation.[211] In an *ex vivo* platelet aggregation model in rhesus monkeys, (**79**) was orally active at 1-3 mg kg^{-1} (45-90%



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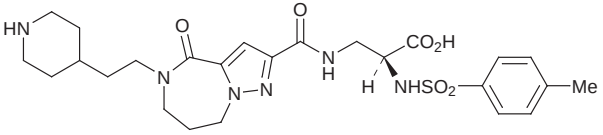
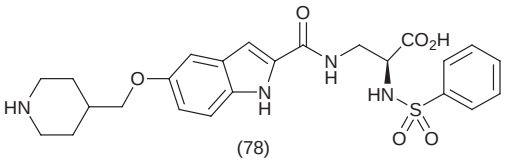


(77) L-738,167



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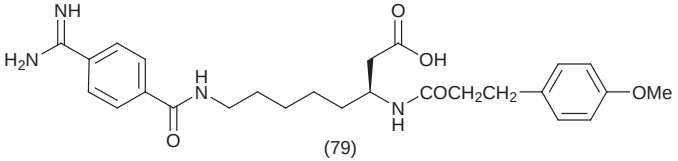

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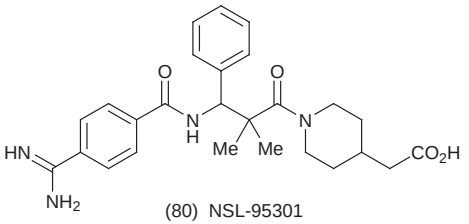
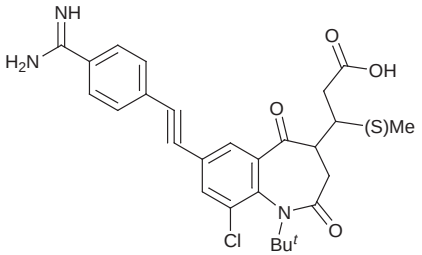
inhibition after 5 hours). However, a similar compound [MeO-Ph-CH_2-CH_2-CO-group replaced by $(CH_3)_2CH$-CH_2CO-] had a better therapeutic profile because it had a small effect on bleeding time (2-3 times control) at a dose of 3 mg kg^{-1}.

Other IIb/IIIa non-peptide antagonists include compounds like (**80-86**).[212–221] XR299 (**82**) was a potent antiplatelet agent when dosed intravenously in a canine model. The administration of an oral dose (1 mg kg^{-1}) of the ethyl ester of (**82**) (XR300) in dogs gave 90-100% inhibition of *ex vivo* ADP (100 μM)-mediated platelet aggregation which declined to approximately 70% over 6 hours. XR299 did not have significant effects against other integrins. The IC_{50} values against Huvec-vitronectin, Huvec-fibronectin, Mac-1, VLA-4/VCAM-1, Mac-1/ICAM were 200, 180, >100, >100 and 100 μM, respectively.[215] In the 3,4-dihydroisoquinolone series of compounds (**83**), alkyl substituents [R^1 = -CH_2CH_3, -$(CH_2)_2CH_3$, -$(CH_2)_3CH_3$, -$(CH_2)_4CH_3$, -$(CH_2)_5CH_3$, -$(CH_2)_3OCH_2CH_3$,] afforded a 10-fold increase in intrinsic activity (IC_{50} values 24-61 nM) while aryl substituents (R^1 = Ph, *p*-$C_6H_4COOCH_3$ and *p*-$C_6H_4OCH_3$) yielded a 40-fold improvement (IC_{50} values 6-8 nM) compared to the unsubstituted compound. All the compounds were less potent in the plasma-based systems probably due to the increased interaction with plasma proteins.[217] The indazole analogue (**84**) (receptor binding IC_{50} 0.02 μM, ADP-induced platelet aggregation IC_{50} 0.14 μM) did not compete with vitronectin binding to $\alpha_v\beta_3$ receptors up to a concentration

of 100 μM. However, (**84**) or similar analogues did not display good oral bioavailability.[218] Oral activity was observed in the dipeptide series of compounds. For example, 4-(4-amidinophenoxy)-butanoyl-Asp-Val (FK633, reported previously) was not active orally in rats and dogs but additional C-terminal modifications led to compounds like (**85**) (IC_{50} for ADP-induced human platelet aggregation 30 nM) which displayed oral activity in rats (95% inhibition of platelet aggregation up to 2 hours at a dose of 3.2 mg kg^{-1}) and dogs (45% inhibition of platelet aggregation up to 3 hours at a dose of 3.2 mg kg^{-1} and 60% inhibition up to 6 hours at a dose of 10 mg kg^{-1}). Many other C-terminal modifications [e.g. $-NH-CH_2CH(CH_3)_2$, $-NHCH_2CH_2Ph(4-OCH_3)$ and $-NHCH_2CH_2Ph(3,4\text{-di-}OCH_3)$ groups] resulted in less potent compounds.[220]



(80) NSL-95301



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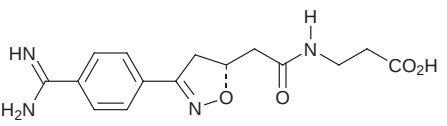
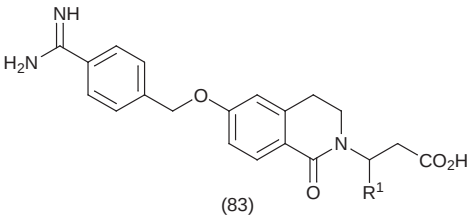


(82) (XR299)



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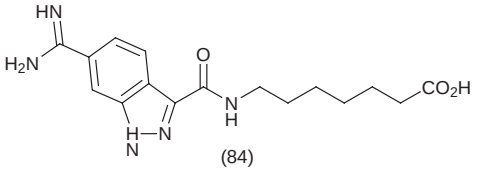

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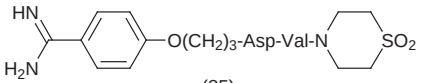

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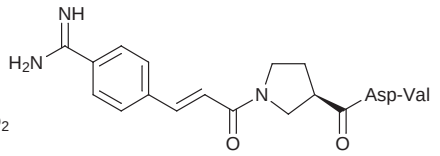

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4.12 LHRH Analogues – Some aspects of LHRH work have been reviewed.[222] The primary structures of two forms of GnRH from tunicate (*Chelyosoma productum*) have been determined. Both tunicate GnRH-1 [Pyr-His-Trp-Ser-Asp-Tyr-Phe-Lys-Pro-Gly-NH_2] and GnRH-2 [Pyr-His-Trp-Ser-Leu-Cys-His-Ala-Pro-Gly-NH_2, a Cys-Cys linked dimer] resulted in a doubling of the gonadal content of oestradiol after 24 hours. A synthetic analogue of tunicate GnRH-1 containing an amide bond between the side chains of Asp^5 and Lys^8 was found to double and triple oestradiol content after 6 and 24 hours, respectively.[223]

No endogenous hypothalamic follicle-stimulating hormone-releasing peptide has yet been isolated. However, lamprey LHRH-III [Pyr-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH_2] has been reported to have a dose-related FSH- but not LH-releasing action on incubated hemipituitaries of male rats. Lamprey LHRH-I [Pyr-His-Tyr-Ser-Leu-Glu-Trp-Lys-Pro-Gly-NH_2], on the other hand, had little activity to release either LH or FSH. The lamprey LHRH-III also caused a significant increase in plasma FSH when injected to rats between 10-100 pmol dose levels.[224] Analogues of lamprey GnRH-III were evaluated as antitumour agents. All of the analogues, [Lys^5]-, [N^{ε}-Fmoc-Lys^5]-, [Lys^5, c(Asp^6, Lys^8)]-, [c(Asp^6, Lys^8)]-, [Lys^4, N^{ε}-Fmoc-Lys^8]-, [Lys^4]-, [N^{ε}-Ac-Lys^4]-, [Glu^6]-, [Phe^7]-, [Trp(N-in-For)^{3,7}, $\Delta^{3,4}$-Pro^9]-, [D-Ala^{10}]-, Ac-[D-Trp^1, D-Ala^{10}]-, [Asu^6]-, [$\Delta^{3,4}$-Pro^9]-, [Trp(N-in-For)^{3,7}, (^{3}H)Pro^9]-, [(^{3}H)Pro^9]-GnRH-III and GnRH-III(1-9)-ethylamide, were less potent than the parent peptide in cell proliferation assays using human tumour cell lines (MCF-7, MDA-MB-231, PC3 and LNCaP). Although on individual cell lines (e.g. MCF-7) some of the analogues, e.g. [Lys^5, c(Asp^6, Lys^8)]- and [c(Asp^6, Lys^8)]-GnRH-III, were similar in potency to the parent peptide.[225]

Various other analogues of LHRH have been reported.[226,227] Betidamino acids in which each N′-acyl/alkyl group may mimic naturally occurring amino acid side chains or introduce novel functionalities were incorporated in acyline (Ac-D-2-Nal-D-Phe(p-Cl)-D-3-Pal-Ser-4-Aph(Ac)-D-4Aph(Ac)-Leu-Ilys-Pro-D-Ala-NH_2). Many of the analogues, e.g. [Agl(2-naphthoyl)^1]acyline, [Agl(nicotinoyl)^3]acyline, [Agl(isonicotinoyl)^3]acyline, [Agl(formyl)^4]acyline, [Agl(4-hydroxy-

benzoyl)^5]acyline, [Agl(Ac)^7]acyline, [Agl(isobytyryl)^7]acyline, [Agl(isopropyl-β-Ala)^8]acyline, [Agl(guanidinoacetyl)^8]acyline, [Agl^{10}]acyline and [Agl(formyl)^{10}]-acyline, were similar in potency to acyline in the ovulation inhibition assay. [Agl(4-chlorobenzoyl)^2]acyline, [Agl(Pca)^2]acyline (Pca = 2-pyrazinecarboxylic acid), [Agl(Apc)^2]acyline (Apc = 3-amino-4-pyrazolecarboxylic acid), [Agl(hydroxyacetic acid)^4]acyline, [Agl(4-(acetylamino)benzoyl)^5]acyline and [Agl(4-(acetylamino)benzoyl)^6]acyline were less potent than acyline in the ovulation inhibition assay. Betidamino acid containing analogues showed increased hydrophilicity but a shorter duration of action in the castrated male rats.[226]

4.13 α-MSH Analogues – Melanocortin peptides [ACTH, α-, β-, and γ-melanocyte stimulating hormone (MSH), and fragments thereof] derived from propiomelanocortin have a diverse array of biological activities. The recent cloning of a family of five distinct melanocortin receptors through which these peptides act has provided the tools for understanding the functions of these peptides. Early work on melanocortin peptides focused on their roles in pigmentation, adrenocortical function, the immune, central and peripheral nervous systems. However, the more recent information has opened up several lines of evidence for important roles in the development of obesity, insulin resistance and type II diabetes.[228–232] Some of the work has been reviewed.[228,233] Using deletion constructs, the C-terminal Val^{83}-Cys^{131} region of the agouti protein was found to be important for the activity. The data indicated that Val^{83}, Arg^{85}, Pro^{86} and Pro^{89} were important for inhibition of binding to melanocortin receptors 3, 4 and 5 while Val^{83} was also important for inhibition of MC1R.[234]

Most of the agonist and antagonist analogues of melanocyte stimulating hormone have been discovered based on the α-MSH amino acid sequence [Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2]. α-MSH was most potent at the hMC_1R (IC_{50} 5.9 nM), 6-8-fold less potent at the hMC_3R (IC_{50} 50.4 nM) and hMC_4R (IC_{50} 38.7 nM) and about 100-fold less potent at the hMC_5R (IC_{50} 557 nM).[235] The linear peptide [Nle^4, D-Phe^7]-α-MSH was a more potent agonist than α-MSH and nearly equipotent at all the receptor subtypes (IC_{50} 0.51-1.17 nM). C-Terminally modified analogues of α-MSH and γ-MSH (Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-NH_2) were tested at the MC_1 and MC_3 receptors for their binding and cAMP stimulation activities.[236] The D-Phe^7 analogues, [Nle^4, D-Phe^7, Phe^{12}]α-MSH, [Nle^4, D-Phe^7, Ser^{12}]α-MSH and [Nle^4, D-Phe^7, Leu^{12}]α-MSH, were more potent than α-MSH in both the test systems. The other α-MSH analogues, [Phe^{12}]α-MSH, [Asp^{10}]α-MSH, [Asp^{10}, Phe^{12}]α-MSH, [Ala^{11}, Ala^{12}]α-MSH, [Ala^{11}, Ala^{13}]α-MSH, [Ala^{12}, Ala^{13}]α-MSH and [Ala^{11}, Ala^{12}, Ala^{13}]α-MSH, were much less potent at the MC_1 receptor preparations. However, in the MC_3 receptor cAMP stimulation test, the multiple Ala analogues, [Ala^{11}, Ala^{12}]α-MSH, [Ala^{11}, Ala^{13}]α-MSH, [Ala^{12}, Ala^{13}]α-MSH and [Ala^{11}, Ala^{12}, Ala^{13}]α-MSH, were similar or somewhat more potent than α-MSH. All the γ-MSH analogues, [Nle^3, Pro^{11}]γ-MSH, [Nle^3, Ser^{11}]γ-MSH, [Nle^3, Leu^{11}]γ-MSH and [Nle^3]γ-MSH, were much less potent.[236]

The side chain to side chain linked cyclic peptides, Ac-Nle-c[Asp-His-D-Phe-Arg-Trp-Lys]-NH_2, and Ac-Nle-c[Asp-His-D-Phe-Arg-Trp-Ala-Lys]-NH_2, were

more potent at the hMC_1R and hMC_4R (IC_{50} values 0.25-0.89 nM) and 10-50-fold less potent at the hMC_3R and hMC_5R (IC_{50} values 8.5-43.6 nM). The L-Phe^7 cyclic peptide Ac-Nle-c[Asp-His-Phe-Arg-Trp-Ala-Lys]-NH_2 was less potent than the corresponding D-Phe^7 analogue in all the receptor preparations (IC_{50} 22.4-2400 nM) but the selectivity pattern of the two analogues was similar.[235] Both the peptides were agonists in a cAMP release assay. Ac-Nle-c[Asp-His-D-Phe-Arg-D-Trp-Ala-Lys]-NH_2 was the most selective hMC_1R agonist (IC_{50} value 0.91 nM) and 70-150-fold less potent at the hMC_2R, hMC_4R and hMC_5R preparations (IC_{50} values 71.5-141 nM).[235]

Additional modifications at the D-Phe^7 position in one of the above-mentioned agonist analogues, Ac-Nle^4-c[Asp-His-D-Phe^7-Arg-Trp-Lys^{10}]-α-MSH(4-10)-NH_2 (lactam ring between Asp^5 and Lys^{10}), generated agonists and antagonists.[237,238] [D-Phe(p-Cl)^7] and [D-Phe(p-F)^7] analogues were agonists but [D-Phe(p-I)^7] and [D-Nal(2)^7] analogues were potent antagonists (pA_2 10.3). Ac-Nle^4-c[Asp-His-D-Nal(2)^7-Arg-Trp-Lys^{10}]-α-MSH(4-10)-NH_2 was a potent antagonist at the MC_4R (pA_2 8.3) with minimal agonist activity, a less potent antagonist of the MC_3R, and a full agonist of the MC_1 and MC_2 receptors.[237] Ac-Nle^4-c[Asp-His-D-Phe(p-I)^7-Arg-Trp-Lys^{10}]-α-MSH(4-10)-NH_2 (lactam ring between Asp^5 and Lys^{10}) was found to be a potent agonist at the cloned human MC_1R (EC_{50} 0.055 nM) and mouse MC_1R (EC_{50} 0.19 nM) but had potent antagonist activities at the human MC_4R (pA_2 9.7) and human MC_3R (pA_2 8.3) with significant partial agonist activities (EC_{50} values 0.57 and 0.68 nM, respectively). In the disulfide series of cyclic compounds, [Cys^4, D-Phe^7, Cys^{10}]MSH and [Cys^4, D-Phe^7, Cys^{10}]MSH(4-13) were similar to [D-Phe^7]α-MSH at MC_1, MC_3 and MC_4 and much less potent at the MC_5 receptor subtype.[237,238]

In addition to the larger peptides (7-13 amino acid) mentioned above, a number of smaller tri- and tetrapeptide derivatives have also been reported as agonists of α-MSH.[239] Four peptides, based on [Nle^4, D-Phe^7]-α-MSH, were discovered to only bind to the hMC_1 and hMC_4 receptor subtypes. The tetrapeptide Ac-His-D-Phe-Arg-Trp-NH_2 possessed 0.61 μM binding affinity at the hMC_1R, 1.15 μM binding affinity at the hMC_4R (>10 μM at hMC_3R and hMC_5R subtypes), and agonist activity (cAMP release) at both receptor subtypes. The tripeptides Ac-D-Phe-Arg-Trp-NH_2 and Ac-D-Phe-Arg-D-Trp-NH_2 possessed 2.0 and 9.1 μM binding affinities, respectively, only at the hMC_4R, and both compounds effected agonist activity. The tetrapeptide Ac-His-Phe-Arg-D-Trp-NH_2 possessed 6.3 μM affinity and full agonist activity at the hMC_1R, while only binding 7% at the hMC_3R, 36% at the hMC_4R, and 11% at the hMC_5R at a maximal concentration of 10 μM. A number of other tri- and tetrapeptide derivatives, e.g. Ac-His-D-Phe-D-Arg-D-Trp-NH_2, Ac-D-Phe-D-Arg-Trp-NH_2, Ac-Gly-D-Phe-Arg-D-Trp-NH_2, Ac-D-Phe-D-Arg-D-Trp-NH_2, Ac-D-Phe-D-Arg-Trp-NH_2, Ac-Phe-Arg-D-Trp-NH_2 and Ac-Phe-D-Arg-D-Trp-NH_2, were inactive up to a concentration of 10 μM in both the binding and cAMP accumulation assays.[239] A number of other smaller linear and cyclic peptides like Met-Asn-His-D-Phe-Arg-Trp-Gly, c(Met-Asn-His-D-Phe-Arg-Trp-Gly), Asn-His-D-Phe-Arg-Trp-Gly, c(Asn-His-D-Phe-Arg-Trp-Gly), His-D-Phe-Arg-Trp-

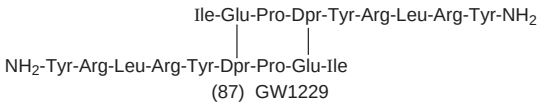
Gly, c(His-D-Phe-Arg-Trp-Gly) and His-D-Phe-Arg-Trp were less potent than [Nle^4, D-Phe^7]-α-MSH at all the receptor subtypes. The cyclic peptides were less potent than the corresponding linear peptides on all the receptor subtypes.[240]

Some agonist and antagonist analogues have also been obtained by screening phage libraries and by chemical modifications of ACTH.[241–243] A 13-amino acid peptide obtained from the phage library, Ser-Ser-Ile-Ile-Ser-His-Phe-Arg-Trp-Gly-Leu-Cys-Asp-NH_2 (MS-04), bound to the MC_1R receptor with an affinity of 7 nM. However, the peptide exhibited very weak agonistic activity when compared with α-MSH and [Nle^4, D-Phe^7]α-MSH in a cAMP accumulation assay on B16F1 cells. The IC_{50}s for the peptide in other receptor binding assays were 21 μM, >50 μM and >50 μM in MC_3R, MC_4R and MC_5R, respectively.[241] Other peptides identified from the phage library e.g Ser-Ser-Leu-Glu-Cys-Ser-Phe-Arg-Trp-Gly-Pro-Glu-His-NH_2 (MS-01) (MC_1R K_i 3.7 μM), Ser-Val-Thr-Val-Pro-Phe-Arg-Trp-Tyr-Ser-Cys-Ser-NH_2 (MS-02) (MC_1R K_i 43 μM) and Ser-Leu-Asp-Phe-Asn-Ser-Phe-Arg-Trp-Cys-Ser-Ala-Leu-NH_2 (MS-03) (MC_1R K_i 15.9 μM) were weakly binding ligands [α-MSH (MC_1R K_i 0.21 nM)].[241]

Antagonists obtained by the modifications of ACTH are: [Phe(p-I)^7]-ACTH(4-10) [MC_3R pA_2 7.4, MC_4R pA_2 8.4, MC_5R pA_2 7.9], [Ala^6]-ACTH(4-10) [MC_3R pA_2 6.5, MC_4R pA_2 <6, MC_5R pA_2 8.7], [Pro^{8,10}, Gly^9]-ACTH(4-10) [MC_4R pA_2 8.6, MC_5R pA_2 6.5] and [D-Arg^8]-ACTH(4-10) [MC_4R pA_2 8.2, MC_5R pA_2 8.1].[242] [D-Ala^4, Gln^5, Tyr^6]ACTH(4-10) did not displace [^{125}I][Nle^4, D-Phe^7]α-MSH bound to the MC_1, MC_3, MC_4 and MC_5 receptors.[243]

Ac-Nle^4-c[Asp-His-D-Phe-Arg-Trp-Lys^{10}]-NH_2 [agonist on the rat MC_3R (EC_{50} 0.27 nM) and mouse MC_4R (EC_{50} 0.057 nM)] was found to produce a potent inhibition of feeding within one hour of administration.[244] At the highest dose (3 nmol) food intake was significantly inhibited for up to 4 hours. Inhibition of feeding with 3 nmol of the cyclic peptide was blocked by co-administration of 6 nmol Ac-Nle^4-c[Asp-His-D-Nal(2)-Arg-Trp-Lys^{10}]-NH_2 [SHU-9119, antagonist on the rat MC_3R (IC_{50} 4.5 nM)] and mouse MC_4R (IC_{50} 0.36 nM). The administration of MT-II also inhibited food intake in three other models of hyperphagia, involving C57BL/6J-*Lep^{ob}*, C57BL/6J-A^y, and neuropeptide Y-injected C57BL/6J mice. In the C57BL/6J-*Lep^{ob}* model, the peptide was effective when administered intraperitoneally at a dose of 100 nM. MT-II also inhibited normal nocturnal food intake.[244] Agonist activities of β-MePhe, β-MeTrp and 1,2,3,4-tetrahydro-β-carboline (Tca) analogues of Ac-Nle^4-c[Asp-His-Xaa^7-Arg-Yyy^9-Lys]-NH_2 are reported in the frog and lizard skin assays.[245]

4.14 Neuropeptide Y (NPY) Analogues – Work on neuropeptide Y including receptor subtypes, agonists and antagonists and NMR studies on some analogues has been reviewed.[246,247] The role of various receptor subtypes in NPY-induced feeding, renal effects of NPY, vasoconstrictor effects and adrenocorticotrophic hormone release has been discussed.[248–251] Using several selective ligands, it has been suggested that NPY receptor involved in ACTH release may be distinct from the feeding receptor but the profile of the NPY_{ACTH} receptor appears to be most similar to that of the NPY_5 receptor.[250] In a hamster cheek pouch microcirculation model, (**87**) (GW1229) did not affect basal vascular conductance



(87) GW1229

but inhibited the reduction in arteriolar diameter and vascular conductance induced by NPY and [Leu^{31}, Pro^{34}]neuropeptide Y (both 100 nM), and that of neuropeptide Y(13-36) (300 nM) but had no effect on the vasoconstriction induced by noradrenaline.[251]

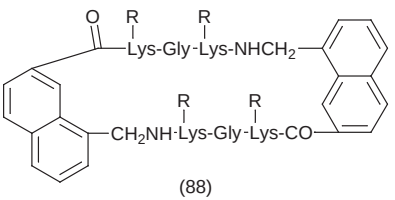
Several centrally truncated linear and disulfide bridge containing analogues in which amino acid sequences of various lengths were replaced by a spacer group (Gly-Pro-Gly) were tested for increase in blood pressure and attenuation of cardiac vagal action. Most of the analogues, e.g. Tyr-Pro-Cys-Lys-Pro-Asp-Asn-Gly-Pro-Gly-Leu-Arg-His-Tyr-Ile-Asn-Leu-Cys-Thr-Arg-Gln-Arg-Tyr, Tyr-Pro-Ser-Lys-Pro-Asp-Cys-Gly-Pro-Gly-Leu-Arg-His-Tyr-Ile-Asn-Leu-Cys-Thr-Arg-Gln-Arg-Tyr, Tyr-Pro-Cys-Lys-Pro-Asp-Asn-Pro-Gly-Gly-Pro-Gly-Leu-Arg-His-Tyr-Ile-Asn-Leu-Cys-Thr-Arg-Gln-Arg-Tyr, Tyr-Pro-Ser-Lys-Pro-Asp-Cys-Pro-Gly-Gly-Pro-Gly-Leu-Arg-His-Tyr-Ile-Asn-Leu-Cys-Thr-Arg-Gln-Arg-Tyr, Tyr-Pro-Cys-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Gly-Pro-Gly-Leu-Arg-His-Tyr-Ile-Asn-Leu-Cys-Thr-Arg-Gln-Arg-Tyr and Tyr-Pro-Ser-Lys-Pro-Asp-Cys-Pro-Gly-Glu-Asp-Gly-Pro-Gly-Leu-Arg-His-Tyr-Ile-Asn-Leu-Cys-Thr-Arg-Gln-Arg-Tyr, showed some activity in both the *in vivo* models.[252]

Conformationally restricted, centrally truncated [Gly^6, des-amino acids 7-24] analogues of NPY, were synthesised by forming lactam bridges (amide bonds between γ-carboxyl of Glu and ε-amino of Lys residues) between residues i to i+3 or i to i+4.[253] The parent peptide, des-AA$^{7-24}$c(25/29)[Gly^6, Glu^{25}, Lys^{29}]-NPY was only 8-fold less potent than NPY at the Y_2 receptors (IC_{50} 2.6 nM) and about 90-fold less potent at the Y_1 receptors (IC_{50} 70 nM). Only two of the cyclic peptides, des-AA$^{7-24}$c(26/29)[Gly^6, Glu^{26}, Lys^{29}]- (K_i Y_1 85 nM and Y_2 104 nM) and des-AA$^{7-24}$c(26/29)[Gly^6, Glu^{26}, Lys^{29}, Pro^{34}]-NPY (K_i Y_1 44 nM and Y_2 14400 nM), were more potent at the NPY Y_1 receptors. Most of the other analogues, des-AA$^{7-24}$c(25/29)[Gly^6, Glu^{25}, Lys^{29}]-, des-AA$^{7-24}$c(26/30)[Gly^6, Glu^{26}, Lys^{30}]-, des-AA$^{7-24}$c(27/31)[Gly^6, Glu^{27}, Lys^{31}]-, des-AA$^{7-24}$c(28/32)[Gly^6, Glu^{28}, Lys^{32}]-, des-AA$^{7-24}$c(25/28)[Gly^6, Glu^{25}, Lys^{28}]-, des-AA$^{7-24}$c(27/30)[Gly^6, Glu^{27}, Lys^{30}]- and des-AA$^{7-24}$c(28/31)[Gly^6, Glu^{28}, Lys^{31}]-NPY, were more potent at the Y_2 receptors. The most selective Y_2 peptide was AA$^{7-24}$c(26/30)[Gly^6, Glu^{26}, Lys^{30}]-NPY (K_i Y_1 1010 nM and Y_2 0.7 nM). Two cyclic peptides, des-AA$^{7-24}$c(26/29)[Gly^6, Glu^{26}, D-Tyr^{27}, Lys^{29}]- and des-AA$^{7-24}$c(26/29)[Gly^6, Glu^{26}, D-Ile^{28}, Lys^{29}]-NPY, were nearly equipotent at both the receptor subtypes (IC_{50} values 700-2320 nM).[253]

Deletion analogues of the Y_1-selective ligand des-AA$^{7-24}$c(26/29)[Gly^6, Glu^{26}, Lys^{29}, Pro^{34}]-NPY (K_i Y_1 44 nM and Y_2 14400 nM) were studied as Y_1 receptor ligands. Many of the analogues, des-Tyr^1, des-Pro^2, des-Ser^3, des-Lys^4, des-Pro^5, des-Gly^6, des-AA$^{2-6}$, des-AA$^{2-5}$, des-AA$^{2-4}$, des-AA^{2,5} and des-AA^{2,3,5} were similar in Y_1 binding affinity to the parent peptide (K_i values 13-100 nM). Four other deletion analogues, des-Arg^{25}, des-AA$^{1-6,25}$, N-acetyl, des-AA$^{1-6,25}$, des-

$AA^{2-6,25}$, were much less potent (Ki values 700-3720 nM). Many of the Pro^{34} analogues, des-AA^{7-24}c(26/29)[Gly^6, Glu^{26}, Lys^{29}, Pro^{34}]-, des-$AA^{1,7-24}$c(26/29)[Gly^6, Glu^{26}, Lys^{29}, Pro^{34}]-, des-$AA^{2,5,7-24}$c(26/29)[Gly^6, Glu^{26}, Lys^{29}, Pro^{34}]- and des-$AA^{2,3,5,7-24}$c(26/29)[Gly^6, Glu^{26}, Lys^{29}, Pro^{34}]-NPY behaved as agonists (inhibition of cAMP accumulation) in norepinephrine-stimulated SK-N-MC cells (EC_{50} values 12-55 nM). One of the more potent analogues des-AA^{7-24}c(26/29)[Gly^6, Glu^{26}, Lys^{29}, Pro^{34}]-NPY failed to produce significant activity *in vivo* in both food intake and growth hormone secretion assays.[253]

Based on the concept of template-assembled synthetic proteins, a cyclic template consisting of two β-turn mimetics was used to attach four C-terminal tetrapeptide α-Arg-Gly-Arg-Tyr-NH_2) units at the side chain amino groups of the lysine residues by oxime linkages.[254] The peptide (**88**) bound to the Y_2 receptor (IC_{50} 67.2 nM) but showed no agonist activity (inhibition of forskolin-stimulated cAMP in LN319 cells at 10 μM) (IC_{50} for NPY = 2.5 nM). At presynaptic Y_2 receptors modulating adrenaline release, the peptide inhibited the response induced by NPY(13-36), a Y_2-selective fragment, with a pA_2 value of 8.48. Non-peptide antagonist of NPY have been obtained based on a screening lead (K_i human Y_1 receptor 2.1 μM).[255] The most potent of the trisubstituted indoles, (**89**, human Y_1 receptor K_i 0.75-1.4 nM) displayed a much poorer affinity for Y_2, Y_4 and Y_5 receptors (K_i values >10 μM). When coadministered with NPY (230 pM, icv) into lateral ventricle, (**89**) blocked the increase in food consumption elicited by NPY (ED_{50} 17 nM). Serum levels of (**89**) (LY357897) were poor after oral and subcutaneous administration.



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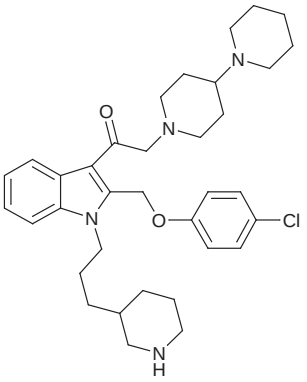

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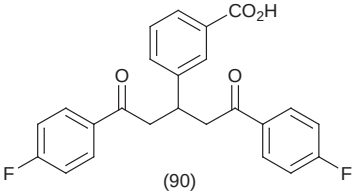
4.15 Neurotensin Analogues – SAR studies of peptidic and non-peptidic neurotensin [Pyr-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu] analogues are reviewed.[256] Biological activities of some of the neurotensin receptor ligands described previously have been reported.[257–259] The proposed neurotensin(8-13) binding site to the transmembrane receptor is primarily composed of eight residues (Phe^{326}, Ile^{329}, Trp^{334}, Phe^{337}, Tyr^{339}, Phe^{341}, Tyr^{342}, and Tyr^{344} in the

human receptor; Phe^{331}, Ile^{334}, Trp^{339}, Phe^{342}, Phe^{344}, Phe^{346}, Tyr^{347}, and Tyr^{349} in the rat receptor) located in the third extracellular loop.[260] In the bound conformation of neurotensin(8-13), the backbone of Arg^{9}-Pro^{10}-Tyr^{11}-Ile^{12} forms the proline type I turn, and the hydroxy group of Tyr^{11} interacts with the two guanidinium groups of Arg^{8} and Arg^{9}.

Non-peptide antagonists of neurotensin have been reported.[261–263] Further SAR studies (substituents on each aromatic ring and the linking 1,5-pentanedione chain) on a random screening lead which displayed poor affinity (IC_{50} 450 nM) at the neurotensin receptor led to compounds like (**90**) (IC_{50} 302 nM) which inhibited calcium mobilisation induced by neurotensin in HT29 cells (IC_{50} 2.2 μM). None of the compounds reversed the hyperthermia induced by a small peptide neurotensin agonist.[263] Another non-peptide antagonist (**91**) (SR 142948A) inhibited [^{125}I-Tyr^{3}]neurotensin binding to the human high affinity neurotensin receptor cloned from the HT29 cell line and stably expressed in CHO cells. It also antagonised effects of neurotensin in several *in vivo* models including turning behaviour, hypothermia and analgesia.[264]



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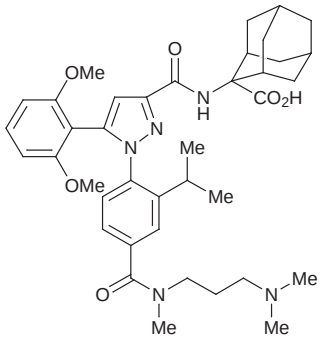
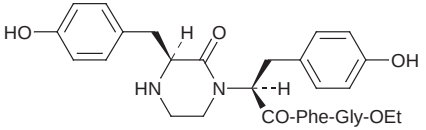


(91) SR 142948A

4.16 Opioid (Enkephalin, β-Casomorphin, Morphiceptin, Deltorphin and Dynorphin) Peptides – Reviews on the δ-selective opioid peptides of amphibian skin, nociceptin/orphanin FQ and the opioid receptor like ORL1 receptor have appeared.[265,266] In addition to the established opiate peptides, new opiate-like peptides including the two decapeptides [Glu-Gln-Trp-Gly-Arg-Glu-Glu-Gly-Glu-Glu and Ala-Leu-Gly-Glu-Arg-Lys-Tyr-His-Phe-Gln-NH_2] were isolated from rainbow trout pituitary and canine brain.[267,268] Stability and permeability studies of enkephalin analogues across the blood-brain barrier and Caco-2 cells have been carried out.[269–271] The stability of [Met^{5}]-enkephalin-Arg^{6}-Gly^{7}-Leu^{8} to guinea pig ileum or striatum membrane fraction indicated that dipeptidyl carboxypeptidase and aminopeptidase activities were responsible for the degradation of the peptide to Tyr-Gly-Gly-Phe-Met-Arg, Tyr-Gly-Gly-Phe and Tyr. In the presence of three peptidase inhibitors (captopril, amastatin and phosphoramidon), about 95% of the octapeptide remained intact.[269] Administration of [D-Pen^{2,5}]enkephalin [10, 60 or 100 mg kg^{-1} (i.v. bolus dose)] to mice indicated that

a small amount entered the brain and most of the peptide (>80%) was excreted by the biliary route.[270]

A number of enkephalin analogues have been reported.[272–275] Peptidomimetic derivatives like (**92**) were much less potent than Leu-enkephalin and were 2-60 times more potent at the δ receptor (mouse vas deferens IC_{50} values 4-165 μM) than at the μ receptor (guinea pig ileum IC_{50} values 31-1198 μM). Analogues of enkephalin containing a mono or a disaccharide at the C-terminus retained significant binding affinity at the μ and δ receptors. The most potent and selective glycopeptide (**93**) was 2.7 times more potent than Leu-enkephalin at the μ receptor and 35.4 times more potent than Leu-enkephalin at the δ receptor. More lipophilic adamantane derivatives of [D-Ala^2]Leu-enkephalin, e.g. [D-Ala^2]Leu-enkephalin-OAda, [D-Ala^2]Leu-enkephalin-O-CH_2-Ada, [D-Ala^2]Leu-enkephalin-O-CH_2-CH_2-Ada and [D-Ala^2]Leu-enkephalin-NH-Ada, were moderately potent in the binding and analgesic assays. The N-terminally modified analogues, Ada-CO-[D-Ala^2]Leu-enkephalin and Ada-OCO-[D-Ala^2]Leu-enkephalin were inactive. [D-Ala^2]Leu-enkephalin-OAda showed significant dose-related analgesic activity at 5, 10, 20 and 50 mg kg^{-1} dose levels.[274]



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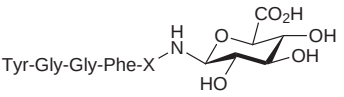


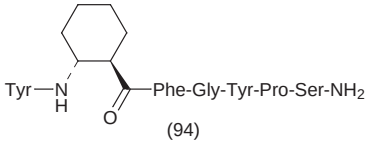
(93) X = Leu

Based on some of the earlier publications, 2′,6′-dimethyl-Tyr derivatives were synthesised as μ and δ receptor ligands.[276–278] The D-Phe containing di- and pentapeptide derivatives, 2′,6′-dimethyl-Tyr-D-Phe-NH_2, and 2′,6′-dimethyl-Tyr-D-Phe-Gly-Val-Val-NH_2 (K_i 0.53 and 2.32 nM at μ and δ receptors, respectively) acted as antagonists in the guinea pig ileum preparation (pA_2 7.2-7.3). The corresponding D-Tyr-D-Phe analogues, 2′,6′-dimethyl-D-Tyr-D-Phe-NH_2 and 2′,6′-dimethyl-D-Tyr-D-Phe-Gly-Val-Val-NH_2 were weaker antagonists in the guinea pig ileum preparation (pA_2 values 4.9-5.2).[276] The di- and tripeptide 2′,6′-dimethyl-Tyr derivatives containing a Tic residue, Dmt-Tic [K_i^{δ} 0.022 nM; K_i^{μ}/K_t^{δ} 150,000; δ antagonism pA_2 8.2; K_e 5.7 nM] and Dmt-Tic-Ala [K_i^{δ} 0.285 nM; K_i^{μ}/K_i^{δ} 20400; δ antagonism pA_2 8.4; K_e 4.0 nM] were δ selective. In each series, replacement of Dmt residue by D-Dmt residue gave less potent analogues. N-Methylation of the Dmt residue gave mixed results. MeTyr-Tic, D-MeDmt-Tic, MeDmt-Tic-Ala and D-MeDmt-Tic-Ala were more potent than the corresponding Dmt analogues but MeDmt-Tic was about 10-fold less potent than Dmt-Tic.[277] Conformationally constrained β-methyl-Dmt-Tic analogues were more selective. The most potent and selective analogue (2S,SR)-β-methyl-Dmt-Tic showed >3500-fold selectivity for the δ-receptors (IC_{50}s 9.3 nM at the δ receptor and 35000 nM at the μ receptor). The (2R,3S)- and (2R,3R)-β-methyl-Dmt-Tic analogues were inactive at both δ and μ receptors (IC_{50} values >10,000 and >80,000 nM, respectively).[278]

Enzymic stability (brain homogenates and blood) and blood-brain permeability of several deltorphin analogues was studied.[279,280] In comparison to [D-Ala^2]deltorphin I, [Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2], and [D-Ala^2]deltorphin II, [Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2], which permeated blood-brain barrier and were stable to the enzymes present in mice brain homogenate and blood ($t_{1/2}$ 2-10 hours), analogues containing a Ser or Gln residue in position 4 and another D-amino acid in position 5, [D-Ala^2, Ser^4, D-Val^5]-, [D-Ala^2, Ser^4, D-Ala^5]-, [D-Ala^2, Gln^4, D-Val^5]- and [D-Ala^2, Gln^4, D-Ala^5]deltorphin, were much more stable than the parent peptide ($t_{1/2}$ >15 hours). The N-terminally extended analogues, Arg-Arg-[D-Ala^2]-, Arg-[D-Ala^2]-, Lys-Lys-[D-Ala^2]-, Lys-[D-Ala^2]-, Ala-Pro-[D-Ala^2]-, Pro-Pro-[D-Ala^2]- and Abu-Abu-[D-Ala^2]deltorphin II, were quickly degraded to the parent peptide. In case of the proline analogues, the N-terminal dipeptide was cleaved but in all the other analogues the N-terminal amino acids were cleaved one at a time by an aminopeptidase.[279] Antinociception studies indicated that deltorphins I and II were the peptides with the highest blood-brain barrier penetration rate amongst the opioid peptides.[280] These peptides permeated blood-brain barrier 4-12 times better than typical μ-opioid receptor agonists such as DAMGO and dermorphin, though 160-220 times less effectively than morphine and 2-3 times less than [Lys^7]dermorphin, an opioid peptide with an unusually high blood-brain barrier penetration rate.

A number of SAR studies are reported on deltorphin analogues.[281–284] α-Aminoisobutyric acid replacements in various positions in deltorphin-1 gave compounds like [Aib^2]-, [Aib^3]- and [Aib^2, Aib^3]deltorphin which exhibited high δ-receptor affinity ($K_i\delta$ 0.12-3.6 nM; $K_i\mu$ 4.5-15.2 μM) and selectivity ($K_i\mu/K_i\delta$ 4200-8460). Replacement of the Asp^4 by Aib nearly lost all the selectivity. Tyr-D-Ala-Phe-Aib-Val-Val-Gly-NH_2 was only 2-fold more potent at the δ-receptor ($K_i\delta$ 0.20 nM; $K_i\mu$ 0.43 nM) and Tyr-D-Ala-Phe-Asp-Val-Val-Gly-OH was about 40-fold more potent at the δ-receptor ($K_i\delta$ 0.11 nM; $K_i\mu$ 4.48 nM).[281] Various X^3 Gly^4 dual-substitution analogues like [4-thiazolylalanine^3, Gly^4]-, [3-benzothienylalanine^3, Gly^4]-, [Trp^3, Gly^4]-, [Phe(*p*-Cl)^3, Gly^4]-, [Tyr^3, Gly^4]- and [cyclopentylglycine^3, Gly^4]-deltorphin were less potent than [Gly^4]deltorphin. Some other analogues, e.g. [phenylglycine^3, Gly^4]-, [4-Pal^3, Gly^4]-, [Phe(*m*-CF_3)^3, Gly^4]-, [Tyr(OMe)^3, Gly^4]-, [Val^3, Gly^4]-, [Abu^3, Gly^4]- and [Nva^3, Gly^4]-deltorphin, displayed some improvement in δ-selectivity (2-3-fold), but again the analogues were less potent than [Gly^4]deltorphin.[282] Conformationally restricted analogues of deltorphin containing 2-aminotetralin-2-carboxylic acid (Ate) residue instead of Phe (also containing Ile in place of Val residues), Tyr-D-Ala-(R)-Atc-Asp-Ile-Ile-Gly-NH_2, Tyr-D-Ala-(S)-Atc-Asp-Ile-Ile-Gly-NH_2, Tyr-D-Ala-(R)-Atc-Glu-Ile-Ile-Gly-NH_2 and Tyr-D-Ala-(S)-Atc-Glu-Ile-Ile-Gly-NH_2 were nearly equipotent in the mouse vas deferens assay (IC_{50} 0.038-0.270 nM). In the guinea pig ileum assay, the compounds were less potent (IC_{50} 2162-31270 nM). The most selective analogue Tyr-D-Ala-(R)-Atc-Glu-Ile-Ile-Gly-NH_2 was >200,000-fold more potent on the vas deferens.[283] A number of Thr^4 or Thr^7 glycosylated analogues of deltorphin [Tyr-D-Ala-Phe-Thr(β-D-Glc)-Val-Val-Gly-NH_2, Tyr-D-Ala-Phe-Asp-Val-Thr[β-D-Glc$(OAc)_4$-Gly-NH_2 and Tyr-D-Ala-Phe-Asp-Val-Thr(β-D-Glc)-Gly-NH_2] were less potent than the parent peptide.[284]

Analogues of dermorphin [Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2] have been reported.[285,286] Heptapeptide Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NHC_3H_7(*i*) (IC_{50} values at the mouse vas deferens and guinea pig ileum 27.2 and 0.31 nM, respectively) and the corresponding benzyl amide derivative, Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-$NHCH_2C_6H_5$ (IC_{50} values at the mouse vas deferens and guinea pig ileum 17.4 and 0.1 nM, respectively) were nearly equipotent *in vitro*.[285] However, in the *in vivo* analgesic tests, the *i*-propyl analogue was about three times more potent than dermorphin and the benzyl amide analogue was >100 times less potent. All the other analogues containing the C-terminal *i*-propyl group [Tyr-D-Ala-MePhe-Gly-Tyr-Pro-Ser-NHC_3H_7, Tyr-D-Ala-Phe-Sar-Tyr-Pro-Ser-NHC_3H_7, Tyr-D-Ala-Phe-Glu(Tyr-Pro-Ser-NH_2)-NHC_3H_7, Tyr-D-Ala-Phe-Glu(Tyr-Pro-Ser-NHC_3H_7)-NH_2, Tyr-D-Ala-Phe-Gly-Tyr-Pro-Gly-NHC_3H_7, Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ala-NHC_3H_7, Tyr-D-Ala-MePhe-Gly-Tyr-Pro-Thr-NHC_3H_7, Tyr-D-Ala-Phe-Gly-Tyr-Pro-Lys-NHC_3H_7 and Tyr-D-Ala-MePhe-Gly-Tyr-Pro-Arg-NHC_3H_7] were less potent than dermorphin in the *in vitro* and *in vivo* assays. Conformationally constrained analogues of dermorphin containing a β-amino-cycloalkane or cycloalkene carboxylic acid in place of the D-Ala residue (e.g. **94**) were much less potent (K_i values 1170-3390 nM at the two receptor subtypes) than the parent peptide.[286]



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Human pharmacology, stability, blood-brain barrier permeability and conformational studies of dynorphin A(1-13) and some analogues are reported.[287–291] In comparison to dynorphin A(1-8) (degraded in human and rhesus monkey plasma in <90 minutes, cleavage of N-terminal Tyr and Arg^6-Arg^7 peptide bond), less than 5% of [$MeTyr^1$, $MeArg^7$, D-Leu^8]-dynorphin A(1-8)-NHEt was degraded in 24 hours. The analogue was also stable *in vivo* and no degradation products could be detected in any of the blood samples collected at various time intervals up to 6 hours after the i.v. injection.[290] Levels of [$MeTyr^1$, $MeArg^7$, D-Leu^8]-dynorphin A(1-8)-NHEt (9 to 58 times less than the plasma levels) could be detected in the cerebrospinal blood of rhesus monkeys after intravenous administration between 3-180 minute intervals.[291]

A number of new analogues of dynorphin have been reported.[284,292–294] N-terminal mono and di-substituted (allyl, benzyl, and cyclopropylmethyl) derivatives of [D-Pro^{10}]dynorphin A(l-11) were studied for antagonist *vs* agonist activity at κ-opioid receptors.[292,293] All of the N-monoalkylated derivatives exhibited much higher affinity (K_i < 0.05 nM) and selectivity [K_i κ/μ > 200] for κ-receptors in comparison to the N,N-dialkyl [D-Pro^{10}]dynorphin A(1-11) analogues, although one disubstituted analogue, N,N-diCPM[D-Pro^{10}]dynorphin A(l-11), retained high affinity (K_i 0.19 nM) for κ receptors. The N-allyl and N-CPM analogues were moderately potent agonists in the guinea pig ileum assay, while

the N-benzyl derivative was a weak agonist in this assay. *In vivo* in the phenylquinone abdominal stretching assay, the N-CPM analogue exhibited potent antinociceptive activity (ED_{50} 1.1 μg mouse^{-1}), while N-allyl[D-Pro^{10}]-dynorphin A(l-11) exhibited weak antinociceptive activity (ED_{50} 27 μg mouse^{-1}). For the N,N-dialkyl derivatives the identity of the N-terminal alkyl group affected the efficacy observed in the smooth muscle assays. The N,N-diCPM analogue exhibited negligible agonist activity, and N,N-diallyl[D-Pro^{10}]dynorphin A(1-11) showed weak antagonist activity against dynorphin A(1-13)NH_2 in the GPI. In contrast, the N,N-dibenzyl compound showed appreciable opioid agonist activity in this assay. *In vivo*, the N,N-diallyl analogue exhibited weak antinociceptive activity (ED_{50} 26 μg/mouse).

Several cyclic lactam analogues of dynorphin A(1-13)NH_2 were prepared by incorporating c[D-Asp^{i}, Dap^{i+3}], c[D-Asp^{i}, Dab^{i+3}] and c[D-Asp^{i}, Orn^{i+3}] residues in various parts of the dynorphin A(1-13)NH_2 sequence.[294] The cyclic peptides exhibited marked differences in binding affinities for κ, μ, and δ receptors and in opioid activity in the guinea pig ileum. Cyclic peptides containing an amide bond between the side chains of the amino acid residues in positions 3 and 6, c[D-Asp^3, Dap^6]-, c[D-Asp^3, Dab^6]- and c[D-Asp^3, Orn^6]dynorphin A(1-13)NH_2 showed very weak binding affinity at all opioid receptors (K_i values 200-2800 nM) and less than 5-fold selectivity between various receptor subtypes. Cyclic peptides containing an amide bond between the side chains of the amino acid residues in positions 5 and 8, c[D-Asp^5, Dap^8]-, c[D-Asp^5, Dab^8]- and c[D-Asp^5, Orn^8]dynorphin A(1-13)NH_2, were most potent at the κ-receptors (K_i values 8-14 nM) and least potent at the δ receptors (K_i values 1.9-4.1 μM) (μ-receptor K_i values 62-75 nM). Thus the analogues showed only modest selectivity (<10-fold) between κ and μ receptors and high selectivity between κ/μ and δ receptors. Cyclic peptides containing an amide bond between the side chains of the amino acid residues in positions 6 and 9, c[D-Asp^6, Dap^9]-, c[D-Asp^6, Dab^9]- and c[D-Asp^6, Orn^9]dynorphin A(1-13)NH_2 exhibited very poor selectivity between the κ (K_i values 1.5-2.6 nM) and μ (K_i values 3.2-4.8 nM) receptors and only moderate selectivity between κ/μ and δ receptors (K_i values 28-180 nM). Compared to the corresponding linear peptides, all of the cyclic peptides exhibited decreased μ receptor affinity, while κ receptor affinity was retained or improved.[294]

The heptadecapeptide nociceptin (orphanin FQ) is a novel peptide isolated from brain tissue that has an amino acid sequence [Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asp-Glu] most similar to that of the endogenous opioid peptide dynorphin A. Since its discovery, several biological activities including hyperalgesia, anxiolytic, diuretic and vasorelaxant properties have been associated with this peptide.[295–299] The hyperalgesia induced by nociceptin is dose-dependent and gradually resolves over time, with tail-flick latencies continuing to increase to values significantly above the initial baseline values. The analgesic action of nociceptin is sensitive to opioid antagonists like naloxone. Two fragments of nociceptin, nociceptin(1-11) and nociceptin(1-7), increased the tail-flick latencies, consistent with the analgesic action. Again, the analgesia was antagonised by opioid antagonist diprenorphine. It, therefore,

appears that the hyperalgesic and analgesic effects of nociceptin are mediated by different receptors.[299]

In membranes from recombinant Chinese hamster ovary cells expressing the opioid receptor-like ORL1 receptor (ORL1 receptor), equilibrium binding of [^{3}H]nociceptin is highly specific and of high affinity ($K_d \approx 0.1$ nM).[300] [Tyr^1]nociceptin was nearly as potent as the parent peptide (K_i 0.26 nM) but [des-Phe^1]nociceptin was much less potent (K_i 275 nM). Removal of the C-terminal amino acids also resulted in a substantial loss of affinity. The smallest C-terminal active fragments consisted of nociceptin(6-17) [Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln] (K_i 20 nM, ED_{50} 39 nM) and nociceptin(12-17) [Arg-Lys-Leu-Ala-Asn-Gln] (K_i 49 nM, ED_{50} 790 nM). Other high affinity ligands for the opiate-like receptor ORL1 were identified from a combinatorial library.[301] The most potent peptides were Ac-Arg-Tyr-Tyr-Arg-Trp-Arg-NH_2, Ac-Arg-Tyr-Tyr-Arg-Trp-Lys-NH_2, Ac-Arg-Tyr-Tyr-Arg-Ile-Lys-NH_2, Ac-Arg-Tyr-Tyr-Lys-Trp-Arg-NH_2 and Ac-Arg-Tyr-Tyr-Lys-Trp-Lys-NH_2. In the mouse vas deferens preparation, the nociceptin receptor that inhibits the electrically evoked twitches of the vas deference was demonstrated to be distinct from the δ opioid receptor, since naloxone and Dmt-Tic (a selective δ opioid receptor antagonist) block the δ opioid receptor but have no effect on the nociceptin receptor.[302] SAR studies indicated that the entire sequence of the peptide was not required for the activity, since nociceptin(1-13)-NH_2 was as active as the parent peptide. Replacements of the Arg^{8,12} and Lys^{9,13} by alanine and Phe^1 and Phe^4 by D-Phe residue in the nociceptin(1-13)-NH_2 sequence leads to inactive peptides. N-Acetylation or N-diallylation of nociceptin(1-13)-NH_2 significantly reduced the activity but, unlike enkephalin analogues, the diallyl-nociceptin(1-13)-NH_2 was not an antagonist.

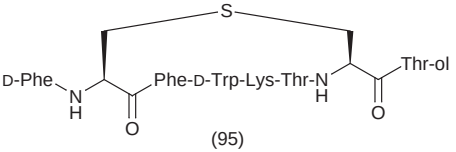
Hybrids of nociceptin and dynorphin A were compared with those of the parent peptides for the binding and biological potencies towards the nociceptin and dynorphin A (κ-opioid) receptors.[303] Replacement of as many as eleven residues in the C-terminus of dynorphin by the corresponding nociceptin sequence had no significant effect on binding and biological activity towards the κ-opioid receptor (ED_{50} values 0.3-1 nM; ED_{50} values $\geqslant 10$ μM at the nociceptin receptor). In marked contrast, replacement of as few as six residues (Arg-Lys-Leu-Ala-Asn-Gln) at the C-terminus of nociceptin by the corresponding dynorphin sequence (Leu-Lys-Trp-Asp-Asn-Gln) impairs both affinity and activity towards the nociceptin receptor. Recombinant peptide approach led to a hybrid Tyr-Gly-Gly-Phe-Leu-Arg-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln which prefers the κ-opioid receptor, another hybrid Tyr-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln which prefers the nociceptin receptor and a third hybrid Tyr-Gly-Gly-Phe-Leu-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln which does not discriminate between the two types of receptor (K_i values 2 nM and 0.8 nM at nociceptin and κ-opioid receptors, respectively).[303]

4.17 Somatostatin Analogues – Five receptor subtypes of somatostatin ($SSTR_1$-$SSTR_5$) have been cloned and characterised.[304] Additional work on the characterisation of various somatostatin receptor subtypes in different tissues and the role

of each receptor subtype in relation to the multiple biological activities of somatostatin has continued.[305–309] Results of various clinical trials using sandostatin (octreotide) have been reported.[310–313]

In addition to the older somewhat less selective compounds, some of the newer compounds have been claimed to be more selective. For example, the lanthionine octapeptide with C-terminal Thr-ol (**95**) showed high affinity for rat $SSTR_5$ compared to somatostatin and sandostatin (IC_{50} values 1.29, 1.04 and 0.86 nM, respectively). However, it displayed about 50 times weaker binding affinity for $mSSTR_{2b}$ than sandostatin (IC_{50} values 13.13 and 0.28 nM, respectively) and was inactive (IC_{50} >1000 nM) at mouse $SSTR_3$ and human $SSTR_4$ receptors. Consistent with its affinity to $SSTR_2$ (believed to be linked to the inhibition of growth hormone release), compounds (**95**) had much lower potency for inhibition of growth hormone secretion than sandostatin (IC_{50} values 48 and 0.52 nM, respectively).[314,315] The affinity of [des-Ala^1, des-Gly^2, des-Asn^5, D-Trp^8, Iamp^9]-somatostatin was studied by competition experiments using the non-selective radioligand ^{125}I-[Leu^{28}, D-Trp^{22}, Tyr^{25}]-somatostatin-28 in areas of the rat brain and pituitary known to express identified receptor subtypes. In the cerebellar nuclei and cerebral cortex, which possess SST_1 receptor subtype, [des-Ala^1, des-Gly^2, des-Asn^5, D-Trp^8, Iamp^9]-somatostatin exhibited a moderate affinity (IC_{50} 10-50 nM). In the hippocampus, immature cerebellum and pituitary which contain $SST_{2\text{-}5}$, the IC_{50} values were >1 μM. The Tyr^3 analogue of [des-Ala^1, des-Gly^2, des-Asn^5, D-Trp^8, Iamp^9]-somatostatin gave similar results.[316]

Antagonists of somatostatin have only been available in the last few years. 4-NO_2-Phe-c(D-Cys-Tyr-D-Trp-Lys-Thr-Cys)-Tyr-NH_2 and [Ac-4-NO_2-Phe-c(D-Cys-Tyr-D-Trp-Lys-Thr-Cys)-D-Tyr-NH_2 (inactive at the SST_1 and SST_4 receptor subtypes and high affinity at the $SSTR_2$ and $SSTR_5$ receptor subtypes) inhibited somatostatin-mediated inhibition of cAMP accumulation in a dose dependent manner.[317] The more potent antagonist, [Ac-4-NO_2-Phe-c(D-Cys-Tyr-D-Trp-Lys-Thr-Cys)-D-Tyr-NH_2], displays a binding affinity (K_i values 0.3, 100 and 2.5 nM on $SSTR_2$, $SSTR_3$ and $SSTR_5$ receptor subtypes, respectively) to $SSTR_2$ comparable with that observed for the native hormone (K_i = 0.2 nM) and reverses somatostatin-mediated inhibition of cAMP accumulation in rat somatomammotroph GH_4C_1 cells, cells transfected with the $SSTR_2$ and $SSTR_5$ subtypes, as well as somatostatin-stimulated growth of yeast cells expressing the $SSTR_2$ subtype. At a concentration of 1 μM, it shifted the dose response curve of somatostatin by 500-fold in the cAMP accumulation assay. BIM-23056 [D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D-Nal-NH_2] (a linear octapeptide antagonist of somatostatin) inhibited somatostatin-induced changes in intracellular calcium ion concentration (pK_B 8.0) and formation of inositol-1,4,5-trisphosphate in CHO-K1 cells transfected with the human recombinant $SSTR_5$ receptor.[318]



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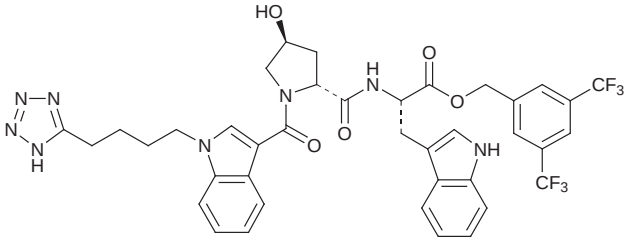
4.18 Tachykinin (Substance P and Neurokinins) Analogues – Work on tachykinin receptors has been reviewed.[319] A novel human NK_3 receptor homologue that binds [^{3}H]senktide and [^{125}I-MePhe^7]NKB, and is responsive to tachykinin peptide agonists has been expressed.[320] Conformational properties of various tachykinin antagonists have been investigated by NMR, computer-assisted modelling and X-ray studies.[321,322] Additional biological studies have been reported on the tachykinin receptor ligands reported in the past.[323–328] More tachykinin-like peptides have been isolated from the skin of Costa Rican phyllomedusid frog *Agalychnis callidryas* [Gly-Pro-Pro-Asp-Pro-Asn-Lys-Phe-Ile-Gly-Leu-Met-NH_2, Gly-Pro-Pro-Asp-Pro-Asp-Arg(Lys)-Tyr-Pro-Gly-Met-NH_2, Pyr-Pro-Asp-Pro-Asp-Arg-Phe-Tyr-Pro-Gly-Met-NH_2 and Gly-Pro-Pro-Asp-Pro-Asn-Lys-Phe-Tyr-Pro-Val-Met-NH_2] and the brain of the Madeira cockroach [Asp-Pro-Ser-Gly-Phe-Leu-Gly-Val-Arg-NH_2, Asp-Pro-Ala-Met-Gly-Phe-Gln-Gly-Val-Arg-NH_2, Asp-Pro-Ala-Ala-Gly-Phe-Phe-Gly-Met-Arg-NH_2, Val-Pro-Ala-Ser-Gly-Phe-Phe-Gly-Met-Arg-NH_2, Gly-Pro-Ser-Met-Gly-Phe-His-Gly-Met-Arg-NH_2, Ala-Pro-Ser-Met-Gly-Phe-Gln-Gly-Met-Arg-NH_2 and Ala-Pro-Glu-Glu-Ser-Pro-Lys-Arg-Ala-Pro-Ser-Gly-Phe-Leu-Gly-Val-Arg-NH_2].[329,330]

In contrast to [D-Arg^1, D-Trp^{5,7,9}, Leu^{11}]Substance P which prevented colony formation in small cell lung cancer cells in a dose-dependent fashion (IC_{50} 5 μM), some other analogues [Arg-D-Trp-MePhe-D-Trp-Leu-D-Met-NH_2, Arg-D-Trp-MePhe-D-Trp-D-Leu-D-Met-NH_2, D-Arg-Pro-Lys-Pro-D-Phe-Gln-D-Trp-Phe-D-Trp-Leu-Leu-NH_2 and Arg-D-Phe-Gln-D-Trp-Phe-D-Trp-Leu-Gly-NH_2] led to 30-60% inhibition of the growth of H-69 small cell lung cancer cells at 25 μM. [D-Arg^1, D-Trp^{5,7,9}, Leu^{11}]Substance P given peritumourally at 35 μg $g^{-1}day^{-1}$ for 7 days produced a marked inhibition of the growth of the H-69 xenograft, which was maintained beyond the duration of the antagonist treatment.[331] A smaller but significant effect was also seen when the compound was administered i.p. to nude mice bearing H-69 tumours. Major metabolites of [D-Arg^1, D-Phe^5, D-Trp^{7,9}, Leu^{11}]Substance P following intraperitoneal administration (12 μg g^{-1}) to nu/nu mice bearing the H69 small-cell lung cancer xenograft were identified as [D-Arg^1, D-Phe^5, D-Trp^{7,9}, Leu^{11}]Substance P acid and [D-Arg^1, D-Phe^5, D-Trp^{7,9}]Substance P(1-10).[332]

A number of monocyclic and bicyclic peptides and dipeptide derivatives have been reported as neurokinin receptor antagonists.[333–335] Monocyclic pseudopeptide analogues, c(Leuψ(CH_2NH)Leu-Gln-Trp-Phe-β-Ala), c(Leuψ(CH_2NH)Asp(OBzl)-Gln-Trp-Phe-β-Ala), c(Leuψ(CH_2NH)Lys(Z)-Gln-Trp-Phe-β-Ala), c(Leuψ(CH_2NH)Cha-Gln-Trp-Phe-β-Ala), c(Leuψ(CH_2NH)Nal-Gln-Trp-Phe-β-Ala), c(Leuψ(CH_2NH)Asp(NHBzl)-Gln-Trp-Phe-β-Ala), and conformationally more rigid bicyclic peptides, c[(Met-Asp-Trp-Phe-Dap-Leu) c(2β-5β)], c[(Cha-Asp-Trp-Phe-Dap-Leu) c(2β-5β)], c[(Met-Dap-Trp-Phe-Asp-Leu) c(2β-5β)], c[(Met-Asp-Trp-Phe-Dap-MeLeu) c(2β-5β)] and c[(Nle-Asp-Trp-Phe-Dap-Leu) c(2β-5β)], were evaluated in NK_2 receptor preparations.[333] Three of the bicyclic peptides, c[(Met-Asp-Trp-Phe-Dap-Leu) c(2β-5β)], c[(Met-Dap-Trp-Phe-Asp-Leu) c(2β-5β)]and c[(Nle-Asp-Trp-Phe-Dap-Leu) c(2β-5β)], showed the highest affinity in human isolated ileum and colon, rabbit isolated pulmonary artery and

hamster isolated trachea preparations (pK_B values 8.1-10.3). Examples of the dipeptide derivatives acting at neurokinin receptors include compounds (**96**) and (**97**). The Hyp-Trp derivative (**96**) is an NK_1 selective receptor antagonist (K_i hNK_1 receptor 2.4 nM, human NK_2 receptor 4200 nM) (pA_2 values on NK_1, NK_2 and NK_3 tissue preparation 9.0, 5.2 and 5.5, respectively) and inhibits SP-induced bronchoconstriction in guinea pigs up to 40 minutes after i.v or aerosol administration.[334] The Nal derivative (**97**) showed high affinity at the NK_1 receptors expressed in human IM9 (K_i 1.0 nM) and U373MG (K_i 2.8 nM) cells and guinea pig lung membranes (K_i 5.9 nM) but showed no affinity for the NK_1 sites present in rat urinary bladder membranes up to 10 μM concentration.[335]



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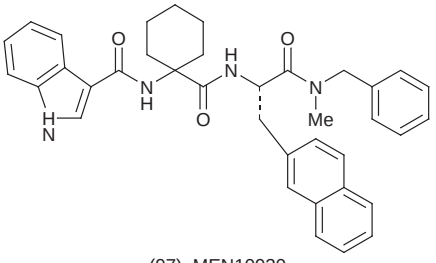
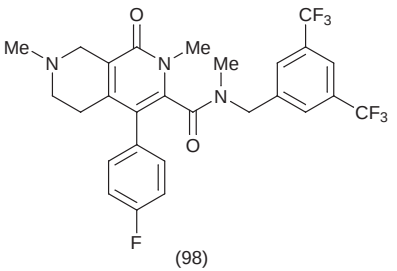


(97) MEN10930

The non-peptide antagonists of tachykinins reported this year include compounds (**98-101**). Many of these showed various levels of selectivity and were active in a number of *in vitro* and *in vivo* tests (oral administration).[336–348] Compound (**98**) blocked capsaicin-induced plasma extravasation in guinea pig trachea (ED_{50} 0.050 mg kg^{-1}, iv and 0.27 mg kg^{-1}, po).[339] Compound (**99**), obtained by chemical modifications to a chemical library lead using NK_1 and NK_2 receptors, was a potent inhibitor of SP-induced bronchoconstriction in guinea pigs (ED_{50} 0.03-0.1 mg kg^{-1}, iv) and mice (ED_{50} 3 mg kg^{-1}, po).[340] The triazole derivative (**100**) was an NK_1 antagonist (IC_{50} 0.18 nM) which antagonised the extravasation induced by the vannilloid sensorotoxin resiniferatoxin when administered orally (ID_{50} 0.34 mg kg^{-1}). At a dose of 1 mg kg^{-1}, the effect lasted for about 8 hours (55% inhibition). The corresponding triazolinone analogue gave a much longer duration of action at the same dose (66% inhibition after 24 hours).[343] In mice, oral administration of (**101**) (SB223412) produced



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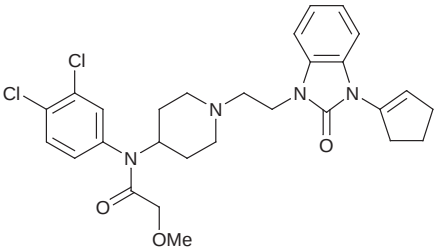

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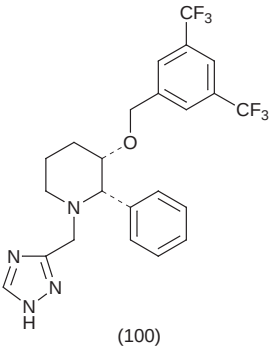

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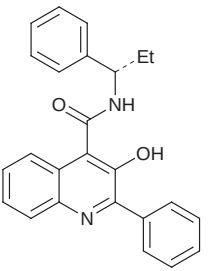
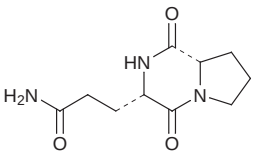


(101) SB223412

dose-dependent inhibition of behavioural responses induced by the NK_3 receptor selective agonist, senktide (ED_{50} 12.2 mg kg^{-1}).[347]

4.19 Thyrotropin-releasing Hormone Analogues – Conformationally restricted analogues of [Gln^2]-TRH are reported. Compounds (**102**) and (**103**) were active in the antidepressive and antiamnesic models in rats after intraperitoneal administration.[349]



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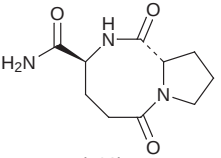

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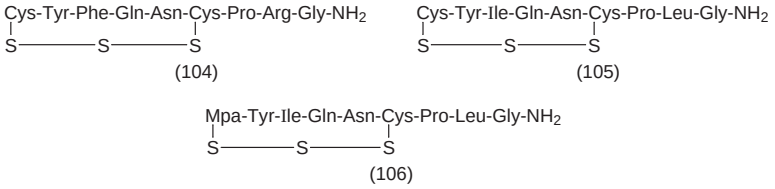
4.20 Vasopressin and Oxytocin Analogues – Since the discovery of multiple vasopressin receptors, a number of selective ligands have been reported. [β-Mercapto-β,β-pentamethylenepropionic acid[1], D-Tyr(OEt)[2], Val[4], Tyr^9-NH_2] was shown to be a potent antidiuretic, antivasopressor and antioxytocic peptide with pA_2 values of 7.69-7.94 and affinities of 1.12-11.0 nM. When radioiodinated at Tyr^9, the peptide bound to V_2 and V_{1a} receptors with a dissociation constant

of 0.22-0.75 nM.[350] A C-terminally extended octapeptide analogue, phenylacetyl-D-Tyr(Et)-Phe-Gln-Asn-Lys-Pro-Arg-Tyr-NH_2 was a strong V_1 antagonist (pA_2 8.64) and a very weak V_2 antagonist. The iodinated phenylacetyl-D-Tyr(Et)-Phe-Gln-Asn-Lys-Pro-Arg-Tyr-NH_2 derivative bound specifically to the V_{1a} vasopressin receptor with an apparent K_d value of 0.168 nM.[351]

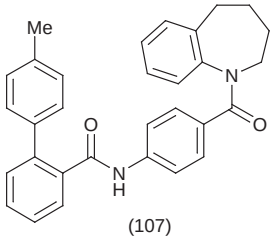
Ligand binding characteristics of the human V_3 (V_{1b}) receptor have been investigated in detail.[352] In general, most of the agonist and antagonist analogues were less potent at the V_3 (pituitary) receptors than at the V_1 (vascular) or V_2 (renal) receptors. Arg^8-vasopressin [K_i values 1.73, 1.1 and 1.1 nM at V_1, V_2 and V_3 receptors, respectively], Lys^8-vasopressin [K_i values 2.3, 3.3 and 2.87 nM at V_1, V_2 and V_3 receptors, respectively] and Arg-vasotocin [K_i values 5.0, 6.2 and 10.2 nM at V_1, V_2 and V_3 receptors, respectively] bind with much higher affinity to the three receptor subtypes than oxytocin [K_i values V_1, V_2 and V_3 receptors 64, 167 and 1782 nM, respectively]. In comparison, many of the analogues, e.g. phenylacetyl-D-Tyr(Et)-Phe-Val-Asn-Lys-Pro-Tyr-NH_2 [K_i values V_1, V_2 and V_3 receptors 2.37, 1805 and 798 nM, respectively], phenylacetyl-D-Tyr(Et)-Phe-Gln-Asn-Lys-Pro-Arg-NH_2 [K_i values V_1, V_2 and V_3 receptors 0.8, 302 and 31 nM, respectively] and phenylacetyl-D-Tyr-Phe-Val-Asn-Arg-Pro-Arg-Arg-NH_2 [K_i values V_1, V_2 and V_3 receptors 0.58, 259 and 15.9 nM, respectively], were more selective for the V_1 receptor subtypes. Two of the oxytocin antagonists, $d(CH_2)_5$[$Tyr(OMe)^2$, Thr^4, Orn^8]- [K_i values V_1, V_2 and V_3 receptors 7.6, 5964 and 28700 nM, respectively] and $d(CH_2)_5$[$Tyr(OMe)^2$, Thr^4, Orn^8 ,Tyr^9-NH_2]-vasotocin [K_i values V_1, V_2 and V_3 receptors 3.9, 929 and 10229 nM, respectively], were the most selective ligands at the V_1 receptor. The most potent V_1/V_3 analogue was 4-OH-phenylacetyl-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-NH_2 [K_i values V_1, V_2 and V_3 receptors 0.45, 428 and 2.2 nM, respectively].[352]

Trisulfide analogues of vasopressin and oxytocin are reported.[353,354] In general, the analogues like (**104**-**106**) were either equipotent or less potent than the parent disulfide peptides. For example, (**104**) was equipotent to Arg vasopressin at the V_1 receptor (IC_{50} values 9.5 and 10 nM, respectively) and about 3-fold less potent than the parent peptide at the V_2 receptor subtype (IC_{50} values 9.5 and 3.3 nM, respectively).[353] Two of the oxytocin analogues (**105**) and (**106**) were less potent than the parent peptides.[354]



A number of papers on the chemistry and biology of non-peptide vasopressin antagonists have appeared.[355–358] Compound (**107**) (based on OPC-31260 reported earlier) [V_{1A} pK_i 8.07, V_2 pK_i 8.61 and oxytocin pK_i 7.47] and the corresponding compound containing a -NMe_2 group in place of the methyl group

were slightly more potent at the V_2 receptors.[355] Compound (**108**) (YM087) showed high affinity for V_{1a} receptors from rat liver and V_2 receptors from rat kidney with K_i values of 0.48 and 3.04 nM, respectively. YM087 also inhibited [^{3}H]oxytocin binding to rat uterus plasma membranes with a K_i value of 44.4 nM, and at 100 μM did not affect the binding of [^{3}H]AVP to anterior pituitary (V_{1b} receptors) plasma membranes. In *in vivo* studies, i.v administration of YM087 inhibited the pressor response to exogenous AVP in pithed rats in a dose dependent manner.[356–358]



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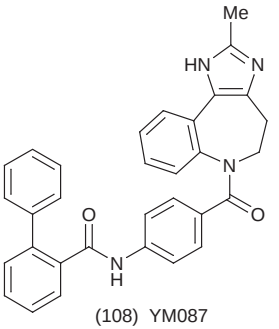


(108) YM087

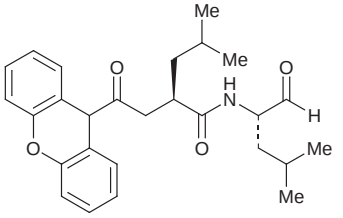
5 Enzyme Inhibitors

Most of the work this year has been on inhibitors of converting enzymes, farnesyltransferase, HIV protease, matrix metalloproteases and thrombin. Only a limited amount of work has been published on the inhibitors of renin, elastase, calpain and cathepsin D.

5.1 Calpain Inhibitors – Calpains (I and II) are calcium-activated neutral proteases belonging to a family of intracellular cysteine proteases. The possible role of calpains in the pathology of a variety of nervous system disorders including stroke, Alzheimer's disease, muscular dystrophy, and epilepsy has led to a search for inhibitors for these enzymes. Dipeptide and tripeptide aldehydes were evaluated as inhibitors of calpain I.[359] In the dipeptide series, Z-*t*-butylGly-Leu-H and Z-Leu-Leu-H, Z-Leu-Abu-H, Z-Leu-Cha-H, Z-Leu-Nle-H, Z-Leu-Phe-H, Z-Leu-His-H, Z-Leu-Tyr(OBzl)-H and Z-Leu-Val-H were similar in potency to the parent peptide (IC_{50} values 4-15 nM). Z-Nle-Leu-H, Z-Ile-Leu-H and Z-Val-Leu-H were somewhat less potent (IC_{50} values 33, 41 and 29 nM, respectively). In the case of Z-Leu-Leu-H, the P_1 leucine residue could be changed by many other hydrophobic residues. Replacement of the Z group in the Z-Leu-Leu-H by 4-nitrobenzyloxycarbonyl, Fmoc, methoxycarbonyl and toluenesulfonyl groups also resulted in compounds equipotent to the parent dipeptide aldehyde. In the tripeptide series, Ac-Leu-Leu-Leu-H, Ac-Leu-Leu-Arg-H, Ac-Leu-Leu-D-Leu-H, Ac-Leu-Leu-Lys(Boc)-H, Ac-Leu-Leu-Met-H, Ac-Leu-Leu-

Nle-H and Ac-Leu-Leu-Phe-H were very similar in potency (IC_{50} values 12-70 nM). Replacement of the P_2 leucine residue in Ac-Leu-Leu-Leu-H by Ala, Phe or Trp residues gave about a 4-fold reduction in potency. Modifications in the P_3 position did not have much effect on the potency. Ac-Leu-Leu-Leu-H, Ac-Ala-Leu-Leu-H, Z-Ala-Leu-Leu-H, Z-Glu(O-Bu^t)-Leu-Leu-H, Z-Leu-Leu-Leu-H, Z-Lys-Leu-Leu-H, Z-Phe-Leu-Leu-H, Z-Ser(But)-Leu-Leu-H, Z-Tic-Leu-Leu-H, Z-Tyr-Leu-Leu-H and naphthyl-2-CO-Leu-Leu-Leu-H were very similar in potency. Incorporation of non-peptidic residues at the N-terminus of some of the dipeptide aldehydes gave less potent compounds like (**109**) and (**110**) (calpain I IC_{50} 30 nM, cathepsin B IC_{50} 60 nM).[360,361]

In addition to the aldehyde derivatives mentioned above, fluoromethyl ketone inhibitors of human calpain I have also been reported.[362,363] SAR studies revealed that the nature of the N-terminal capping group has a significant effect on the inhibitory activity of this series of compounds. Compound (**111**), having a tetrahydroisoquinoline containing urea as the N-terminal capping group, was the most potent dipeptide fluoromethyl ketone inhibitor of the series. In an intact human cell assay, compound (**111**) inhibited calpain I with IC_{50} value of 0.2 μM.



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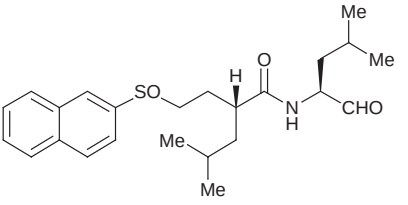

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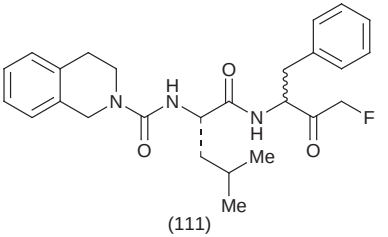


(111)

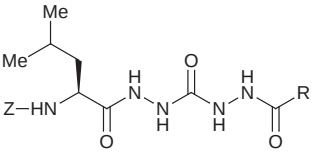
5.2 Caspase Inhibitors – The caspases are a family of cysteine proteases that play regulatory roles in the cell. The inhibitors of these enzymes may be important for the treatment of neurodegenerative diseases, ischaemic injury and cancer. These enzymes require an Asp in P_1 position. The crystal structures of caspase-1 and caspase-3 show that the active enzyme is a heterotetramer, containing two small and two large subunits. In addition to the reversible inhibitors like Ac-Asp-Glu-Val-Asp-H and Ac-Tyr-Val-Ala-Asp-H, irreversible

tripeptide inhibitors like Z-Val-Ala-Asp(OMe) fluoromethyl ketone have been reported. Up to 50 μM, Ac-Asp-Glu-Val-Asp-H, a caspase-3-preferred inhibitor, inhibits caspase-2 activation and DNA fragmentation *in vivo*, but does not prevent loss of mitochondrial function, while higher concentrations of the inhibitor (>50 μM) inhibit both.[364] The tertiary structure and substrate binding site of caspase-8 has been predicted.[365] Some of the work on caspases has recently been reviewed.[366–368] A caspase-like apoptosis-regulatory protein (CLARP) has been identified. The protein contains two amino terminal death effector domains fused to a carboxyl-terminal caspase-like domain. The structure and amino acid sequence of CLARP resemble those of caspase-8, caspase-10 and DCP-2. Expression of CLARP induced apoptosis was blocked by the viral caspase inhibitor p35, dominant negative mutant caspase-8, and the synthetic caspase inhibitor Z-Val-Ala-Asp(OMe)-fluoromethyl ketone.[369]

5.3 Cathepsin Inhibitors – A number of quenched fluorogenic substrates with P_2 variations in the series Ac-Glu-Glu(Edans)-Lys-Pro-Ile-X-Phe-Phe-Arg-Leu-Gly-Lys(Dabcyle)-Glu-NH_2 [X = Cys, Cys(Me), Cys(Et), Cys(t-Bu), Cys(carboxymethyl), Met, Val or Ile; Edans = 5-(2-aminoethyl) aminonaphthalene-1-sulfonic acid; Dabcyl = 4′-dimethylaminoazobenzene-4-carboxylic acid] were reported as substrates for human cathepsin D.[370] Using a 15-residue synthetic peptide library and a native protein as substrates, it was found that positions P_1 and P_1' of the cathepsins D and E (aspartic proteases) substrates must be occupied exclusively by hydrophobic amino acids with aromatic or aliphatic side chains. However, Val and Ile residues are not allowed at position P_1. Position P_2' accepts a broad range of amino acids, including charged and polar ones. Additional requirements concerning the substrate positions P_3' and P_4' were also defined by pool sequencing.[371]

Inhibitors of cathepsin K, a cysteine protease implicated in the process of bone resorption, have been reported. The design process involved X-ray crystallographic studies using papain and thiol protease inhibitor leupeptin (Ac-Leu-Leu-Arg-H) which bound to the S side of the enzyme active site and another similar aldehyde Z-Leu-Leu-Leu-H which bound in the S′ direction.[372,373] One of the more potent inhibitors (**112**, R-CO- = 4-$(CH_3)_2$-NCH_2-Z-Leu) inhibited osteoclast-mediated calcemic response to human parathyroid hormone(1-34) when co-infused (6 hours) with human parathyroid hormone(1-34) at a dose of 13 mg kg^{-1} h^{-1}, i.v.

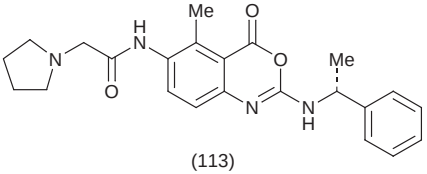
Cathepsin W, a novel human cysteine protease distinct from that of cathepsin L- and B-like proteases, has been identified from the dbEST database. Northern



(112) R–CO = 4-Me_2NCH_2-Z-Leu

blot analysis indicated a specific expression of cathepsin W in lymphatic tissues. Further analysis revealed predominant levels of expression in T-lymphocytes, and more specifically in $CD8^+$ cells.[374]

5.4 Cytomegalovirus Protease Inhibitors – The human cytomegalovirus (serine protease) is a prevalent member of the herpes virus family infecting up to 80% of the general population. This virus is responsible for opportunistic infections in immunocompromised individuals including organ transplant recipients and AIDS sufferers. Crystallographic studies on human cytomegalovirus protease have been reported in the past.[375–377] Modifications of benzoxazinone derivatives (reported previously as mammalian serine protease inhibitors) resulted in compounds like (**113**) as selective inhibitors of cytomegalovirus protease (IC_{50} 2.4 μM; 36-47% inhibition of human leukocyte elastase and chymotrypsin at 100 (M). A number of other compounds containing a Me_2N-, Boc-NH-, *p*-methoxybenzenesulfonamide, nicotinamide or dimethylglycinamide in place of the pyrrolidineacetamide group were slightly more potent than compound (**113**) against cytomegalovirus protease (IC_{50} values 0.24-2.2 μM) but the compounds were less selective.[378] In fact the Me_2N- analogue was more potent against chymotrypsin (IC_{50} 0.065 μM) than against cytomegalovirus protease (IC_{50} 0.24 (M) and human leukocyte elastase (IC_{50} 3.2 μM)



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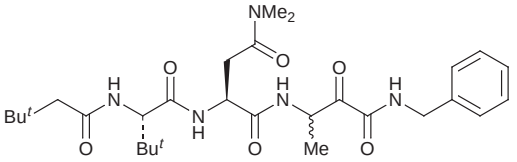
Inhibitors of the human cytomegalovirus protease based on the amino acid sequences of the two cleavage sites are described.[379] Earlier lead compounds like Ac-Ser-Tyr-Val-Lys-NH-CH(CH_3)-CO-CF_3, Ac-Gly-Val-Val-Asn-NH-CH(CH_3)-CO-CF_3 and Ac-Val-Val-Asn-NH-CH(CH_3)-CO-CF_3 (IC_{50} values 1.8-3.0 μM) were weak inhibitors. Further amino acid substitutions in Ac-Val-Val-Asn-NH-CH(CH_3)-CO-CF_3 indicated that the asparagine residue (P_2 position) could be replaced by a number of other amino acids like Gln, Phe, Val, and Asn(Me_2) residues (IC_{50} values 2-11 μM). The P_3 (Val) position could accommodate Abu, t-Leu, t-Bu-Ala and adamantyl residues. Replacement of the acetylvaline residue (P_4 position) by various other groups in one of the more potent compounds, Ac-Val-t-Leu-Asn(Me_2)-NH-CH(CH_3)-CO-CF_3, led to compounds with similar potency, e.g. $(CH_3)_3C$-CH_2-CO-t-Leu-Asn(Me_2)-NH-CH(CH_3)-CO-CF_3 (IC_{50} 1.1 (M). Using $(CH_3)_3C$-CH_2-CO-t-Leu-Asn(Me_2)-NH-CH(CH_3)-CO-CF_3, as an example, the activated carbonyl group of the inhibitors was modified. Several compounds inhibited the enzyme with IC_{50} values of 0.06-11 μM. Compound (**114**) was one of the more potent and selective inhibitors. No significant improvement in the inhibitory activity was observed by extending the binding groups into the S′ site. Many of the above compounds showed good



(114)

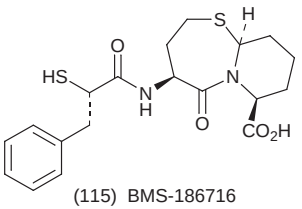
selectivity (>300-fold) against human leukocyte elastase, bovine pancreatic α-chymotrypsin, and human liver cathepsin B.[379]

5.5 Converting Enzyme [Angiotensin (ACE), Neutral Endopeptidase (NEP), Endothelin (ECE) and Interleukin-1β (ICE)] Inhibitors – Many peptides are obtained from their precursors by the action of converting enzymes. For example, angiotensin converting enzyme cleaves a dipeptide from the C-terminus of angiotensin I to generate the pressor peptide angiotensin II. In addition, some of the biologically active peptides (e.g. bradykinin and atrial natriuretic factor) are degraded by the converting enzymes. Thus many physiological and pathological processes are controlled by converting enzymes. Inhibitors of angiotensin, neutral endopeptidase, endothelin and interleukin-1β have been reported.

5.5.1. Angiotensin Converting Enzyme and Neutral Endopeptidase Inhibitors – Inhibitors of angiotensin converting enzyme have been identified by a library approach.[380] Libraries of angiotensin converting enzyme inhibitors involving N-terminal reductive alkylation of -X-Pro, -X-X and -X-X-NH_2 [X = naturally occurring amino acids except cysteine] dipeptides using ethyl-2-oxo-4-phenylbutyrate were synthesised on solid supports. Although compounds containing Ser-Lys, Ser-Gln, Lys-Ser and Gln-Ser dipeptides were identified as angiotensin converting enzyme inhibitors, none of the compounds were more potent than enalapril.

Dual metalloprotease inhibitors have been obtained in the mercaptoacetyl and carboxyalkyl series of compounds. In both series of compounds, oxazepinones and/or thiazepinones were incorporated as conformationally restricted dipeptide surrogates.[381,382] One of the thiazepinone derivatives (**115**) (IC_{50} 8 and 5 nM against NEP and ACE, respectively), at a single oral dose (30 μmol kg^{-1} (12 mg kg^{-1}), decreased mean arterial pressure approximately 40 mmHg below baseline for 10-24 hours. In comparison, fosinopril (an ACE inhibitor) averaged a 20 mmHg decrease during the same period. In a DOCA salt rat hypertension assay (NEP inhibition model), oral administration of (**115**) (100 μmol kg^{-1} day^{-1}) resulted in a 38 mmHg decrease in systolic blood pressure. In comparison, the corresponding oxazepinone analogue had a short duration of action (<1 hour). Temocaprilat (**116**) (active metabolite of temocapril; ethyl ester of hPhe moiety) was excreted predominantly in bile.[382]

Based on the crystal structure of thermolysin, and some modelling studies, *ortho*- and *meta*-substituted benzofused macrocyclic lactams were synthesised as zinc metalloprotease inhibitors.[383,384] The 11-membered lactam (**117**) (S,S



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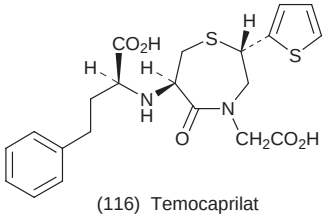
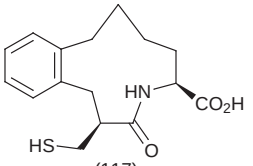


(116) Temocaprilat

isomer) inhibited both thermolysin and neutral endopeptidase (IC_{50} 68 and 0.9 nM, respectively) but was inactive (IC_{50} >10000 nM) against angiotensin converting enzyme. In the case of the 13-membered compounds, the (R,S) isomer (**118**) was more potent (IC_{50} thermolysin 1800 nM and neutral endopeptidase 27 nM) than the (S,S) isomer (IC_{50} thermolysin 41000 nM and neutral endopeptidase 224 nM). Oral administration of a prodrug of (**118**) (Ac-S- and -COOBzl) in conscious rats (10 mg kg^{-1}) significantly increased (>200%) levels of exogenously administered atrial natriuretic peptide. In comparison to the *ortho* derivatives, the *meta* derivatives inhibited neutral endopeptidase and angiotensin converting enzyme. For example, a 13-membered lactam derivative (**119**) was a potent inhibitor of neutral endopeptidase and angiotensin converting enzyme and a poor inhibitor of thermolysin (IC_{50} values 8, 4 and 48000 nM, respectively). The 14-membered (S,S) isomer (**120**) showed improved selectivity against neutral endopeptidase (IC_{50} values, neutral endopeptidase 4 nM, angiotensin converting enzyme 175 nM and thermolysin 34000 nM). Biological activities of a dual metallopeptidase inhibitor, MDL 100,240, have been reported.[385,386]



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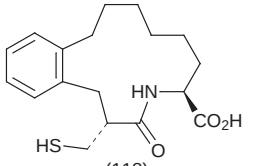

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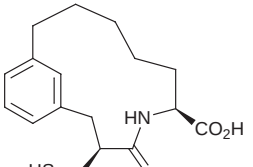

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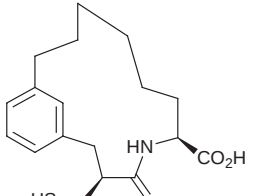

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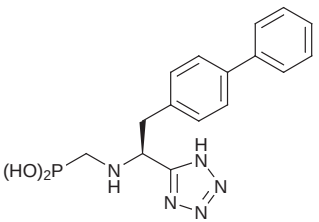
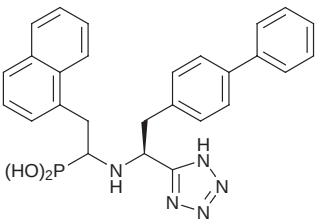
5.5.2. Endothelin Converting Enzyme Inhibitors – SAR studies on GCS 26303 (**121**), reported previously as a dual inhibitor of endothelin converting enzyme and neutral endopeptidase, were attempted to improve inhibitory activity against

endothelin converting enzyme.[387] Replacement of the tetrazole group by triazole, -COOH or -CH_2-COOH groups and replacement of the biphenyl group by Phe, hPhe, Nal and Trp side chains led to much less potent compounds (<20% inhibition at 1 μM). Similarly, replacement of the $(HO)_2PO$-CH_2-NH- group by $(HO)_2PO$-CH_2-CH_2-, -NH-P(O) (OH)-CH_2-CH_2-2-naphthyl, $(HO)_2PO$-CH_2- and HS-CH_2- groups abolished most of the activity (<26% inhibition at 1 μM). Most of the compounds like (**122**) inhibited both neutral endopeptidase (IC_{50} 4.8 nM) and endothelin converting enzyme (IC_{50} 17 nM). Among the big ET-1 analogues, big ET-1(18-34), [Phe^{21}]- and [Ala^{31}]big ET-1(18-34) exhibited a significant inhibition of ECE-1. A kinetic analysis revealed [Phe^{21}]big ET-1(18-34) to be a competitive inhibitor (K_i 20.6 μM) and [Ala^{31}]big ET-1(18-34) to be a non-competitive inhibitor (K_i = 35.6 μM). Many other analogues, big ET-1(17-26), [Ala^{20}]-, [Ala^{21}]-, [Ala^{22}]-, [Phe^{22}]-, [Gln^{27}, Thr^{28}, Ala^{29}]-, [Phe^{21}]-, [D-Trp^{21}, D-Val^{22}]- and [D-Val^{22}]big ET-1(16-34), were inactive.[388]



(121) GCS 26303



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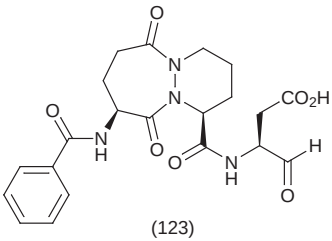
5.5.3 Interleukin 1β Converting Enzyme Inhibitors – In vitro and *in vivo* studies of ICE inhibitors have been reviewed.[389] The substrate specificity of interleukin-1β converting enzyme was defined by using a combinatorial positional scanning approach.[390] Ac-Trp-Glu-His-Asp-aminomethylcoumarin was the best substrate. Hydrophobic amino acids were preferred in S_4 (Trp > Tyr > Nle > Leu) position. Various amino acids were tolerated in P_3 position, but His was favoured by approximately threefold over Val in P_2 position. Based on this substrate sequence, an aldehyde inhibitor, Ac-Trp-Glu-His-Asp-H (K_i 56 nM), was synthesised. In comparison, Ac-Tyr-Val-Ala-Asp-H was >10-fold less potent inhibitor of the enzyme (K_i 760 nM). Various other inhibitors of ICE containing a C-terminal Asp aldehydes or Asp phenyl ketoethers and non-peptidic residues as P_2-P_3 replacements have been reported.[391–393] Examples of these types of compounds include (**123-125**). Compound (**123**) inhibited IL-1β production by >95% in a mouse model of biochemical efficacy at a single 100 mg kg^{-1} dose given by i.p. administration.[391] The pyridone 6-benzyl substituent in (**125**) (IC_{50} 10 nM) could be replaced by n-butyl group with only a slight reduction in potency (IC_{50} 16 nM). Other replacements like Me, Et, n-Pr, n-hexyl or Ph gave less potent compounds (IC_{50} values 25-150 nM).[393] Other reported inhibitors of ICE include compounds like (**126**) and (**127**).[394,395]



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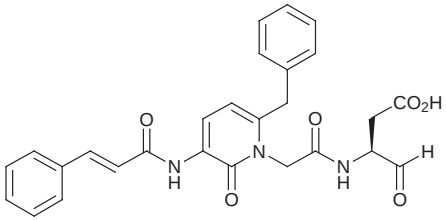

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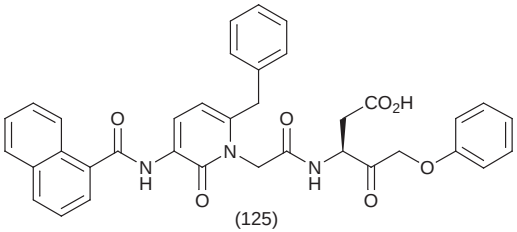

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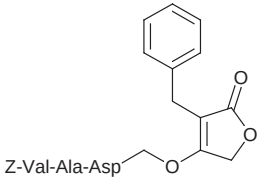

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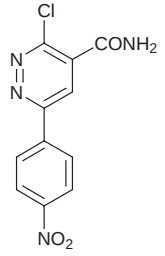

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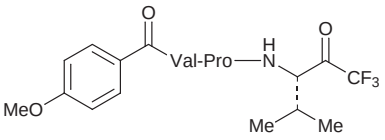
5.6 Elastase Inhibitors – Based on some of the earlier work on N-substituted Val-Pro-Val-trifluoromethyl ketones, orally active elastase inhibitors were identified.[396,397] Various substituents at the N-terminal end of Val-Pro-Val-trifluoromethyl ketone, e.g. 4-$MeOC_6H_5$-, C_6H_5-CH_2-O-, C_6H_5-O-, cyclopentyl-O-, isobutyl-O-, isopropyl-O-, EtO-, MeO- and C_6H_5-O-CH_2-, resulted in potent and selective inhibitors of leukocyte elastase (K_i 1.5-44 nM; 8-93% inhibition of elastase-induced haemorrhage in hamsters at a dose of 10 mg kg^{-1}, po). The most potent analogues (**128**) and (**129**) prevented elastase-induced haemorrhage when administered intravenously (ED_{50} values 0.59 and 0.51 mg kg^{-1}, respectively) or orally (ED_{50} values 4.9 and 2 mg kg^{-1}, respectively). The half life for the degradation of the chloromethyl ketone inhibitor of elastase, Boc-Tyr-Leu-Val-CH_2Cl, in an aqueous solution (pH 7.4) at 37 °C was found to be about 36 h.[398] The degradation products in aqueous solution were Boc-Tyr-Leu-Val-CH_2OH at pH 7.4 and Tyr-Leu-Val-CH_2Cl at pH 2.0. In the rat plasma, the half-life of Boc-Tyr-Leu-Val-CH_2Cl was 42.4 min.



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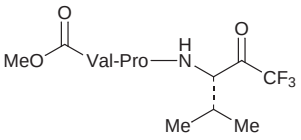


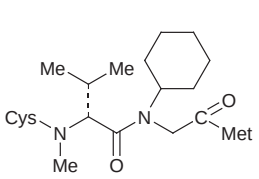
(129)

5.7 Farnesyltransferase Inhibitors – Reviews on farnesyltransferase inhibitors have appeared.[399–401] Amino acid residues that convert the protein substrate specificity of farnesyltransferase into that of geranylgeranyltransferase type I have been identified. A single amino acid change at one of the three residues [Ser^{159}, Tyr^{362}, or Tyr^{366}] of its β-subunit appear to be involved.[402] Tetrapeptides derived from the C-terminus of p21 Ras were the first reported peptidic farnesyltransferase inhibitors, the most potent being Cys-Val-Phe-Met.[403] Locally constrained analogues containing a Tic residue in place of the Phe resulted in a more potent analogue Cys-Val-Tic-Met (IC_{50} 10 nM). Introduction of lipophilic chains at the N-terminal amino group [CH_3-$(CH_2)_8$-CO-, CH_3-$(CH_2)_{10}$-CO-, CH_3-$(CH_2)_{12}$-CO-, CH_3-$(CH_2)_{14}$-CO-, CH_3-$(CH_2)_{16}$-CO- or Boc] resulted in less potent compounds (IC_{50} 23-125 nM). Prodrug derivatives containing a hSer lactone or hCys thiolactone at the C-terminal end also led to less potent inhibitors [HS-$(CH_2)_2$-CO-MeVal-Tic-hSer lactone, HS-$(CH_2)_2$-CO-MeVal-Tic-hCys lactone, HS-$(CH_2)_2$-CO-MeVal-Tic-D-hCys lactone, HS-$(CH_2)_2$-CO-Val-Phe-hCys lactone and HS-$(CH_2)_2$-CO-Val-Phe-D-hCys lactone] (IC_{50}s 350-83,000 nM) which did not show significant activity in the cell-based assays. Replacement of the peptide bond between the Cys-Val and Val-Tic residues by an aminomethylene group gave more potent compounds [Cys-ψ(CH_2NH)Val-Tic-Met and Cysψ(CH_2NH)Valψ(CH_2NH)Tic-Met] in the *in vitro* enzyme inhibitory assays but the compounds were poor inhibitors in the cell based assays.[403]

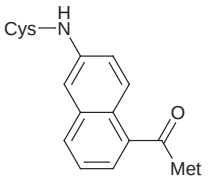
Other conformationally restricted analogues were synthesised by incorporating N-alkyl amino acid residues or non-peptide scaffolds. In one approach the Cys and Met residues were retained and the central two amino acid residues are replaced by N-substituted glycines.[404] Many of the compounds, e.g. Cys-Val-N(CH_2Ph)-CH_2CO-Met, Cys-Val-N(CH_2-CH_2Ph)-CH_2CO-Met, Cys-Val-N[CH_2-CH_2Ph(3,4-OMe)]-CH_2CO-Met, Cys-Val-N(C_6H_{11})-CH_2CO-Met, Cys-N($CHMe_2$)-CH_2-CO-N(CH_2Ph)-CH_2CO-Met, Cys-N($CHMe_2$)-CH_2-CO-N[CH_2CH_2Ph(3,4-OMe)-CH_2CO-Met, Cys-N(CH_2-CH_2-NMe_2)-CH_2-CO-N(CH_2Ph)-CH_2CO-Met, Cys-N($CHMe_2$)-CH_2-CO-Phe-Met, Cys-N(CH_2-CH_2-NMe_2)-CH_2-CO-Phe-Met and Cys-N(CH_2-$CHMe_2$)-CH_2-CO-Phe-Met, inhibited farnesyltransferase with IC_{50} values ranging between 0.07 and 75 μM. The most potent compound (**130**) (HR-11) had an IC_{50} of 1.2 nM against farnesyltransferase and was >750 times less potent against geranylgeranyltransferase (IC_{50} of 910 nM). The methyl ester of HR-11 inhibited ras processing in v-ras transformed NIH3T3 cells (IC_{50} of 10 μM).

In the search for hydrophobic scaffolds capable of orienting the cysteine and methionine residues in appropriate positions, 1,5-naphthyl and 1,6-naphthyl

scaffolds were investigated.[405] Compounds like (**131**) and (**132**) (IC_{50} 60 and 1.8 nM, respectively) and the Met methyl ester derivative of compound (**132**) (RPR 114334) inhibited the anchorage-independent growth of several cell lines. Activated Ha-*ras* and Ki-*ras* transformed cell lines were both inhibited in their ability to form colonies in soft agar (IC_{50} values 5-10 μM). Cysteine-derived compounds containing a diary lether framework (**133**, R = H, CH_3, COOMe) were less potent inhibitors of farnesyltransferase (IC_{50} values 460-650 nM) than the tetrapeptide Cys-Val-Ile-Met (IC_{50} 165 nM), but reduced the size and number of colonies of Ha-*ras* transformed Rat1 cells by 90% at 10-30 μM concentrations.[406]



(130) HR11



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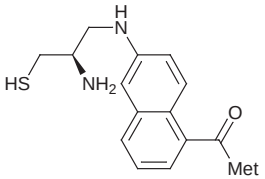
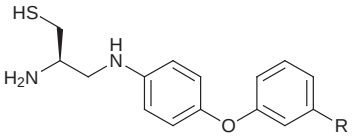


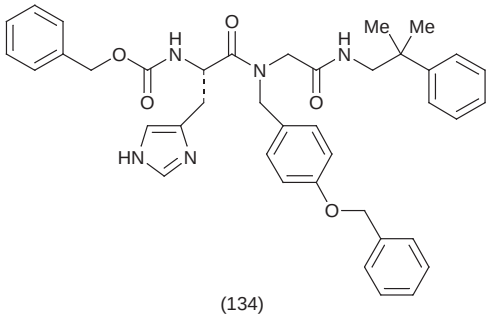
(132) RPR 113829



(133)

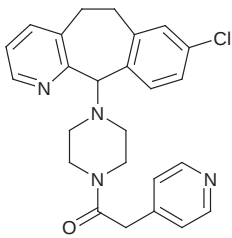
SAR studies on the random screening lead Z-His-Tyr(OBn)-Ser(OBn)-Trp-DAla-NH_2 (PD083176) (IC_{50} of 20 nM) including the replacement of the N-terminal Z group by acetyl, cyclohexylcarboxy, cyclobutyloxycarbonyl, Pyr or Fmoc group (IC_{50} values 1.6 to >20 μM) and the histidine residue by D-His, Cys, Ala, Phe, Orn, and Trp led to less potent peptides (IC_{50} values 4.7 to >20 μM).[407] Substitution of the Tyr(OBn) by D-Tyr(OBn), D-Tyr, Tyr, Tyr(OPO_3H_2), Phe(*p*-$CH_2PO_3Et_2$), Phe, D-Phe, hPhe, Asp, Glu, Gln or Cha gave compounds with very similar potency (IC_{50} values 0.018-0.17 μM). Other replacements (Ala, Lys, Cys) led to >50-fold reduction in potency. Similar to the Tyr(OBn) residue, the Ser(OBn) was also not essential for the activity. Thr(OBn), Cys, Phe and Cys(SBn) analogues were similar in potency to the parent hexapeptide and the Ser, D-Ser(OBn) and Ala analogues were >50-fold less potent. Replacement of the Trp residue by Ala, Phe or D-Trp resulted in 10, 100 and 430-fold, respectively, reduction in potency. Replacement of the D-Ala-NH_2 residue by Ala-NH_2 gave a 2-fold more potent analogue but the D-Ala, D-Ala-OMe, D-Ala-NH-NH_2 and D-Ala-NHEt analogues were about 2-8-fold less potent.[407] By truncating the C-terminus of Z-His-Tyr(Bzl)-Ser(Bzl)-Trp-Ala-NH_2, followed by further chemical modifications, a Z-His derivative (**134**) was obtained which inhibited isolated farnesyltransferase with an IC_{50} value of 4 nM (18000 nM against geranylgeranyltransferase-1), ras farnesylation in transformed cells at a

concentration of 50 nM and colony formation of transformed cells in soft agar with an IC_{50} of 180 nM.[408] Compound (**134**) was also shown to be active in athymic mice implanted subcutaneously with H-ras-F cells. When administered intraperitoneally at a dose of 150 mg kg^{-1} day^{-1} (once a day) for 14 consecutive days after tumour implantation, the tumour growth was inhibited by 88% relative to untreated controls.



(134)

In addition to (**134**), several other non-peptide inhibitors of farnesyltransferase were also shown to be active in *in vivo* tumour models.[409–411] For example, when dosed orally as a suspension in corn oil at 100 mg kg^{-1}, twice daily for 5 days a week in nude mice growing a human colorectal tumour SW-620, Sch 54429 (**135**) inhibited tumour growth by 42% after 35 days. The 3-bromo substituted pyridyl N-oxide amide analogue (**136**) reduced tumour growth by 81% when administered four times a day at 50 mg kg^{-1} and 52% at 10 mg kg^{-1} (half-life 40 min). Various other classes of non-peptide inhibitors of farnesyltransferase were obtained by using other approaches (**137-139**).[412–415] For example, (**137**) was obtained by three-dimensional database searching methods followed by SAR studies and (**138**) was obtained from natural sources.



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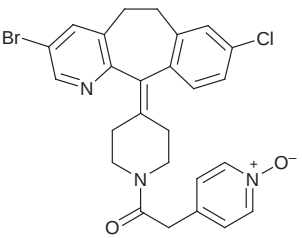

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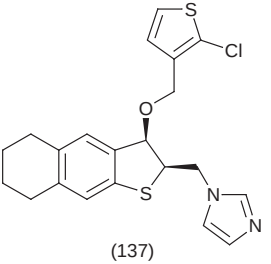
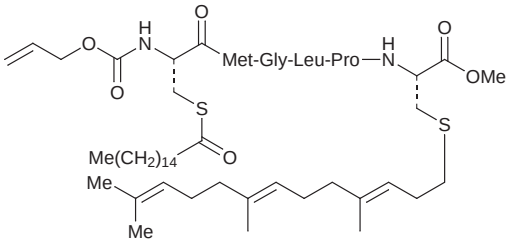


(137)

5.8 HIV Protease Inhibitors – Antibody approaches to HIV-1 have been reviewed.[416] Several publications on antibody-related work have appeared.[417–420] One of the murine monoclonal antibodies (F11.2.32) bound to HIV protease with an affinity of 5 nM.[420] Epitope mapping studies identified the peptide P36-P46



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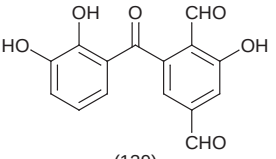
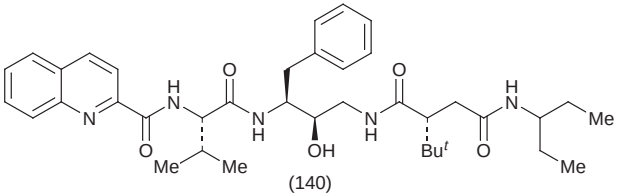


(139)

(Met-Ser-Leu-Pro-Gly-Arg-Trp-Lys-Pro-Lys-Met) as the shortest fragment of HIV-1 protease sequence capable of competing with the native enzyme in binding to F11.2.32. Crystallographic studies on the enzyme bound to the P36-P46 peptide indicated that the antigen binding site includes several aromatic side chains in direct contact with the peptide and the bound peptide form a type II β turn around Leu^{38}, Pro^{39}, Gly^{40} and Arg^{41} residues. Linear peptides derived from the membrane proximal region of the gp41 ectodomain are effective inhibitors of HIV type 1-mediated fusion events.[421] The parent peptide DP178 [Tyr^{643}-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe^{678}] was active in this assay but truncation of the peptide led to an inactive peptide [Tyr^{643}-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp^{669}]. However, the monocyclic derivative of the truncated peptide [Tyr^{643}-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu^{652}-Glu-Ser-Gln-Asn-Gln-Gln-Glu^{659}-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp], obtained by linking the two glutamic acid residue side chains [Glu^{652} and Glu^{659}] with a diamine $NH_2(CH_2)_5$-NH_2, and a bicyclic derivative [Tyr^{643}-Thr-Glu^{645}-Leu-Ile-His-Ser-Leu-Ile-Glu^{652}-Glu-Ser-Gln-Asn-Gln-Gln-Glu^{659}-Lys-Asn-Glu-Gln-Glu-Leu-Glu^{666}-Glu-Leu-Asp], obtained by linking Glu^{645} to Glu^{652} and Glu^{659} to Glu^{666} with the same amine, were active in the infectivity assay.[421] The bicyclic peptide was nearly equipotent to the linear peptide DP178. The $P2^{gag}$ peptide Ala-Glu-Ala-Met-Ser-Gln-Val-Thr-Asn-Thr-Ala-Thr-Ile-Met processed from HIV-1 $Pr55^{gag}$ by HIV-1 protease was shown to be a suicide inhibitor of the enzyme (K_i 30 μM and IC_{50} 10 μM for the synthetic peptide substrate, succinyl-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln) and inhibited the proteolytic cleavage of the viral precursor protein ($Pr55^{gag}$) into functional structural units ($p17^{gag}$ and $p24^{gag}$) *in vitro*.[422] The N-Terminal truncated nonapeptide Ala-Glu-Ala-Met-Ser-Gln-Val-Thr-Asn was nearly as potent as the patent peptide. Many other fragments like Ala-Glu-Ala-Met-Ser-Gln, Ala-Glu-Ala-Met-Ser-Gln-Val, Ala-Glu-Ala-Met-Ser-Gln-Val-Thr, Val-Thr-Asn and Val-Thr-Asn-Thr-Ala-Thr-Ile-Met were inactive. N-Terminal glyoxylyl (CHOCO-) derivatives like CHO-CO-Pro-Ile-Val-NH_2 and CHO-CO-Phe-Pro-Ile-Val-NH_2 (K_i 0.5-0.7 mM) and Fmoc-amino acids like N^{α}-Fmoc-N^{ω}-Tos-Arg (IC_{50} 5 μM) have been reported as weak inhibitors of the enzyme.[423,424] Crystal structure of N^{α}-Fmoc-N^{ω}-Tos-Arg-NH_2 bound to HIV-1 protease indicated that the inhibitor binds in

an extended conformation that results in occupancy of the S_2, S_1', and S_3' subsites of the active site.

A series of compounds, based on Boc-Pheψ[CH_2NH]Phe-Glu-Phe-NH_2, varying in the type of scissile bond replacements as well as in the P_1, P_1' and P_2' side chains were investigated for inhibition of HIV-1 and HIV-2 protease.[425] While inhibitors containing reduced amide bond, hydroxyethylamine and statine isosteres inhibited HIV-1 protease (K_i values 0.1-1.0 nM), these compounds were poor inhibitors of HIV-2 protease. Glutamic acid was identified to be the optimal P_2' residue for both HIV-1 and HIV-2 protease. For example, Boc-Phe(ψCH_2NH]Phe-Glu-Phe-NH_2, was a potent inhibitor of both the enzymes (K_i values 0.2 and 1.0 nM for HIV-1 and HIV-2 protease, respectively). Replacement of the Glu residue by Gln, Cys, Lys, Ile, Val or Asp residue resulted in less potent compounds against both the enzymes. Unlike the reduced amide bond containing peptides, analogues containing ψ[CH_2-CH_2NH] and ψ[CH(OH)CH_2NH] replacements, Boc-Pheψ[CH_2-CH_2NH]Phe-Glu-Phe-NH_2 (K_i values 0.05 and 4.1 nM for HIV-1 and HIV-2 protease, respectively) and Boc-Pheψ[CHOH-CH_2NH]Phe-Glu-Phe-NH_2 (K_i values 0.1 and 40 nM for HIV-1 and HIV-2 protease, respectively), were more potent against HIV-1 protease. Using this data hydroxyethylene isostere containing inhibitors equipotent against both the enzymes were synthesised. One such compound Boc-Pheψ[CHOH-CH_2]Phe-Glu-Phe-NH_2 (K_i values 0.02 and 0.05 nM for HIV-1 and HIV-2 protease, respectively) was nearly equipotent against both the enzymes.[425] A series of HIV protease inhibitors containing a (hydroxyethyl)amidosuccinoyl core (**140**) inhibited HIV-1 and HIV-2 protease. Several analogues of (**140**) containing Asn or Thr in place of Val or a 2-quinoxaline group in place of the 2-quinolinecarbonyl group inhibited HIV-1 replication in cell culture assays (EC_{50} 3.7-35 nM). However, the compounds exhibited poor bioavailability (<10%) in the rat, following oral administration.[426] Other examples of compounds containing various isosteres include compounds (**141**) and (**142**). The tetrazole based isostere (**141**) (IC_{50} 51 μM) was a weak inhibitor of the enzyme.[427] The sulfonamide isostere (**142**) was an irreversible inhibitor (IC_{50} 6.6 nM). A number of analogues with replacements in quinoline-2-carbonyl, N-isopropyl or N-ethyl positions were less potent (IC_{50} values 10-20 nM).[428]



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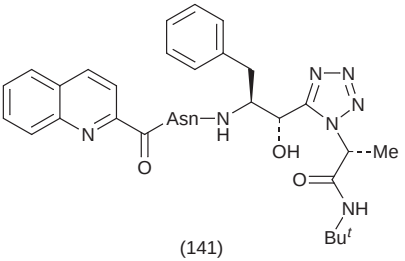
Examples of the more recently reported inhibitors which contain some of the structural features present in HIV-1 protease inhibitors that have reached the market (e.g. saquinavir, indinavir and ritonavir) include compounds like (**143-145**).[429–433] Compound (**143**) (IC_{50} 2 nM, CIC_{95} 100 nM) was orally bioavailable



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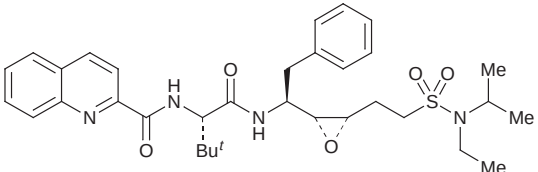
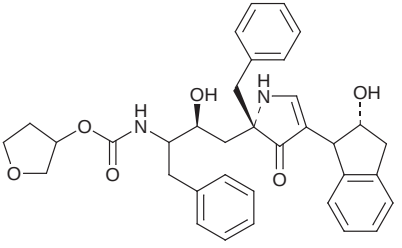


(142)

in dogs (13%) but had a short duration of action ($t_{1/2}$ 30 min).[429] AG1343 (**144**) is a potent enzyme inhibitor (K_i 2 nM) and antiviral agent (HIV-1 ED_{50} 14 nM) with oral bioavailability in the range of 17-47% in rats, dogs, marmosets and cynomolgus monkeys. An X-ray cocrystal structure of the enzyme and AG1343 complex revealed that the thiophenyl ether and phenol amide substituents interact with the S_1 and S_2 subsites of HIV-1 protease, respectively. Palinavir (**145**) is a potent inhibitor of the human HIV type 1 (K_i 0.031 nM) and type 2 proteases (K_i 0.134 nM).[432]



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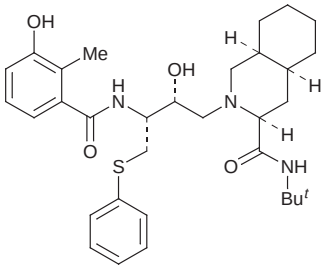
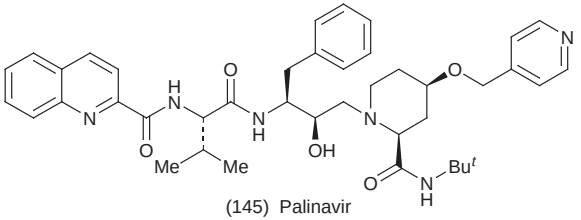
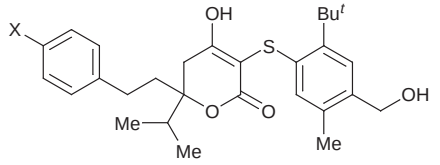


(144) Viracept, AG1343



(145) Palinavir

Many other non-peptide inhibitors of HIV protease have been reported. Chemically, most of these inhibitors are based on additional SAR studies on the earlier reported dihydropyrone, cyclic urea based and sulfamide series of compounds.[434–452] The main purpose behind the design was to obtain compounds with improved pharmacokinetic profile and more resistant to viral mutations. Compounds (**146-149**) represent some of the examples of different structural types. After oral administration in mice at a dose of 25 mg kg^{-1}, (**146**) (X = OH or NH_2) showed oral bioavailability between 80-96%.[434] Compound (**147**) (PNU-140690) showed activity against a variety of HIV type 1 laboratory strains, clinical isolates and other variants resistant to other protease inhibitors.[436] One of the cyclic urea-based compound (**148**) (IC_{90} = 8.7 nM) provided modest bioavailability in dogs at a dose of 10 mg kg^{-1}. However, no blood levels could be detected in rats at the same dose.[440] The observed half-life for (**149**) was 5.6 hours after i.v and 10 hours after oral dosing in the female Beagle dog. The corresponding benzimidazole analogue showed similar *in vitro* and *in vivo* profile.[444]



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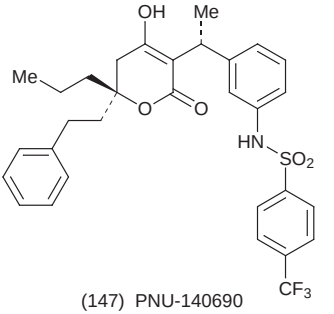
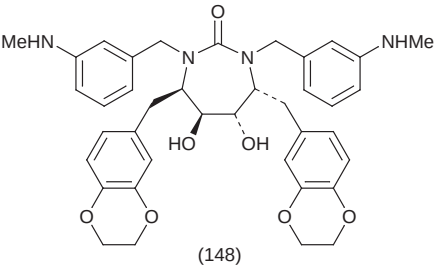


(147) PNU-140690



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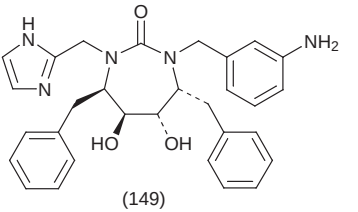

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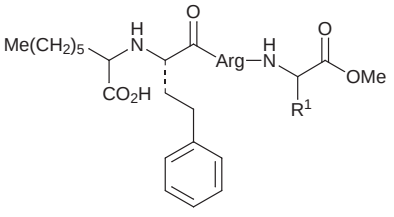
5.9 Matrix Metalloproteinase Inhibitors – As in previous years, inhibitor design has been based on thiol, carboxyalkyl or hydroxamate derivatives. Mercaptoacyl derivatives were reported as inhibitors of MMP-3, MMP-8 and MMP-9. Replacement of the HS-CH_2-CO- group in HS-CH_2-CO-Leu-Phe-NHMe (IC_{50} MMP-3 2.6 μM, MMP-8 0.05 μM and MMP-9 0.09 μM) by Me-S-CH_2-CO-, HS-CH_2-CH_2-CO- and CH_3-CH_2-CO- groups resulted in much less potent compounds.[453]

Replacement of the P_1' leucine residue by Thr reduced potency. However, the P_1' Met and P_2' Trp analogues were somewhat more potent. HS-CH_2-CO-Met-Trp-NHMe was similar in potency (IC_{50} MMP-3 0.81 μM, MMP-8 0.02 μM and MMP-9 0.012 μM) to HS-CH_2-CO-Met-Phe-NHMe and HS-CH_2-CO-Leu-Trp-NHMe analogues. When administered orally (40 mg kg^{-1}), HS-CH_2-CO-Leu-Phe-NHMe inhibited (40%) the hind paw swelling in an *in vivo* adjuvant arthritic rat model of rheumatoid arthritis.[453] Additional modifications of the mercaptoacyl moiety in the HS-CH_2-CO-Val-Phe-NHMe type of inhibitors generated HS-CH(R)-CO-Val-Phe-NH-Me type of compounds. Like the parent Val-Phe compound [MMP-8, MMP-3 and MMP-9 IC_{50} values 2.60, 0.05 and 0.09 μM, respectively], the compounds containing a -CH_2-CH_2-NPhth, -$(CH_2)_3$-NPhth, -$(CH_2)_4$-NPhth, -$(CH_2)_3$-COOMe or a -$(CH_2)_3$-NH-SO_2Me substituent inhibited all the three enzymes [MMP-8, MMP-3 and MMP-9 IC_{50} values 0.49-0.86, 0.014-0.125 and 0.001-0.031 μM, respectively].[454]

Using a combinatorial library of N-carboxyalkyl tripeptides [HOOC-CH(COOH)-tripeptide-OMe], compounds like (**150**) [R1 = Nle, Ile, hPhe, Trp and Gln side chains] were identified as moderately potent inhibitors of MMP-3 (stromelysin) (IC_{50} values 0.4-0.9 μM).[455] The design of other similar compounds was based around compounds reported previously as MMP-3 inhibitors.[456–458] A combination of a biphenylethyl moiety at P_1', a *tert*-butyl group at P_2', and a methyl group at P3′ led to orally bioavailable inhibitors of stromelysin-1 (MMP-3) and gelatinase-A (MMP-2), but poor inhibitors of collagenase (MMP-1). Compound (**151**) (R = 4-F) was one of the more potent inhibitors (K_i MMP-3 10 nM and MMP-2 17 nM) and showed no activity against MMP-1 (K_i >10 μM). Other analogues containing a 3-NH_2, 4-CF_3, 3-CF_3, 4-Cl, 2,4, 4-Ph, 3-Cl,4-F, 4-Br, 3-F, 2-F, 4-CN, 4-SCH_3, 4-SO_2CH_3, 4-OCH_3 or a 4-(2-imidazolyl) groups in place of the 4-F in compound (**151**) were also moderately potent inhibitors of MMP-3 and MMP-2 (K_i values MMP-3 5-39 nM and MMP-2 25-280 nM). Although some of the above compounds were 2-10-fold more potent against MMP-3 than against MMP-2, many of the compounds (R = 4-CF_3, 4-SO_2CH_3, 4-CHO, 4-COOH, 4-$COOCH_3$, 4-$CONHCH_3$ and 4-$CON(CH_3)_2$ were nearly equipotent against both the enzymes. Given orally, compound (**151**) was active in



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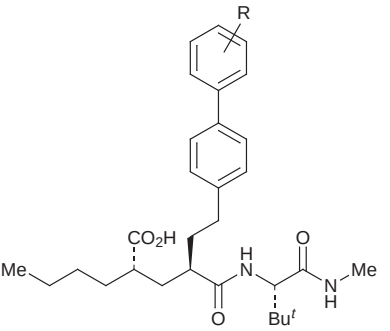
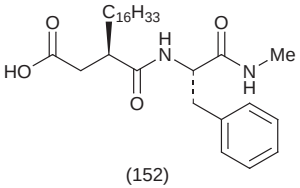


(151) R = 4-F L-758,354

the mouse pleural cavity assay (ED_{50} 11 mg kg^{-1}, inhibition of the degradation of radiolabelled transferrin by human MMP-3). In other cartilage destruction and inflammatory arthritis (adjuvant-induced or collagen-induced) models, compound (**151**) did not show any activity up to a dose of 50 mg kg^{-1}.[456]

Peptidomimetic carboxylate- and hydroxamate-based inhibitors containing extended P_1' groups (C_6H_{13} to $C_{16}H_{33}$) were reported to be selective for MMP-2 inhibition.[459] Phe derivative (**152**) was one of the most potent carboxylate containing inhibitor [IC_{50}s MMP-1 30 μM, MMP-2 50 nM, MMP-3 50 μM]. All the other compounds (C_6H_{13} to $C_{15}H_{31}$) were poor inhibitors of all three enzymes (IC_{50} values >200 nM). The corresponding hydroxamate derivative of (**152**) appeared to be somewhat less selective [IC_{50}s MMP-1 (20% inhibition at 100 μM), MMP-2 20 nM, MMP-3 300 nM, matrilysin (MMP-7) 20 nM]. Unlike the carboxylate series of compounds, the hydroxamate compounds containing a Phe in P_2' position with C_6H_{13} to $C_{16}H_{33}$ side chain in the P_1' position retained good MMP-2 inhibitory activity (IC_{50} values <10 nM). In the hydroxamate series containing a t-butyl group in the P_2' position, (**153**) was one of the most potent and selective inhibitor [IC_{50}s MMP-1 5 μM, MMP-2 0.6 nM, MMP-3 90 nM, matrilysin 5 μM]. In this series, several of the carboxylate analogues ($C_{13}H_{27}$ to $C_{16}H_{33}$) retained significant MMP-2 inhibitory activity (IC_{50} <100 nM).[459]



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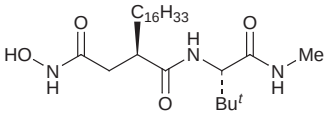
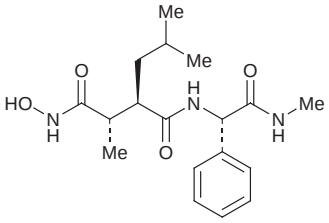


(153)

In a series of hydroxamic acid derivatives containing an isobutyl group in the P_1' position, modifications in the P_2' position and at the α-substituent of the hydroxamic acid moiety were attempted in an attempt to improve oral activity. A large number of substituents in both positions gave potent inhibitors of MMP-1 and MMP-9 (IC_{50} values 0.2-15 nM). The phenylglycine analogues like (**154**) showed oral activity.[460] Oral administration of (**154**) to adjuvant arthritic rats (100 mg kg^{-1} twice a day) for 20 days inhibited increases in hind foot pad swelling on days 17 and 20. Non-peptide hydroxamate derivatives like (**155**) were obtained by SAR studies around a targeted screening lead.[461] In an *ex vivo* rat model and an *in vivo* rabbit model of stromelysin-induced cartilage degradation, compounds devoid of any substituent adjacent to the hydroxamic acid were short acting after oral administration. In comparison, the more potent D-valine derived inhibitor (**155** GCS 27023) prevented cartilage degradation for up to 8 hours. In the human recombinant assays, (**155**) was found to be active against a variety of metalloproteases (K_i values, stromelysin, 43 nM; MMP-1, 33 nM; MMP-2, 20 nM; MMP-9, 8 nM). Some of the conformationally restricted hydroxamate derivatives were poor inhibitors of gelatinase A and gelatinase B.[462]



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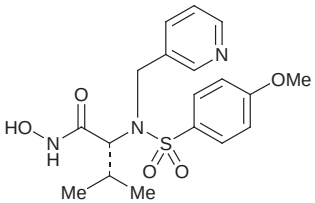
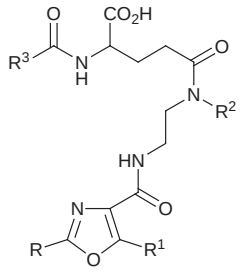


(155)

5.10 Phosphatase Inhibitors (Ser/Thr or Tyr) – Many cellular processes are regulated by reversible phosphorylation of proteins containing Ser, Thr or Tyr residues. Phosphorylation of these amino acids is catalysed by protein kinases, whereas dephosphorylation is catalysed by the protein phosphatases. Various aspects of these enzymes including endogenous protein and peptide inhibitors are reviewed.[463–466] Using crystallography studies on crystals of protein-tyrosine phosphatase 1B bound either to a high affinity ligand [bis-(*p*-phosphophenyl) methane] or phosphotyrosine, a new aryl phosphate binding site (in addition to the active site) was identified adjacent to the active site.[467]

Protein-tyrosine phosphatase inhibitors based on a hexapeptide, Asp-Ala-Asp-Glu-Xxx-Leu (Xxx = non-hydrolysable phosphotyrosine mimic phosphonomethyl-Phe, difluorophosphonomethyl-Phe or O-malonyl-Tyr), have been reported previously. Based on this work, cyclic peptide analogues of Asp-Ala-Asp-Glu-Tyr(malonyl)-Leu are reported.[468] In comparison to the linear peptide, Ac-Asp-Ala-Asp-Glu-Tyr(malonyl)-Leu-NH_2 (K_i 13 μM), and the cyclic peptide containing one glycine residue, c(Asp-Ala-Asp-Glu-Tyr(malonyl)-Leu-Gly) (K_i 25.2 μM), the ring enlarged compound containing two glycine residues, c(Asp-Ala-Asp-Glu-Tyr(malonyl)-Leu-Gly-Gly) (K_i 2.6 μM), was more potent. The most potent cyclic peptide, c(Asp-Ala-Asp-Glu-Tyr(malonyl)-Leu-Cys(S-CH_2-CO-) (K_i 0.73 μM), was obtained by linking the Cys side chain to the N-terminal Asp residue. Cyclic peptides containing a Tyr residue in place of the O-malonyl-Tyr, c(Asp-Ala-Asp-Glu-Tyr-Leu-Gly-Gly) and c(Asp-Ala-Asp-Glu-Tyr-Leu-Cys(S-CH_2-CO-), were inactive (K_i >800 μM).[468]

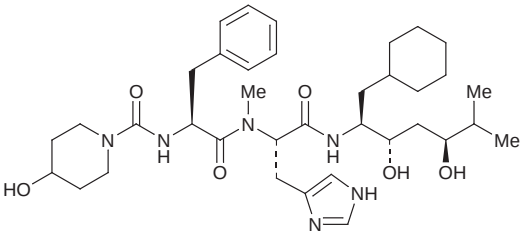
Based on functional groups present in natural product serine/threonine protein phosphatase inhibitors, a pharmacophore model (**156**) was used to synthesise a library of compounds using glutamic acid as a template (R = Ph, R^1 = CH_3 or Ph, R^2 = CH_3, *n*-C_6H_{13} or benzyl and R^3 = -CH_2-CH_2-Ph, CH=CH-Ph or *n*-C_9H_{19}).[469,470] With the exception of a few compounds, all lacked significant growth inhibitory activity. Compound (**156**, R = Ph, R^1 = CH_3, R^2 = benzyl and R^3 = -CH_2-CH_2-Ph) caused 50% growth inhibition at 20 μM but had no further cytotoxicity at higher concentrations. A similar compound (**156**, R = Ph, R^1 = CH_3, R^2 = benzyl and R^3 = *n*-C_9H_{19}) caused 50% growth inhibition at 100 μM and had a clear concentration-dependency.[470] Another similar compound (**156**, R = Ph, R^1 = Ph, R^2 = , R^3 = n-C_6H_{13}) had a K_i of approximately 10 μM for recombinant human Cdc25A, -B, and -C, and a K_i of 0.85 μM for the PTP1B.



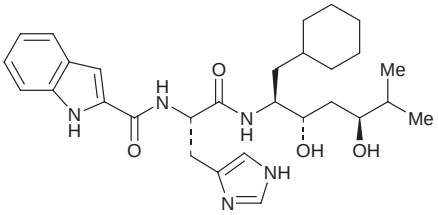
(156)

5.11 Renin Inhibitors – A series of renin inhibitors containing the (2S,3S,5S)-2-amino-1-cyclohexyl-6-methyl-3,5-heptanediol fragment as a transition state mimic were reported.[471,472] One of the more potent compounds (**157**, IC_{50} 0.68 nM) with a 4-hydroxypiperidine residue at the P_4 position lowered blood pressure in salt-depleted, conscious marmosets after oral administration at a dose of 10 mg kg^{-1} for a period of 5 hours. The 4-hydroxypiperidine group in the Phe-MeHis derivative (**157**) could be replaced by a number of other groups with retention of the renin inhibitory activity. The hydroxy group of the 4-hydroxy-piperidine moiety could be replaced by a methoxy or an acetoxy group with slight increase in *in vitro* activity but both the compounds were less potent *in vivo* in marmosets. Compound (**157**) did not inhibit pepsin, cathepsin D and human ACE (IC_{50} >100 μM).[471] The N-terminal P_4 and P_3 substituents in (**157**) were replaced with smaller heterocyclic groups. Four of the more potent analogues containing an indole-2-carbonyl-, 5-F-indole-2-carbonyl-, benzimidazole-2-carbonyl- or isoquinolin-1-one-3-carbonyl groups at the N-terminus gave moderately potent inhibitors of human plasma renin (IC_{50} values 13-48 nM). The indole-2-carbonyl- analogue (**158**) inhibited human, cynomolgus monkey and marmoset renin (IC_{50} values 42-93 nM). It was weakly active against dog renin (IC_{50} 3500 nM) and a very poor inhibitor of rat renin, porcine pepsin, bovine cathepsin D and human converting enzyme (2-40% inhibition at >10 μM). In salt-depleted, conscious marmosets, the blood pressure lowering activities of the more potent analogues were not always parallel to their *in vitro* enzyme inhibitory activity. For example, the most potent benzimidazole analogue (IC_{50} 13 nM) was only weakly active in the marmosets at a dose of 10 mg kg^{-1}, po. At the same dose, the indole-2-carbonyl- (**158**, JTP-3072) and 5-F-indole-2-carbonyl- derivatives caused significant reduction in blood pressure up to a period of three hours.[472] Non-peptide inhibitors like (**159**) (R = $-OCH_2COOCH_3$, $-OCH_2CONH_2$ or $-OCH_2SO_2CH_3$) inhibited human renin (IC_{50} values 6, 20 and 13 nM, respectively).[473]

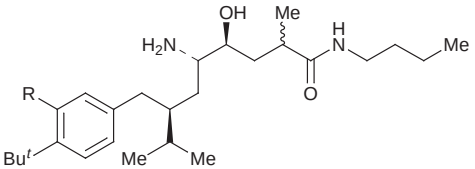
5.12 Thrombin Inhibitors (Serine Protease) – Many additional SAR studies directed towards improving the pharmacokinetic profiles of the inhibitors reported in previous years have been described. A library of substrates carrying substitutions at P_1, P_2 and P_3 positions were used to study the requirements of S_1,



(157) JTP2724



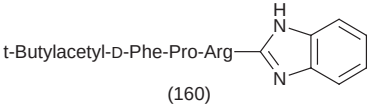
(158) JTP-3072



(159)

S_2 and S_3 sites of thrombin.[474] Thrombin catalysed hydrolysis of N-ethoxycarbonyl-D-Phe-Pro-α-AzaLys *p*-nitrophenyl ester, Z-Pro-α-AzaLys *p*-nitrophenyl ester and N-α-(N,N-dimethylcarbamoyl)-α-AzaLys *p*-nitrophenyl ester was studied. Decarboxylation following the enzymatic hydrolysis of these peptides gave the corresponding hydrazines which were competitive inhibitors of thrombin.[475] Several other D-Phe-Pro-Arg derivatives containing P_1-argininoyl heterocycle (benzoxazole, benzimidazole or thiazole rings) were reported.[476,477] The benzimidazole analogue (**160**) retained thrombin inhibitory activity (IC_{50} 0.092 μM) but showed much reduced potency against factor Xa (IC_{50} >2.5 μM), trypsin (IC_{50} 1.01 μM) and plasmin (IC_{50} >2.5 μM).[476] The corresponding benzoxazole derivative inhibited thrombin (IC_{50} 0.068 μM), factor Xa (IC_{50} 0.11 μM), trypsin (IC_{50} 0.0023 μM) and plasmin (IC_{50} 1.32 μM). Replacement of the t-butylacetyl-D-Phe-Pro by Ac-D-Phe-α-naphthyl-Ala resulted in loss of thrombin inhibitory activity (IC_{50} >2.5 μM), but improvement in factor Xa (IC_{50} 0.029 μM), and plasmin (IC_{50} 0.014 μM) inhibitory activities. Thiazole compounds like (**161**) inhibited both thrombin and trypsin.[477] $Ph(CH_2)_3CO$-Pro-Arg-thiazole was an inhibitor of thrombin (IC_{50} 2 μM) and trypsin (IC_{50} 0.23 μM) and a poor inhibitor of prolyl endopeptidase and elastase (IC_{50}s 28 μM and >1000 μM, respectively). The corresponding lysine derivative $Ph(CH_2)_3CO$-Pro-

Lys-thiazole inhibited prolyl endopeptidase, thrombin and trypsin (IC_{50}s 7.8, 46 and 2.4 μM, respectively). Three other analogues, $Ph(CH_2)_3CO$-Pro-Pro-thiazole, $Ph(CH_2)_3CO$-Pro-Ala-thiazole and $Ph(CH_2)_3CO$-Pro-Val-thiazole, inhibited prolyl endopeptidase (IC_{50}s 4.4 nM, 5.0 nM and 7.7 μM, respectively) but were inactive against elastase, thrombin and trypsin (IC_{50} >1000 μM).



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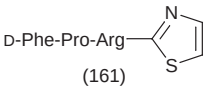
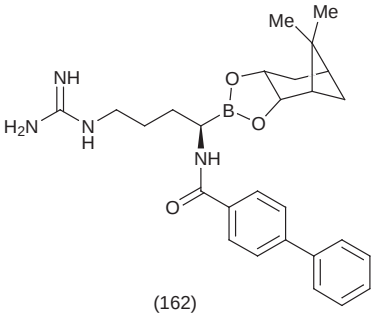


(161)

Various boroArg derivatives have been reported.[478,479] One of the more potent compounds (**162**) inhibited thrombin and trypsin (K_i values 0.94 and 33 nM, respectively). Replacement of the *p*-substituted biphenyl by the *m*-substituted biphenyl derivative led to a 100-fold reduction in thrombin inhibitory potency but a 5-fold improvement in trypsin inhibitory activity (K_i values 110 and 7.4 nM, respectively). In the *p*-substituted biphenyl compounds (e.g. **162**), additional substituents (*o*-CH_3, *o*-F, *o*-NO_2, *o*-NH_2, *m*-NO_2, *m*-NH_2, CF_3, SO_2NHEt, SO_2NH-t-Bu, SO_2NH_2 and $SO_2NHCOOCH_3$) on one of the phenyl rings did not lead to any significant enhancement of the thrombin inhibitory potency. Many of the analogues lost most of the selectivity between thrombin and trypsin inhibition.[479] Based on some the P_1-ketoargininamide containing thrombin inhibitors reported earlier (e.g. Boc-Asp-Pro-Arg-CO-NH-CH_2-CH_2-Ph, CVS863), P_2-P_4-azapeptidomimetic derivatives have been synthesised. Most potent thrombin inhibitor of the series was the AzPhe analogue (**163**) (IC_{50} 880 nM). A number of other analogues containing an acetyl group in place of the Boc group or AzAsp or AzhPhe in place of AzPhe were much less potent (IC_{50} values 3.2-22 μM).[480]



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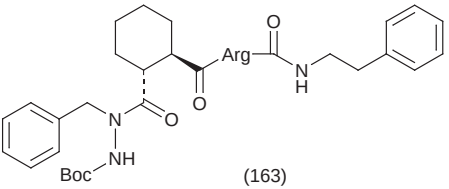
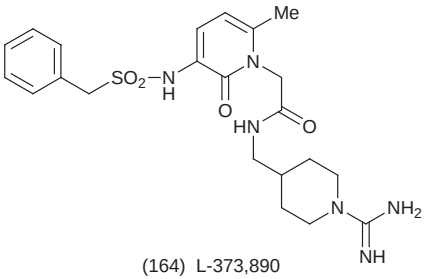


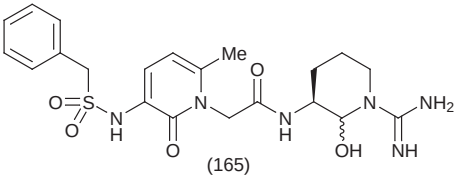
(163)

A combination of 3-amino-2-pyridinone skeleton, used in the case of elastase inhibitors as a Gly-Pro amide bond replacement, and *trans*-cyclohexylamine used in thrombin inhibitors, led to compounds which inhibited thrombin. Further SAR, X-ray and modelling studies have resulted in compounds like (**164**) and (**165**). Compound (**164**) (L-373890) inhibited thrombin (K_i 0.5 nM) and trypsin (K_i 570 nM), but was inactive ($K_i \geq 20$ μM) against plasmin tPA, activated

protein C, plasma kallikrein and chymotrypsin. The corresponding cyclohexylamine derivative was about 10-fold less potent. An intravenous infusion of L-373890 (10 μg $kg^{-1}min.^{-1}$) to rats prevented occlusion in a ferric chloride model of arterial thrombosis. Replacement of the Ph-CH_2-SO_2- group by 4-MePh-SO_2-, PhCO-, Ph$(CH_2)_2$CO-, Ph_2CH-CH_2CO- and Ph-CH_2-O-CO- groups gave less potent compounds.[481] Compound (**165**) inhibited thrombin (IC_{50} 0.46 nM), factor Xa (IC_{50} 446 nM) and trypsin (IC_{50} 10.9 nM) but not plasmin and tPA (IC_{50} >2500 nM). The corresponding des-methyl analogue was less selective (IC_{50} thrombin 0.50 nM, factor Xa 20.9 nM and trypsin 26.2 nM; plasmin and tPA IC_{50} >2500 nM).[482] The des-methyl analogue demonstrated oral efficacy in a rat arterio-venous shunt model of thrombosis with an ED_{50} of 2.9 mg kg^{-1}. Examples of other compounds containing P_2-P_3 dipeptide surrogates include (**166**) and (**167**). Compound (**166**) inhibited both thrombin and trypsin (IC_{50} values 16.4 and 11.6 nM, respectively) but did not have much effect on factor Xa and plasmin (IC_{50} values >2500 and 923 nM, respectively).[483] The tetrahydroquinolyl sulfonamido derivative (**167**) (LR-D/009) inhibited thrombin (IC_{50} = 0.018 μM), with good selectivity over plasmin (IC_{50} 1.04 μM) and trypsin (IC_{50} 0.1 μM). The corresponding naphthalene sulfonamide and benzyl sulfonamide derivatives were much less potent (thrombin IC_{50} 0.1 μM, plasmin IC_{50} 10 μM).[484]

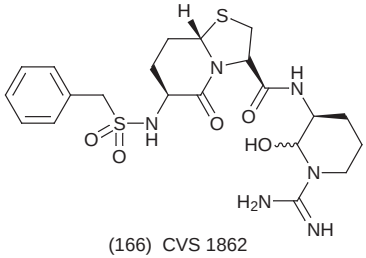


(164) L-373,890

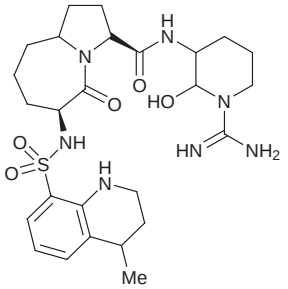


(165)

D-Dicyclohexylalanine, D-diphenylalanine and 3,3-diphenylpropionic acid derivatives have been used as a P_3 ligand in various thrombin inhibitors.[485–487] Many of the compounds were potent inhibitors of thrombin. In some cases, e.g. (**168**), increased hydrophobicity led to enhanced oral absorption but this was not always associated with increased potency in thrombosis models. The des-Boc analogue of (**168**) performed well in an antithrombotic assay (1/5 occlusions at a dose of 6 μg kg^{-1} min^{-1}, i.v.), while the much more lipophilic but essentially equipotent analogue (**168**) performed poorly (4/6 occlusions at the same dose).[485]

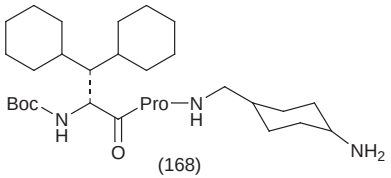


(166) CVS 1862



(167)

A number of compounds like (**169**) exhibited oral bioavailability in rats and dogs, and were efficacious in a rat $FeCl_3$-induced model of arterial thrombosis.[487] Compound (**169**) showed the best overall profile of *in vivo* and *in vitro* activities (K_i thrombin 2 nM, K_i trypsin 353 nM). Other 3,3-disubstituted propionic acid derivatives, e.g. 3,3-dicyclohexyl propionic acid, 3-pyridyl-3-phenyl propionic acid and 3-(2-pyridyl)-3-phenyl propionic acid, were also potent inhibitors of thrombin (K_i 2-30 nM).



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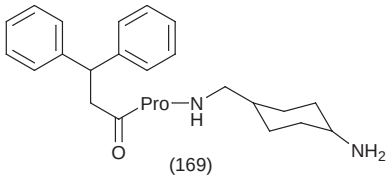
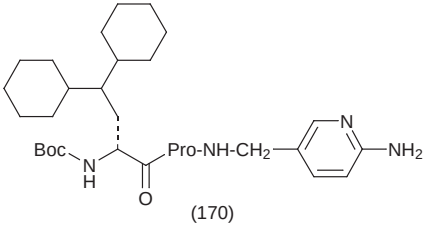


(169)

An aminopyridyl moiety has been shown to be beneficial in the P_1 position. This change, discovered from a random screening lead, was then incorporated in compounds containing D-diphenylalanine, D-dicyclohexylalanine and 3,3-diphenylpropionic acid derivatives in the P_3 position.[488–490] A number of other publications have appeared describing inhibitors containing various substitutions at the N- and C-terminal ends and P_3-P_4 modifications obtained either by SAR studies or by random screening.[491–497] Examples of such compounds include (**170-174**). Biological studies on CVS-1123, [$(CH_3\text{-}CH_2\text{-}CH_2)_2$-CH-CO-Asp($OCH_3$)-Pro-Arg aldehyde] have been reported.[498] Compounds like (**170**) and the corresponding analogues with an unprotected amino group at the N-terminus showed selectivity [(K_i 0.8-12 nM), 300-1500-fold selectivity for thrombin compared with trypsin] and oral bioavailability (40-76%) in rats or dogs. The design of compounds like (**171**) (R = -CH_3 or -NH_2) was based on a comparison of the crystal structures of thrombin and trypsin which indicated that the specificity pockets of the two enzymes were very similar except for the presence of Ala^{190} in thrombin replacing Ser^{190} in trypsin thus making the thrombin specificity pocket slightly bigger and less polar.[493] Replacement of the N-Me guanidino group of compound (**172**) by an ethyl guanidino group led to a much less potent compound. In compounds like (**173**) (piperazide derivatives of 3-amidinophenyl-

alanine), the benzamidine moiety of 3-amidinophenylalanine mimics the guanidinoalkyl side chain of the arginine residue present in most of the thrombin inhibitors.[495] Several of the analogues (**173**, R = -CH_3, -CH_2-CH_2OH, -CHO, -CO-CH_3, -$CON(CH_3)_2$, -SO_2-CH_3, -SO_2-CH_2-CH_3, -SO_2-C_6H_5, SO_2-C_6H_4-4-CH_3, -SO_2-β-$C_{10}H_7$) were potent inhibitors of thrombin (K_i 0.0033 to 0.3 μM) and poor inhibitors of factor Xa and plasmin. Two of the more potent and selective inhibitors of thrombin (**173**, R = -$CON(CH_3)_2$ or -SO_2-CH_3) (K_i 0.0033 and 0.0088 μM) were more than 5,000-fold less potent against factor Xa and plasmin and about 50-fold less potent against trypsin. A number of other thrombin inhibitors (**173**, R = H, Ph, -CO-*n*-C_3H_7, -CO-*n*-C_5H_{11}, -CO-*c*-C_6H_{11}, -CO-Ph, -$COOC_2H_5$, -COOPh) were either equipotent or more potent against trypsin. The L-amidinophenylalanine derivatives were much more potent inhibitors of thrombin than the corresponding D-analogues.



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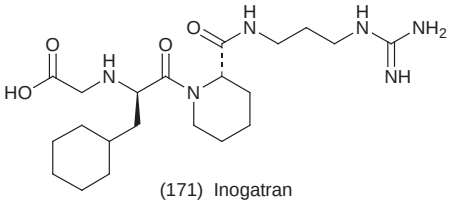
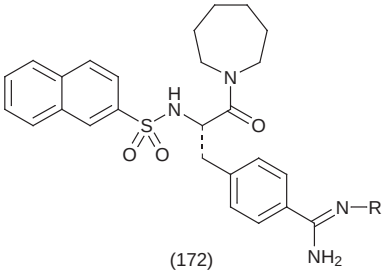


(171) Inogatran



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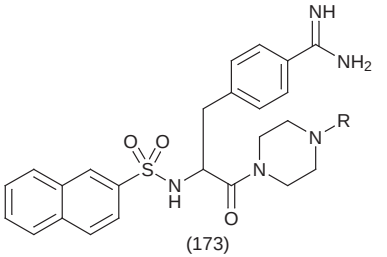
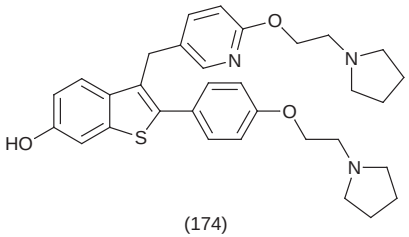


(173)

5.13 Miscellaneous [$p60^{c\text{-}src}$ Protein Tyrosine Kinase, Serine Proteases (Trypsin and Chymotrypsin), Papain and Prolyl Endopeptidase] Inhibitors – The heptapeptide Gly-Ile-Tyr-Trp-His-His-Tyr was identified as a substrate for $p60^{c\text{-}src}$ protein



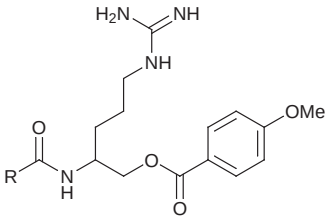
(174)

tyrosine kinase (PTK) by screening a secondary random peptide library. The substrate was modified to obtain several pseudosubstrate-based peptide inhibitors.[498] Some of these peptide inhibitors [e.g. Gly-Ile-Nal(2)-Trp-His-His-D-Nal(2), Gly-Ile-D-Nal(2)-Trp-His-His-D-Nal(2) and Gly-Ile-Nal(2)-Trp-His-His-Nal(2)] were moderately potent inhibitors of the enzyme (IC_{50} values ~ 4 μM). Some other derivatives containing a single Nal(2) residue, Gly-Ile-D-Nal(2)-Trp-His-His-Tyr, Gly-Ile-Nal(2)-Trp-His-His-Tyr, Gly-Ile-Nal(2)-Trp-His-His, Gly-Ile-D-Nal(2)-Trp-His-His, were less potent (IC_{50} values 7-26 μM). Because both Tyr-Ile-Tyr-Gly-Ser-Phe-Lys and Gly-Ile-Tyr-Trp-His-His-Tyr are efficient and specific substrates for $p60^{c\text{-}src}$ PTK, chimeric branched peptides based on these two sequences were synthesised. These branched peptides [Tyr-Ile-Nal(2)-Gly-Lys(N^{ε}-Trp-His-His)-Phe-Lys, Tyr-Ile-Tyr-Gly-Lys(N^{ε}-Trp-His-His)-Phe-Lys, Ile-Nal(2)-Trp-Lys(N^{ε}-Ser-Phe-Lys)-His-His) and Tyr-Ile-Nal(2)-Gly-Lys(N^{ε}-His-His)-Phe-Lys] inhibit $p60^{c\text{-}src}$ PTK with improved potency (IC_{50} values 0.6-3.6 μM), indicating that the enzyme-active site of $p60^{c\text{-}src}$ PTK can accommodate more than a linear motif. The most potent compound of the series Tyr-Ile-Nal(2)-Gly-Lys(N^{ε}-Trp-His-His)-Phe-Lys was selective for $p^{60c\text{-}src}$ protein tyrosine kinase over other Src family protein tyrosine kinases such as Lyn and Lck kinases.[498]

Several publications have appeared on serine proteases like chymotrypsin and trypsin.[499–503] Using DNA recombinant technology, the requirements at the P_1 binding sites of serine proteases [bovine chymotrypsin Aα, porcine pancreatic elastase, subtilisin Carlsberg, *Streptomyces griseus* proteinase A and B and human leukocyte elastase] have been investigated.[499] The most specific coded residue for these six enzymes were Tyr, Leu/Ala, Cys, Cys, Cys and Ile, respectively, and the worst amino acid residue in P_1 position were Pro, Arg, Pro, Pro, Pro and Asp, respectively. The dominant force for interaction at the P_1 position appears to be the hydrophobic interaction. A synthetic library of cyclic peptides [Ser-Cys-X-X-Ser-X-Pro-Pro-Gln-Cys-Tyr-$(Gly)_5$], based on an anti-tryptic loop region of the Bowman-Birk inhibitor, led to the discovery of chymotrypsin inhibitors like Ser-Cys-Thr-Phe-Ser-Ile-Pro-Pro-Gln-Cys-Tyr-$(Gly)_5$, Ser-Cys-Thr-Phe-Ser-Leu-Pro-Pro-Gln-Cys-Tyr-$(Gly)_5$, Ser-Cys-Thr-Phe-Ser-Nle-Pro-Pro-Gln-Cys-Tyr-$(Gly)_5$ and Ser-Cys-Thr-Tyr-Ser-Ile-Pro-Pro-Gln-Cys-Tyr-$(Gly)_5$ (Ki values 17-20 nM).[500] Peptides containing a C-terminal arginol residue linked, via an ester bond, to anisic acid (*p*-methoxy benzoic acid) (**175**) were reported as inhibitors of serine proteinases.[501,502] Boc-Ile-Glu-Gly-Arg$\psi(CH_2O)$-CO-C_6H_4-OMe and Boc-D-Phe-Pro-Arg$\psi(CH_2O)$-CO-C_6H_4-OMe

function as potent time-dependent irreversible inactivators of factor Xa and trypsin, respectively. Boc-D-Phe-Pro-Argψ(CH_2O)-CO-C_6H_4-OMe designed to inactivate thrombin behaves only as a competitive reversible inhibitor. Additionally, Ac-Arg-Gln-Argψ(CH_2O)-CO-C_6H_4-OMe, modelled on a potent chloromethyl ketone inactivator of tryptase, did not inhibit tryptase.

Hepatitis A virus (cysteine protease) is the enzyme responsible for the processing of the viral polyprotein. A peptidyl monofluoromethyl ketone (**176**) based on the peptide substrate of hepatitis A virus was found to be an irreversible inhibitor of the enzyme.[504] Peptidyl boronic acids (based on substrates) were investigated as inhibitors of cysteine protease papain. None of the peptides showed any inhibition up to a concentration of 10 mM.[505]



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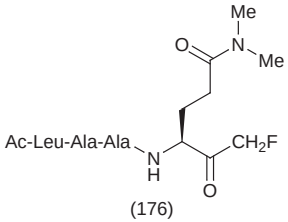
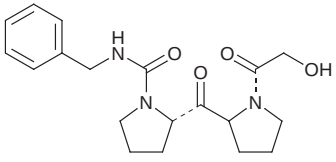


(176)

An orally active prolyl endopeptidase inhibitor (**177**, JTP-4819) has been reported to increase neuropeptides in the brains of old rats resulting in improvements in learning acquisition and memory retention. Oral administration of the compound to healthy male volunteers in single and multiple doses (3 times a day) of 30-120 mg kg^{-1} did not lead to any adverse side effects.[506] Repeated treatment of the senescence-accelerated mouse with another prolyl endopeptidase inhibitor **178** (Y-29794) (1, 10 or 20 mg kg^{-1}, oral) resulted in a significant reduction in the number and density of Aβ-positive granular structures in the hippocampus of the mouse.[507]



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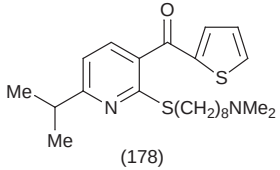


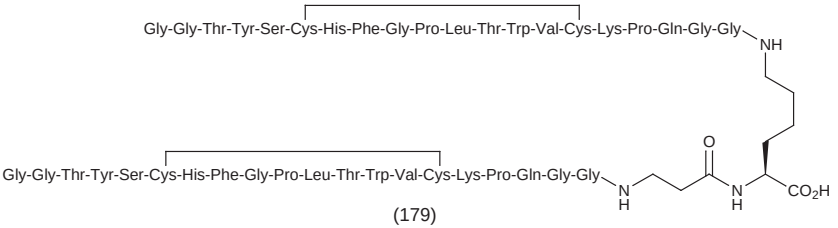
(178)

6 Phage Library Leads

Filamentous bacteriophage can display foreign peptides and proteins on the surface of the virus particles by means of fusion to the coat proteins. The

technology permits the construction of molecular libraries of variants that can be searched *in vitro* by affinity selection and can be used also for receptor mapping and studying the conformational properties of the peptides. Other surface expression systems such as bacterial cell or animal virus have also been devised. A recent publication has described the use of baker's yeast, *Saccharomyces cerevisiae*, as the displaying particle.[508] The phage display technology has been used for detecting agonist/antagonist ligands acting at various receptors, epitope mapping, enzyme inhibitors, protein-protein interaction inhibitors, protein-ligand interaction inhibitors, generating high affinity antibodies and organ targeting of drugs. Some aspects of the work are described in recent reviews.[509–511]

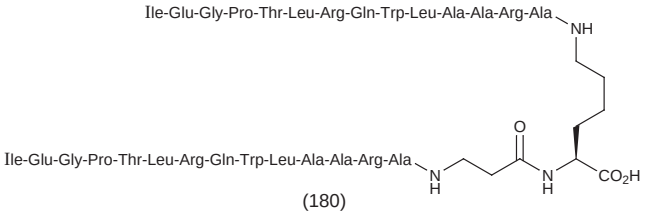
6.1 Erythropoietin and Thrombopoietin Mimetics – Using a soluble form of erythropoietin receptor extracellular domain and Cys-Cys cyclic peptide phage libraries, a new erythropoietin receptor ligand, Gly-Gly-Cys-Arg-Ile-Gly-Pro-Ile-Thr-Trp-Val-Cys-Gly-Gly, was identified (apparent affinity β 10 μM).[512–514] A mutagenesis library containing peptides of increased length led to more potent compounds like Gly-Gly-Thr-Tyr-Ser-Cys-His-Phe-Gly-Pro-Leu-Thr-Trp-Val-Cys-Lys-Pro-Gln-Gly-Gly (EMP1). Some of the high affinity peptides were found to be full agonists (EC_{50}s 200-400 nM) in an erythropoietin responsive cell line assay. Based on the crystal structure of a complex of erythropoietin mimetic peptide and erythropoietin receptor extracellular domain, a dimeric form of erythropoietin mimetic peptide containing two disulfide bridges (one in each chain) was synthesised (**179**). In comparison to EMP1 (about 100,000 times less potent than erythropoietin in stimulating erythropoiesis *in vivo* in mice), the dimeric peptide (**179**) was about 100-fold more potent than EMP-1 in the same assay.



(179)

Phage library screening has also yielded thrombopoietin mimetics.[515,516] Several linear [Gly-Arg-Val-Arg-Asp-Gln-Ile-Met-Leu-Ser-Leu-Gly-Gly (IC_{50} 20 μM), Leu-Ala-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-His-Gly-Asn-Gly-Arg-Asp-Thr (IC_{50} 20 nM)] and disulfide bridge containing cyclic peptides [Gly-Gly-Cys-Thr-Leu-Arg-Glu-Trp-Leu-His-Gly-Gly-Phe-Cys-Gly-Gly (IC_{50} 200 nM) and Gly-Gly-Cys-Ala-Asp-Gly-Pro-Thr-Leu-Arg-Glu-Trp-Ile-Ser-Phe-Cys-Gly-Gly (IC_{50} 60 nM)] competed with thrombopoietin binding. One of the smallest peptides, Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala (IC_{50} 2 nM) (AF12505), was >1000-times less potent (EC_{50} 400 nM) than

thrombopoietin (EC_{50} 100 pM) in a thrombopoietin responsive cell line. A covalently linked dimer of AF12505 (**180**) had an IC_{50} of 0.5 nM and stimulated the thrombopoietin responsive cell line Ba/F3 to proliferate with an EC_{50} of 100 pM, equipotent to thrombopoietin and 4000 times as potent as the monomeric peptide AF12505. Five daily s.c. injections of AF13948 in mice (250 μg kg^{-1}) increased the platelet count by 80% compared to vesicle-treated animals.



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6.2 Angiogenin and Interleukin Antagonists and Epidermal Growth Factor Receptor Ligands – A peptide antagonist of human angiogenin (a tumour-associated angiogenic factor) [Ala-Gln-Leu-Ala-Gly-Glu-Cys-Arg-Glu-Asn-Val-Cys-Met-Gly-Ile-Glu-Gly-Arg, Cys-Cys disulfide bridge] was derived from a phage library.[517] Disulfide-constrained cyclic peptide inhibited the neovascularisation that is induced by angiogenin in the chick chorioallantoic membrane assay. The disulfide bond and Glu^9 were indispensable for its antiangiogenin activity. The Val^9 analogue was much less potent. The peptide blocked the angiogenesis that is induced by the angiogenin-secreting PC3 human prostate adenocarcinoma cells, without any direct effect on the proliferation, as well as the adhesion of PC3 cells to angiogenin.

Three of the peptides, Arg-Leu-Val-Tyr-Trp-Gln-Pro-Tyr-Ser-Val-Gln-Arg, Trp-Glu-Gln-Pro-Tyr-Ala-Leu-Pro-Leu-Glu and Arg-Glu-Tyr-Glu-Gln-Pro-Tyr-Ala-Leu-Trp, blocked binding of IL-1α to the full length IL-1 receptor type I with IC_{50} values of 45-140 μM.[518] Mutagenesis libraries were used to obtain high affinity ligands like Phe-Glu-Trp-Thr-Pro-Gly-Tyr-Trp-Gln-Pro-Tyr-Ala-Leu-Pro-Leu and Glu-Thr-Pro-Phe-Thr-Trp-Glu-Glu-Ser-Asn-Ala-Tyr-Tyr-Trp-Gln-Pro-Tyr-Ala-Leu-Pro-Leu. Further modifications of the leads (directed peptide libraries and synthesis) led to a 15 amino acid peptide, AF12198, Ac-Phe-Glu-Trp-Thr-Pro-Gly-Trp-Tyr-Gln-J-Tyr-Ala-Leu-Pro-Leu-NH_2 (IC_{50} 0.55 nM) (J = 2-azetidine-1-carboxylic acid), with both *in vitro* and *in vivo* IL-1 antagonist activity.[519] AF12198 selectively binds the human type I IL-1 receptor but not the human type II receptor or the murine type I receptor. *In vitro*, AF12198 inhibits IL-1-induced IL-8 production by human dermal fibroblasts (IC_{50} 25 nM) and IL-1-induced ICAM-1 expression by endothelial cells (IC_{50} 9 nM). When given as an intravenous infusion (16 to 72 mg kg^{-1} hr^{-1}) to cynomolgus monkeys, AF12198 blocks *ex vivo* IL-1 induction of IL-6 and down modulates *in vivo* induction of IL-6. Two major metabolites of AF12198 [Ac-Phe-Glu-Trp-Thr-Pro-Gly-Trp-Tyr-Gln-J-Tyr and Ac-Phe-Glu-Trp-Thr-Pro-Gly] were detected in plasma. AF12198 had an *in vitro* plasma $t_{1/2}$ of 2.6 hours.

Two phage libraries of human EGF variants in which Arg^{41} or Asp^{46} were randomised were tested in binding and signal transduction assays.[520] Most mutants containing a modification in position 46 (except Leu, Ile, His or Arg-containing) behaved similarly to the wild-type in both the assays. The Arg^{41} mutants were much less potent. The study has not been extended further to generate peptide agonists/antagonists.

6.3 Enzyme Substrates and Inhibitors [Tissue-type Plasminogen Activator and Urokinase-type Plasminogen Activator Inhibitors, Protein Tyrosine Kinase Substrates and Inhibitors and Calmodulin Kinase Inhibitors] – A plasminogen activator inhibitor 1 mutant phage display library was used to study in detail the interaction between plasminogen activator inhibitor 1 and either thrombin or an essential positively charged "loop" of tissue-type plasminogen activator.[521] Sequence analysis of 16 different cDNAs, encoding PAI-1 mutants that are hampered in the binding to thrombin-variable region 1, revealed that four independent variants shared a mutation of the P_4' residue ($Glu^{350} \rightarrow Lys$), nine independent PAI-1 variants shared a substitution of P_1'($Met^{347} \rightarrow Lys$), whereas three others shared a P_2 substitution ($Ala^{345} \rightarrow Asp$). Kinetic analysis of representative mutants provided evidence that the P_4' residue was essential for the interaction with the variable region 1 domain, whereas the P_1' and P_2 residues conferred thrombin specificity.

Investigation of the substrate specificity of protein tyrosine kinase $p55^{fyn}$ using phage libraries resulted in several leads. One of the synthetic peptides, Glu-Phe-Asn-Val-Tyr-Ser-Met-Met-Thr, most frequently found among the selected clones, was shown to be a good substrate of the enzyme.[522] Screening a 15-amino-acid bacteriophage peptide library for peptide sequences that bound $pp60^{c\text{-}src}$ indicated that more than 60% of the phage virions that bound to this enzyme contained a GXXG sequence motif in which X was frequently a hydrophobic residue (Phe, Val or Leu).[523] One of the synthetic peptides [Phe-Val-Gly-Phe-Leu-Gly-Phe-Leu-Gly] containing glycine residues was a competitive inhibitor of $pp60^{c\text{-}src}$ (K_i 24 μM), whereas the corresponding analogue containing proline residues [Phe-Val-Pro-Phe-Leu-Pro-Phe-Leu-Pro] had 100-fold decreased affinity for the enzyme (K_i 3.1 mM). Replacement of the Phe residues in the more potent inhibitor by Tyr [Tyr-Val-Gly-Tyr-Leu-Gly-Tyr-Leu-Gly] did not lead to a substrate for the enzyme.

Two calmodulin binding peptides, Trp-Asp-Thr-Val-Arg-Ile-Ser-Phe-Gly and Trp-Pro-Ser-Leu-Gln-Ala-Ile-Arg-Gly, were found to bind to calmodulin in a Ca^{2+}-dependent fashion and to differentially regulate the ability of calmodulin kinase I and calmodulin kinase II and calmodulin phosphodiesterase.[524] The first peptide inhibited calmodulin kinase I but not calmodulin kinase II, whereas the second peptide inhibited calmodulin kinase II, but only partially inhibited calmodulin kinase I at a >10-fold higher concentration. None of the peptides had an effect on calmodulin-dependent phosphodiesterase.

6.4 Protein-Protein Interaction Inhibitors [Vinculin Binding Peptides and mdm2 Binding Peptides] – To characterise the binding domains of the focal adhesion

protein vinculin, vinculin-binding peptides were isolated from two phage-displayed random peptide libraries displaying 22- and 26-residue peptides.[525] Altogether, five non-similar vinculin-binding peptides [Ser-Thr-Gly-Gly-Phe-Asp-Asp-Val-Tyr-Asp-Trp-Ala-Arg-Gly-Val-Ser-Ser-Ala-Leu-Thr-Thr-Thr-Leu-Val-Ala-Thr-Arg, Ser-Arg-Gly-Val-Asn-Phe-Ser-Glu-Trp-Leu-Tyr-Asp-Met-Ser-Ala-Ala-Met-Lys-Glu-Ala-Ser-Asn-Val-Phe-Pro-Ser-Arg-Arg-Ser-Arg, Ser-Ser-Gln-Asn-Trp-Asp-Met-Glu-Ala-Gly-Val-Glu-Asp-Leu-Thr-Ala-Ala-Met-Leu-Gly-Leu-Leu-Ser-Thr-Ile-His-Ser-Ser-Ser-Arg, Ser-Ser-Pro-Ser-Leu-Tyr-Thr-Gln-Phe-Leu-Val-Asn-Tyr-Glu-Ser-Ala-Ala-Thr-Arg-Ile-Gln-Asp-Leu-Leu-Ile-Ala-Ser-Arg-Pro-Ser-Arg, Ser-Ser-Thr-Gly-Trp-Val-Asp-Leu-Leu-Gly-Ala-Leu-Gln-Arg-Ala-Ala-Asp-Ala-Thr-Arg-Thr-Ser-Ile-Pro-Pro-Ser-Leu-Gln-Asn-Ser-Arg, and an Arg analogue of the first compound Ser-Thr-Gly-Gly-Phe-Asp-Asp-Val-Tyr-Asp-Trp-Ala-Arg-Arg-Val-Ser-Ser-Ala-Leu-Thr-Thr-Thr-Leu-Val-Ala-Thr-Arg] were identified. Binding and competition studies indicated that all five interact with the talin-binding domain of vinculin and do not disrupt the binding of α-actinin or paxillin to vinculin. The identified peptides and talin bind to vinculin in a comparable manner; both bind to immobilised vinculin, but neither binds to soluble vinculin unless the C-terminus of vinculin has been deleted. An analysis of amino acid variants of one of the peptides revealed three non-contiguous motifs that also occur in the region of talin previously demonstrated to bind vinculin. Amino acid substitutions within a 127-residue segment of talin capable of binding vinculin confirmed the importance of two of the motifs and suggested that residues critical for binding are within a 16-residue region.

Mdm2 is an oncogene that was first isolated from a spontaneously transformed mouse fibroblast cell line. The human homologue of the gene (hdm2) has been shown to be over-expressed in a number of human tumours. Mdm2 and its human homologue bind to the tumour suppresser protein p53 and inactivate its function as a transcription factor. The binding site for mdm2 on p53 has been mapped to a small region in the N-terminus of p53.[526] Several amino acids (Phe^{19}, Trp^{23} and Leu^{26}) which are critical for the interaction of p53 with the transcriptional machinery were shown to be involved in binding to mdm2. The β-strand 326-333 of human p53 is involved in the formation of p53 tetramers. Results on mutant proteins obtained by Ala scanning showed that Phe^{328}Ala, Leu^{330}Ala and Ile^{332}Ala proteins were inactive in oligomerisation and DNA-binding assays, while the Glu^{326}Ala, Tyr^{327}Ala, Thr^{329}Ala, Gln^{331}Ala and Arg^{333}Ala proteins had similar properties to those of the wild-type protein.[527]

Using a phage display library a series of 12 to 15 amino acid peptides were identified which interacted strongly with hdm2. The peptide sequences show homology with the previously established mdm2 binding site on p53 (Pro-Leu-Ser-Gln-Glu-Thr-Phe-Ser-Asp-Leu-Trp-Lys-Leu-Leu-Pro-Glu-Asn-Asn-Val).[528] One of the more potent peptides was Met-Pro-Arg-Phe-Met-Asp-Tyr-Trp-Glu-Gly-Leu-Asn (IP3). Aligning the amino acid sequences of various phages revealed the phage consensus sequence Pro-X-Phe-X-Asp-Tyr-Trp-X-X-Leu. Truncation studies indicated that various hexa- and heptapeptides were much less potent (IC_{50} values >40 μM) but the octapeptide Phe-Met-Asp-Tyr-Trp-Glu-Gly-Leu retained significant activity as inhibitors of hdm2-p53 interaction (IC_{50} 2 μM).

Further SAR studies indicated that Phe and Trp were essential for the activity. Leucine could be replaced by Ile, Met or Val with 3-5-fold reduction in potency.

6.5 Brain and Tumour Targeting of Peptides – Sequencing of the inserts from 48 brain-localising phage from library pool I revealed three dominant amino acid sequence motifs. Peptides containing an Ser-Arg-Leu motif represented 54% of the clones, followed by a Cys-Glu-Asn-Trp-Trp-Gly-Asp-Val-Cys motif (29%). Other motifs that appeared more than once included Cys-Lys-Asp-Trp-Gly-Arg-Ile-Cys, Cys-Val-Leu-Arg-Gly-Gly-Arg-Cys and Cys-Thr-Arg-Ile-Thr-Glu-Ser-Cys. From the library pool II phage, 25 sequences revealed only one motif, Trp-Arg-Cys-Val-Leu-Arg-Glu-Gly-Pro-Ala-Gly-Gly-Cys-Ala-Trp-Phe-Asn-Arg-His-Arg-Leu, which comprised 40% of the sequence.[529] When tested as isolated phage, the Cys-Leu-Ser-Ser-Arg-Leu-Asp-Ala-Cys, Cys-Asn-Ser-Arg-Leu-His-Leu-Arg-Cys, Cys-Glu-Asn-Trp-Trp-Gly-Asp-Val-Cys and Trp-Arg-Cys-Val-Leu-Arg-Glu-Gly-Pro-Ala-Gly-Gly-Cys-Ala-Trp-Phe-Asn-Arg-His-Arg-Leu phage each targeted the brain several-fold more effectively than the kidney. Synthetic peptide Cys-Leu-Ser-Ser-Arg-Leu-Asp-Ala-Cys inhibited the preferential localisation into the brain of the phage carrying the same peptide sequence and of the Trp-Arg-Cys-Val-Leu-Arg-Glu-Gly-Pro-Ala-Gly-Gly-Cys-Ala-Trp-Phe-Asn-Arg-His-Arg-Leu phage. Thus the two peptides, which were obtained from different libraries in two independent experiments, seem to bind to the same target molecule. Coupling the peptide onto the surface of red blood cells resulted in their accumulation in the brain to a greater extent than in the kidney. Moreover, the brain localisation of the red blood cells was inhibited by coinjection of the synthetic peptide.

Phage displaying an Arg-Gly-Asp containing cyclic nonapeptide containing two disulfide bonds [Cys-Asp-Cys-Arg-Gly-Asp-Cys-Phe-Cys] (disulfide positions not identified) with a high affinity for α_v integrins homed to tumours when injected intravenously into tumour-bearing nude mice.[530] A substantially higher amount of α_v-directed Arg-Gly-Asp phage than control phage was recovered from malignant melanomas and breast carcinoma. Antibodies detected the α_v-directed Arg-Gly-Asp phage in tumour blood vessels, but not in several normal tissues. Another Arg-Gly-Asp phage, which carries a peptide selective for the $\alpha_5\beta_1$ integrin [Cys-Arg-Gly-Asp-Gly-Trp-Cys], did not show any preferential accumulation into the MDA-MB-435-derived tumours. Immunostaining experiments also indicated that $\alpha_v\beta_3$-directed Arg-Gly-Asp phage was accumulated around the small blood vessels within the tumour. When $\alpha_v\beta_3$-directed Arg-Gly-Asp phage was allowed to circulate for 24 hours prior to harvesting of the tissue for immunostaining, about 90% of the phage had been eliminated from the circulation. In another study, a V_H fusion phage library containing V_H domain unassociated with V_L domain was compared with single-chain F_V fusion phage library as a source of melanoma-specific clones. Both libraries contained the same V_H domain from the vaccinated melanoma patient.[531]

6.6 Epitope Mapping [Human Galactin-3 Epitopes and Antigenic Epitopes of Streptokinase] – Random epitope and peptide libraries were constructed and

compared for their efficiencies in the mapping. The galectin-3 cDNA was randomly digested by DNase I to make random epitope libraries.[532] The libraries were screened by affinity selection using a microtitre plate coated with monoclonal antibodies (A1D6 and 1H11). Direct DNA sequencing of the selected clones defined two distinct epitope sites consisting of nine and eleven amino acid residues. Affinity selection of random peptide libraries recovered a number of sequences that were similar to each other but distinct from the galectin-3 sequence.

To identify the structures of immunodominant epitopes in streptokinase, 15 and 6-mer random phage libraries were used.[533] Repeated panning and selection experiments against a 15-mer peptide phage library, using a representative mAb (A2.5) to this epitope, identified a dominant structural motif [Gly-Pro-Arg/Leu-Trp-Leu] corresponding to amino acids 3 to 7 of native streptokinase [-Ile-Ala-Gly^3-Pro-Glu-Trp-Leu^7-Leu-Asp-Arg-]. Synthetic peptide spanning the epitope of A2.5 (Ala-Gly-Pro-Glu-Trp-Leu-Leu) specifically inhibited the binding of [^{125}I]streptokinase to mAb A2.5 in a concentration-dependent manner, with about 2 μM of the peptide required to inhibit 50% of the binding of radiolabelled streptokinase to the antibody. Even at high concentrations (312 μM), the peptide did not inhibit the binding of six other mAbs, which were directed against different non-overlapping epitopes of streptokinase. Similar studies of the second epitope in streptokinase, which is immunodominant for murine but not human antibodies, identified a consensus sequence Lys-Ser-Lys/Leu-Pro-Phe/Tyr corresponding to amino acids 59-63 of streptokinase.

6.7 Ion Channel Receptor Binding Peptides – Oligomeric N-methyl-D-aspartate receptor (NMDAR) in brain is a ligand-gated ion channel that becomes selectively permeable to ions upon binding to ligands. To study NMDAR structure and function, peptide modulators of NR1 (amino acids 1-561 of an essential subunit of NMDA receptors) were searched using random peptide bacteriophage libraries. One group of cyclic peptides with a consensus sequence of Cys-Asp-Gly-Leu-Arg-His-Met-Trp-Phe-Cys was identified.[534] Two of the peptides, [Ser-Asp-Trp-Cys-Glu-Gly-Leu-Gln-His-Met-Trp-Phe-Cys-Ser-Ser-Leu] and [Tyr-Pro-Asp-Cys-Asp-Gly-Leu-Arg-His-Leu-Trp-Phe-Cys-Leu-Asp-Ile], reduced NMDA-induced channel activity in a dose dependent manner. Two other channel receptor binding peptides, [Leu-Asn-Asp-Trp-Phe-Ile-Thr-Tyr-Ile μMag-4.1) and Leu-Glu-Ala-Trp-Phe-Leu-Gln-Tyr-Ile, were used to study the binding of phage particles to the receptors. Using anti-phage antibody, Mag-4.1 phage was shown to interact with human embryonic kidney cells transiently expressing functional NMDA receptor through assembly of NR1 and NR2A subunits.[535] The Mag-4.1 phage binding was completely inhibited in the presence of 500 μM synthetic Mag-4.1 peptide.

6.8 NMR Studies of Phage Peptides – An NMR approach for structure determination of short peptides displayed on the surface of filamentous bacteriophage virions was demonstrated using the hexapeptide Gly-Pro-Gly-Arg-Ala-Phe [part of the V3 loop present in the third hypervariable region of the envelope

glycoprotein gp120] that constitutes the principal neutralising determinant of HIV-1. The peptide was inserted near the N-terminus of the major coat protein of bacteriophage fd. NMR studies of the recombinant protein solubilised in detergent micelles showed that the inserted peptide adopts a double bend S-shaped conformation that is similar to the antibody bound structure determined by X-ray crystallography.[536]

7 Protein-Protein Interaction Inhibitors

As with integrins (discussed above), the well known example of the involvement of protein-protein interactions in various physiological and pathological conditions, many other such interactions are involved in various diseases. One such example (mdm2/p53) has been mentioned above in the phage library leads section. Some of the other examples are listed below.

7.1 Inhibitors of Fas and FAP-1 Interaction – Fas (APO-1/CD95) [a member of the tumour necrosis factor receptor family] and its ligand have been identified as important signal-mediators of apoptosis. FAP-1 is a protein that associates with a negative regulatory domain (C-terminal 15 amino acids) of Fas and inhibits Fas-induced apoptosis. The 15 amino acid fragment, Asp-Ser-Glu-Asn-Ser-Asn-Phe-Arg-Asn-Glu-Ile-Gln-Ser-Leu-Val, and truncated fragments Arg-Asn-Glu-Ile-Gln-Ser-Leu-Val, Asn-Glu-Ile-Gln-Ser-Leu-Val, Glu-Ile-Gln-Ser-Leu-Val, Ile-Gln-Ser-Leu-Val, Gln-Ser-Leu-Val and Ser-Leu-Val were nearly equipotent in inhibiting Fas/FAP-1 binding. The minimal sequence peptide Ac-Ser-Leu-Val induced Fas-mediated apoptosis in a colon cancer cell line DLD-1 (expressing both Fas and FAP1 and resistant to Fas-induced apoptosis).[537]

7.2 Inhibitors of Bcl2-Bax (and Other Members of this Family) Interaction – The role of Bcl-2 and its partners in human cancers has been mentioned in recent publications and reviews.[538–540] The proto-oncogene bcl-2 encodes a protein that inhibits apoptosis. It forms heterodimers with Bax, an apoptosis inducing member of the Bcl-2 multigene family. Thus inhibition of this interaction may release Bax leading to tumour cell death. Recent studies have demonstrated that the BH3 region was required for the apoptosis inducing activity of Bax, whereas BH1, BH2 and the N-terminus of Bax were dispensable.[541] Using various mutant proteins, homodimerisation and heterodimerisation studies on Bcl2/Bax and other family members (Bcl2/BAD) have been reported.[542,543] Crystal structure studies on rat Bcl-x_L, a deamidated form of Bcl-X_L and Bcl-x_L complexed to a Bax peptide have been reported.[544,545] The Bak peptide, Gly^{72}-Gln-Val-Gly-Arg-Gln-Leu-Ala-Ile-Ile-Gly-Asp-Asp-Ile-Asn-Arg^{87}, forms an α-helix when complexed to Bcl-X_L and binds in a hydrophobic cleft formed by the BH1, BH2 and BH3 regions of Bcl-X_L. The amino terminal residues of the peptide interact with several residues in the BH1 region of Bcl-X_L (Val^{126}, Glu^{129}, Leu^{130}, and Phe^{146}). The carboxyl terminal portion of the Bak peptide interacts predominantly with residues in the BH2 and BH3 regions (Phe^{97}, Arg^{100}, Tyr^{101}, and Phe^{105}). The

hydrophobic side chains of the peptide (Val^{74}, Leu^{78}, Ile^{81}, and Ile^{85}) point into a hydrophobic cleft of Bcl-X_L and stabilise complex formation. In addition to these hydrophobic interactions, the charged side chains of the Bak peptide (Arg^{76}, Asp^{83}, and Asp^{84}) are close to oppositely charged residues of Bcl-X_L (Glu^{129}, Arg^{139}, and Arg^{100}, respectively).[545] A smaller fragment, Gln^{77}-Leu-Ala-Ile-Ile-Gly-Asp-Asp-Ile-Asn-Arg^{87}, was not active in the binding assay.

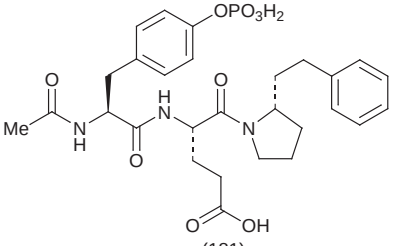
7.3 SH2 Domain Ligands – SH2 domains of various proteins ((100-amino acid stretches of proteins that bind to other proteins containing phosphotyrosine residues) mediate protein-protein interactions and are involved in a wide range of intracellular signalling events. Such proteins can be divided in two categories: the ones with intrinsic enzymatic activity and those without known catalytic domains, which may act as adapters to couple tyrosine phosphorylated proteins to downstream targets. SH2-containing enzymes include kinases such as Src, Lck, phosphatases, phospholipase-C-γ1, ras GTPase-activating protein, and others. SH2-containing adapter proteins which have no known catalytic activity include, for example, the p85 subunit of PI 3′-kinase, SHC, and GRB2/Sem5. Binding interactions of a series of peptide ligands of the $pp60^{c\text{-}src}$ SH2 domain were investigated by using thermodynamic measurements, structural determinations, and molecular computations approaches.[546]

Various studies attempting to identify selective ligands for SH2 domain proteins have been reported. The consensus sequence (Ile/Val)X-Tyr-XX(Leu/Val) was found to be present in the cytoplasmic tails of several lymphocyte receptors that interact with the second SH2 domain of SHP-1, a protein-tyrosine phosphatase associated with inhibition of activation pathways in haematopoietic cells. The catalytic activity of SHP-1 is regulated by its two SH2 domains; phosphotyrosine peptides that bind to the SH2 domains activate SHP-1. A biphosphopeptide corresponding to the cytoplasmic tail of a killer cell inhibitory receptor [Glu-Gln-Asp-Pro-Gln-Glu-Val-Thr-pTyr-Ala-Gln-Leu-Asn-His-Ser-Val-Phe-Thr-Gln-Arg-Lys-Ile-Thr-Arg-Pro-Ser-Gln-Arg-Pro-Lys-Thr-Pro-Pro-Thr-Asp-Ile-Ile-Val-pTyr-Thr-Glu-Leu-Pro-Asn-Ala] with the potential to interact simultaneously with both SH2 domains of SHP-1 was the most potent activator of SHP-1.[547]

Many synthetic phosphopeptide analogues of Ac-Val-Pro-Glu-pTyr-Ile-Asn-Gln-NH_2 [Ac-Glu-pTyr-Ile-Asn-Gln-NH_2, Ac-pTyr-Ile-Asn-Gln-NH_2, Ac-pTyr-Ile-Asn-NH_2, Ac-Glu-pTyr-Aib-NH-NH_2, Ac-pTyr-Glu-Asn-Glu-NH_2 and a cyclic peptide Ac-c(Cys-pTyr-Ile-Asn-Cys)-NH_2] were inhibitors of recombinant EGF receptor and GRB2 and Lck SH2 domain interactions. The cyclic pentapeptide was nearly equipotent on GRB2 and Lck SH2 domains (IC_{50} values 0.37 and 0.25 μM, respectively).[548] An N-terminal 3-aminobenzyloxycarbonyl or a 2-aminobenzoyl group in phosphotyrosine peptides improved the inhibitory potency.[549] Examples of some N-terminally modified peptides include 2-aminobenzoyl-Glu-pTyr-Ile-Asn-NH_2 (IC_{50} 0.022 μM) and (3-amino)Z-pTyr-Ile-Asn-NH_2 (IC_{50} 0.065 μM). In comparison, the IC_{50} values for Glu-pTyr-Ile-Asn-NH_2, Ac-pTyr-Ile-Asn-NH_2, pTyr-Ile-Asn-NH_2 and Z-pTyr-Ile-Asn-NH_2 were between 8-57 μM.

Using the three-dimensional structure of Src SH2 domain complexed to the 11-mer phosphopeptide, Glu-Pro-Gln-pTyr-Glu-Glu-Ile-Pro-Ile-Tyr-Leu, a structure-based design approach was employed to discover peptide and non-peptide antagonists of phosphoprotein-SH2 binding.[550–552] For Src the optimal binding sequence was found to be phosphoTyr-Glu-Glu-Ile. Examples of more potent compounds include (**181**) (IC_{50} 0.56 μM). Many other dipeptide derivatives, e.g. [-N(CH_3)-CH_2-CH_2-CH_2-cyclohexyl replaced by D-hPhe, -NH-$(CH_2)_3$-Ph, -NH-$(CH_2)_2$-Ph, -NH-$(CH_2)_2$-Ph(*p*-CH_3), -NH-$(CH_2)_3$-Ph(*p*-O-CH_2-COOH, -NH-$(CH_2)_3$-Ph(*m*-O-CH_2-COOH, -NH-$(CH_2)_3$-Ph(*m*-O-CH_2-$CONH_2$ or -N(CH_3)-CH_2-CH_2-CH_2-Ph], were less potent (IC_{50} values 3-10 μM).

Since phosphotyrosine containing peptides can be rapidly dephosphorylated *in vivo* by non-specific phosphatases, 4-phosphonodifluoromethyl- and 4-phosphono-phenylalanine residues were incorporated in peptides to prevent dephosphorylation.[550] In a protein association assay (disruption of the binding of ^{35}S-labelled SH2-GST fusion proteins of Src and Abl to the phosphorylated intracellular domain of the platelet derived growth factor receptor), the F_2Pmp containing peptide Glu-Pro-Gln-F_2Pmp-Glu-Glu-Ile-Pro-Ile-Tyr-Leu was 10-15-fold less potent than the parent peptide Glu-Pro-Gln-pTyr-Glu-Glu-Ile-Pro-Ile-Tyr-Leu. The smaller peptides, Ac-F_2Pmp-Glu-Glu-Ile-Glu, Ac-F_2Pmp-Glu-D-hCys-NH_2, Ac-F_2Pmp-Glu-NMe-CH_2-CH_2-CH_2-Chx, and Ac-F_2Pmp-Abu-NMe-CH_2-CH_2-CH_2-Chx were less potent (IC_{50} 6-100 μM) than the corresponding pTyr peptides against Src but were 3-20-fold more potent (IC_{50} 1.5-4 μM) than the parent peptide against Abl. In comparison to 4-phosphonodifluoromethyl-phenylalanine analogues, the 4-phosphono-phenylalanine containing analogues Ac-Phe(PO_3H_2)-Glu-Glu-Ile-Glu, Ac-Phe(PO_3H_2)-Glu-NMe-CH_2-CH_2-CH_2-Chx and Ac-Phe(PO_3H_2)-Abu-NMe-CH_2-CH_2-CH_2-Chx were less potent against both Src and Abl. Replacement of the pTyr derivatives in the above compounds by other Tyr mimics led to compounds like (**182**) which were about 9-15-fold less potent (IC_{50} 7-13 μM) than the Ac-Tyr(OPO_3H_2)-Glu-NMe-CH_2-CH_2-CH_2-Chx analogue. The crystal structure of compound (**182**) complexed with Src SH2 indicated significant differences in binding between (**182**) and Ac-Tyr(OPO_3H_2)-Glu-NMe-CH_2-CH_2-CH_2-Chx analogue.[551]



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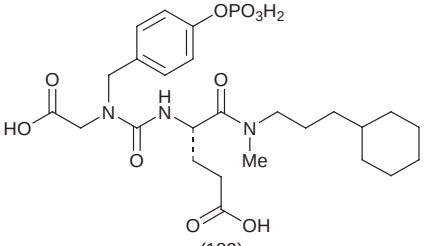

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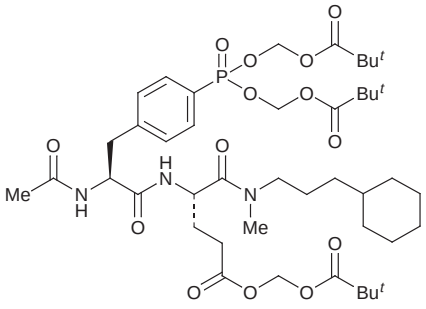
To increase cellular permeability of the compounds, ester derivatives of the F_2Pmp peptides were prepared. The phosphonate monoesters in the 4-phosphonodifluoromethyl-phenylalanine series (**183**) and phosphonate diesters in the



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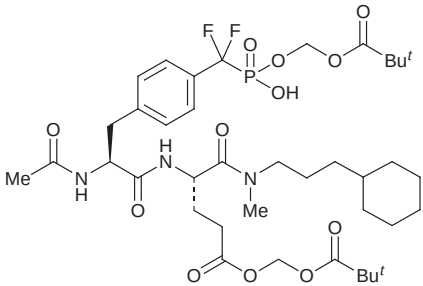

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4-phosphono-phenylalanine series (**184**) and the corresponding Abu analogues were taken up into the Balbc3T3 cells and readily converted into the acids within the cells.[552]

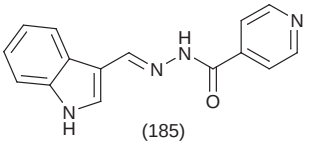
7.4 CD4 Interaction Inhibitors – The interaction between CD4 (a glycoprotein consisting of four Ig-like extracellular domains and expressed on the surface of helper T cells) and major histocompatibility complex class II proteins provides a critical coreceptor function for the activation of $CD4^+$ T cells implicated in the pathogenesis of a number of autoimmune diseases and transplantation responses. In addition to its binding to MHC class II on an antigen presenting cell, CD4 interacts with at least two other T cell surface molecules known to be involved in T cell activation, the T cell receptor and CD3 complex. Evidence also exists suggesting that CD4 is capable of functioning as a signal transduction molecule important for the activation pathway *via* activity of the associated tyrosine kinase $p56^{lck}$. Human CD4 also functions as the primary cellular receptor for human HIV by binding the HIV-1 envelope glycoprotein gp120 with relatively high affinity. Binding of the viral gp120 to CD4 mediates attachment and penetration of HIV particles.

The inhibitors of CD4 and MHC class II interactions have been designed based on the structures of various domains of CD4.[553] One of the cyclic peptides based on the D1 domain, Phe-Cys^2-Tyr-Ile-Cys-Glu-Val-Glu-Asp-Gln-Cys^{11}-Tyr (Cys^2 to Cys^{11} disulfide bridge), inhibited CD4-MHC II binding, antagonised

CD4 function and inhibited rosette formation between CD-4 expressing COS cells and MHC II positive B lymphocytes (IC_{50} 25 μM). Two other peptides, Phe-Cys^2-Tyr-Lys-Gly-Pro-Ser-Lys-Cys^9-Tyr and Phe-Cys^2-Glu-Val-Glu-Asp-Gln-Lys-Glu-Cys^{10}-Tyr, could not inhibit sCD4 interaction with MHC II. The Cys^2 to Cys^{11} cyclic peptide also inhibited the binding of gp120 to sCD4 (IC_{50} 20 μM) and inhibited HIV-1 infection and syncytium formation (80% inhibition at 2 μM).[554] Another peptide Phe-Cys-Ser-Ile-Gln-Phe-His-Trp-Cys-Tyr ($Cys^{2,9}$ disulfide bridge) was only a weak inhibitor of the gp120 and sCD4 interaction (30% inhibition at 125 μM).

Based on the computer analysis of the surface structural features of the D4 domain, a series of linear and cyclic peptide analogues derived from the FG and CC' loops of CD4 were synthesised.[555] Two linear peptides, Leu-Ser-Asp-Ser-Gly-Gln-Val-Leu-NH_2 (FG 347-354) and Lys-Leu-Glu-Asn-Lys-Glu-Ala-NH_2 (CC' 318-324) inhibited CD4 dependent T cell proliferation (34-41% inhibition at 100 μM). Three cyclic peptides based on the above linear peptides, Cys-Ser-Asp-Ser-Gly-Gln-Val-Cys-NH_2, Ac-Cys-Asp-Ser-Gly-Gln-Cys-NH_2, and c(Pro-Leu-Glu-Asn-Lys-Tyr) were weaker inhibitors (11-14% inhibition at 100 μM). Two other cyclic peptides Cys-Lys-Leu-Glu-Asn-Lys-Glu-Cys-NH_2, Cys-Leu-Glu-Asn-Lys-Cys-NH_2 (stable in serum up to 50 hours) were somewhat more potent inhibitors (26 and 36% inhibition, respectively). A small synthetic cyclic heptapeptide [Cys-Asn-Ser-Asn-Gln-Ile-Cys, disulfide bridge containing cyclic peptide] was shown by high resolution NMR spectroscopy to closely mimic the CD4 domain 1 CC′ surface loop.[556] This peptide effectively blocked stable CD4-major histocompatibility complex class II interaction, possessed significant immunosuppressive activity *in vitro* and *in vivo*, and resisted proteolytic degradation (25% degradation in human serum in 72 hours).

A CD4 surface pocket was identified as a functional epitope implicated in CD4-MHC class II interaction and T cell activation.[557] A computer based strategy was then used to screen non-peptide collection to identify a group of compounds as ligands of the proposed CD4 surface pocket. Two of the examples are shown below. These compounds (**185** and **186**) have been shown to block stable CD4-MHC class II binding and exhibit significant inhibition of immune responses in animal models of autoimmune disease and allograft transplant rejection.



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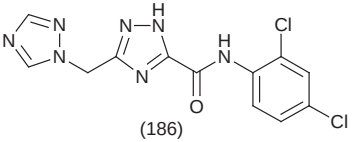

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8 Advances in Formulation/Delivery Technology

Prodrug strategies to enhance the intestinal absorption of peptides have been reviewed.[558] Synthesis of a novel esterase-sensitive cyclic prodrug of a hexapep-

tide obtained by using an (acyloxy)alkoxy promoiety (see Vol. 29, page 243 for structure) has been reported.[559] In a physiological buffer system, cyclic prodrug degraded to the linear hexapeptide ($t_{1/2}$ 206 min.). The rate of hydrolysis in human blood was faster ($t_{1/2}$ 132 min.). The hydrolysis in blood was inhibited by an inhibitor of serine-dependent esterases. The permeability of the prodrug in a Caco-2 cell model was shown to be higher than the linear peptide.

The transepithelial transport of oligopeptides was studied by using human intestinal Caco-2 cell monolayers.[560] The apical-to-basolateral transport mechanism was investigated by using bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), β-casomorphin (Tyr-Pro-Phe-Pro-Gly), ovokinin (Phe-Arg-Ala-Asp-His-Pro-Phe-Leu), Pro-Phe-Gly-Lys and Gly-Gly-Tyr-Arg. The transport rate was greatest for Gly-Gly-Tyr-Arg and smallest for β-casomorphin. The concentration of bradykinin in the apical solution after 60 min of incubation with the Caco-2 monolayer was unchanged, while those of ovokinin and β-casomorphin was decreased, suggesting that these peptides were rapidly cleaved by the brush border peptidases. Thus digestion by cellular peptidase was one of the main factors inhibiting intact peptide flux across the epithelial cell layer. The transport studies using bradykinin analogues [Ac-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, Ac-Pro-Pro-Gly-Phe-Ser-Pro-Phe, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe, Arg-Pro-Pro-Gly-Arg-Ser-Pro-Phe-Arg and Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu] indicated that the number of positively charged amino acids was negatively correlated with the flux. On the other hand, the hydrophobicity of the peptides was positively correlated with the flux, suggesting that such peptides as bradykinin adsorbed to the apical cell membrane surface mainly through hydrophobic interactions, by which the peptides were efficiently internalised by the cells and transported across the cell layer.[560] The effect of stereochemistry on the transport of Aca-linked β-turn peptidomimetics, c($NH(CH_2)_5CO$-Ala-Ala) and c($NH(CH_2)_5CO$-Ala-D-Ala), and the corresponding linear peptides, CH_3-CH_2-CO-Ala-Ala-NH-CH_2-CH_2-CH_3 and CH_3-CH_2-CO-Ala-D-Ala-NH-CH_2-CH_2-CH_3, across a human intestinal cell line was investigated using Caco-2 cells.[561]

Biodegradable polymeric formulations for proteins like insulin and recombinant human growth hormone have been reported.[562,563] Insulin was incorporated (5% and 10%) in polylactic acid polymers by an emulsion solvent evaporation method. The microspheres exhibited burst release in the initial stage. After three weeks, nearly 80% of the insulin remained undegraded in the microspheres. When administered subcutaneously to normal rats, a decrease in serum glucose was observed.[562] Recombinant human growth hormone was incorporated (15% w/w) in polylactic-polyglycolic microspheres and the formulated product was administered in juvenile rhesus monkeys. The formulations provided sustained elevated levels of growth hormone and two other proteins (insulin-like growth factor-1 and IGF binding protein-3) induced by growth hormone. After three monthly injections of one of the formulations, both the human growth hormone and insulin-like growth factor levels remained elevated for 90 days.[563]

A novel biodegradable system for protein and peptide delivery has been reported based on gelatin nanoparticles and poly(lactic-co-glycolic) microspheres.[564] Gelatin nanoparticle-poly(lactic-co-glycolic acid) microsphere compo-

sites were prepared by encapsulating protein-loaded gelatin nanoparticles in poly(lactic-co-glycolic acid) microspheres. Protein (BSA) release experiments indicate that this new composite system possesses sustained release characteristics. In comparison to the gelatin nanoparticle-poly(lactic-co-glycolic acid) microsphere composite which released the protein over an extended period of time (66% over 18 days), the poly(lactic-co-glycolic acid) microspheres containing protein without gelatin released the protein over a very short period of time (90% in 3.5 days). A thermosensitive biodegradable hydrogel consisting of blocks of poly(ethyleneoxide) and poly(L-lactic acid) has been reported for the sustained release of proteins.[565] Aqueous solutions of these copolymers exhibit temperature-dependent reversible gel-solution transitions. The hydrogel can be loaded with biologically active compounds in an aqueous phase at elevated temperatures (around 45 °C). On subcutaneous injection and subsequent cooling to body temperature, the loaded copolymer forms a gel that can act as a sustained-release matrix for drugs.

9 Other Peptides (from Insects, Molluscs etc.)

More than 100 insect neuropeptides have been identified. The amino acid sequences of various myostimulating, myoinhibiting, diuretic and adipokinetic peptides and enzyme inhibitors are included in a recent review.[566] More recently, two neuropeptides, schistomyotropin-1 (Gly-Ala-Ala-Pro-Ala-Ala-Gln-Phe-Ser-Pro-Arg-Leu-NH_2) and schistomyotropin-2 (Thr-Ser-Ser-Leu-Phe-Pro-His-Pro-Arg-Leu-NH_2), were isolated from the brains of the desert locust, *Achistocerca gregaria*.[567] Schistomyotropin-2 was about 10-fold less potent than schistomyotropin-1 in stimulating cockroach hind-gut motility. A D-amino acid containing cardio-excitatory peptide, Asn-D-Trp-Phe-NH_2, was isolated from the hearts of *Aplysia kurodai*. The peptide increased the amplitude of contractions of the perfused *Aplysia* heart with little effect on beating frequency.[568] The peptide also potentiated spontaneous contractions of the anterior aorta. The synthetic peptide containing an L-Trp in place of the D-Trp was about 1000-fold less potent.

SAR studies (myoactivity) of flatworm Phe-Met-Arg-Phe-NH_2 related peptides are reported on isolated muscle fibres of the human blood fluke, *Schistosoma mansoni*.[569] The peptides Tyr-Ile-Arg-Phe-NH_2, Gly-Tyr-Ile-Arg-Phe-NH_2 and Arg-Tyr-Ile-Arg-Phe-NH_2 induced muscle contraction (EC_{50} values 4, 1 and 7 nM, respectively) more potently than Gly-Asn-Phe-Phe-Arg-Phe-NH_2 (EC_{50} 500 nM). Using a series of synthetic analogues of the flatworm peptides Tyr-Ile-Arg-Phe-NH_2, Gly-Tyr-Ile-Arg-Phe-NH_2 and Arg-Tyr-Ile-Arg-Phe-NH_2, the SAR of the muscle Phe-Met-Arg-Phe-NH_2-related peptide receptor was examined. Nearly all of the amino acids were important for the activity. Alanine scans resulted in analogues which were either inactive or demonstrated much reduced potencies. Similarly, the truncated analogues Phe-Arg-Phe-NH_2, Tyr-Arg-Phe-NH_2, His-Arg-Phe-NH_2, Arg-Phe-NH_2 and Phe-NH_2 were inactive and Ile-Arg-Phe-NH_2 and Met-Arg-Phe-NH_2 were less potent. One of the tetrapeptides containing an N-terminal Trp residue, Trp-Ile-Arg-Phe-NH_2, was marginally

more potent (EC_{50} 0.5 nM) than the starting tetrapeptides Tyr-Ile-Arg-Phe-NH_2, Gly-Tyr-Ile-Arg-Phe-NH_2 and Arg-Tyr-Ile-Arg-Phe-NH_2.

In addition to the above studies, a number of publications on similar peptides were published in 1996.[570–575] These studies include analogues of peptides like Lys-Pro-Asn-Phe-Ile-Arg-Phe-NH_2, Lys-Asn-Glu-Phe-Ile-Arg-Phe-NH_2, Pro-Asp-Val-Asp-His-Val-Phe-Leu-Arg-Phe-NH_2, Gly-Tyr-Ile-Arg-Phe-NH_2, Tyr-Ile-Arg-Phe-NH_2 and Arg-Tyr-Leu-Pro-Thr (proctolin). In the case of proctolin, agonist, partial agonist and antagonist analogues were obtained by SAR studies.[574,575]

References

1. A. S. Dutta, *Specialist Periodical Reports, Amino Acids, Peptides and Proteins*, 1998, **29**, 175.
2. Y. Sasaki and J. Abe, *Chem. Pharm. Bull.*, 1997, **45**, 13.
3. M-D. Piulachs, L. Vilaplana, J-M. Bartolome, C. Carreno, D. Martin, R. Gonzales-Muniz, M-T. Garcia-Lopez, D. Andreu and X. Belles, *Insect. Biochem. Molecular Biol.*, 1997, **27**, 851.
4. P. Wipf and T. C. Henninger, *J. Org. Chem.*, 1997, **62**, 1586.
5. M. Noda, T. Ibuka, H. Habishita and N. Fujii, *Chem. Pharm. Bull.*, 1997, **45**, 1259.
6. J. I. DeGraw, R. G. Almquist, C. K. Hiebert, A. K. Judd, L. Dousman, R. L. Smith, W. R. Waud and I. Uchida, *J. Med. Chem.*, 1997, **40**, 2398.
7. J. I. DeGraw, R. G. Almquist, C. K. Hiebert, W. T. Colwell, J. Crase, T. Hayano, A. K. Judd, L. Dousman, R. L. Smith, W. R. Waud and I. Uchida, *J. Med. Chem.*, 1997, **40**, 2386.
8. R. L. Smith, L. Dousman, W. R . Waud and J. I. DeGraw, *J. Med. Chem.*, 1997, **40**, 2407.
9. J-P. Briand, N. Benkirane, G. Guichard, J. F. E. Newman, M. H. V. Van Regenmortel, F. Brown and S. Muller, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 12545-12550.
10. S. E. Gibson (nee Thomas), N. Guillo, R. J. Middleton, A. Thuiliez and M. J. Tozer, *J. Chem. Soc. Perkin I*, 1997, 447.
11. M. S. Wolfe, D. Dutta and J. Aube, *J. Org. Chem.*, 1997, **62**, 654.
12. I. S. Weitz, M. Pellegrini, D. F. Mierke and M. Chorev, *J. Org. Chem.*, 1997, **62**, 2527.
13. P. W. Baures, W. H. Ojala, W. J. Costain, M. C. Ott, A. Pradhan, W. B. Gleason, R. K. Mishra and R. L. Johnson, *J. Med. Chem.*, 1997, **40**, 3594.
14. H. Bekele, C. L. Nesloney, K. W. McWilliams, N. M. Zacharias, P. Chitnumsub and J. W. Kelly, *J. Org. Chem.*, 1997, **62**, 2259.
15. T. Kline, E. Sieber-McMaster, W. F. Lau and S. Natrajan, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1017.
16. D. Muller, I. Zeltser, G. Bitan, and C. Gilon, *J. Org. Chem.*, 1997, **62**, 411.
17. G. Bitan, D. Muller, R. Kasher, E. V. Gluhov and C. Gilon, *J. Chem. Soc. Perkin I*, 1997, 1501.
18. G. Bitan, I. Sukhotinsky, Y. Mashriki, M. Hanani, Z. Selinger and C. Gilon, *J. Peptide Res.*, 1997, **49**, 421.
19. H. Morita, A. Shishido, T. Matsumoto, K. Takeya, H. Itokawa, T. Hirano and K. Oka, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1269.

20. T. J. Gaymes, M. Cebrat, I. Z. Siemion and J. E. Kay, *FEBS Letters*, 1997, **418**, 224.
21. H. Morita, A. Gonda, K. Takeya, H. Itokawa and Y. Iitaka, *Tetrahedron*, 1997, **53**, 1607.
22. H. Morita, A. Gonda, K. Takeya, H. Itokawa and O. Shirota, *Chem. Pharm. Bull.*, 1997, **45**, 161.
23. H. Morita, T. Kayashita, K. Takeya, H. Itokawa and M. Shiro, *Tetrahedron*, 1997, **53**, 1617.
24. H. Morita, Y. S. Yun, K. Takeya and H. Itokawa, *Bioorg. Med. Chem.*, 1997, **5**, 2063.
25. H. Morita, Y. S. Yun, K. Takeya and H. Itokawa, *Chem. Pharm. Bull.*, 1997, **45**, 883.
26. H. Morita, Y. S. Yun, K. Takeya, H. Itokawa and O. Shirota, *Bioorg. Med. Chem.*, 1997, **5**, 631.
27. H. J. Shin, H. Matsuda, M. Murakami, K. Yamaguchi, *J. Nat. Prod.*, 1997, **60**, 139.
28. Y. Hitotsuyanagi, Y. Anazawa, T. Yamagishi, K. Samata, T. Ichihara, K. Nanaumi, N. Okado, S. Nakaike, M. Mizumura, K. Takeya and H. Itokawa, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 3125.
29. J. P. Dirlam, A. M. Belton, N. C. Birsner, R. R. Brooks, S-P. Chang, R. Y. Chandrasekaran, J. Clancy, B. J. Cronin, B. P. Dirlam, S. M. Finegan, S. A. Froshauer, A. E. Girard, S. F. Hayashi, R. J. Howe, J. C. Kane, B. J. Kamicker, S. A. Kaufman, N. L. Kolosko, M. A. LeMay, R. G. Linde II, J. P. Lyssikatos, C. P. MacLelland, T. V. Magee, M. A. Massa, S. A. Miller, M. L. Minich, D. A. Perry, J. W. Petitpas, C. P. Reese, S. B. Seibel, W-G. Su, K. T. Sweeney, D. A. Whipple and B. V. Yang, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1139.
30. J. P. Lyssikatos, S-P. Chang, J. Clancy, J. P. Dirlam, S. M. Finegan, A. E. Girard, S. F. Hayashi, D. P. Larson, A. S. Lee, R. G. Linde II, C. P. MacLelland, J. W. Petitpas, S. B. Seibel and C. B. Vu, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1145.
31. R. G. Linde II, N. C. Birsner, R. Y. Chandrasekaran, J. Clancy, R. J. Howe, J. P. Lyssikatos, C. P. MacLelland, T. V. Magee, J. W. Petitpas, J. P. Rainville, W-G. Su, C. B. Vu and D. A. Whipple, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1149.
32. H. Uchida, T. Kato and K. Achiwa, *Chem. Pharm. Bull.*, 1997, **45**, 1228.
33. O. J. Felician and T. A. Sandson, *Drugs of Today*, 1997, **33**, 555.
34. O. J. Felician and T. A. Sandson, *Drugs of Today*, 1997, **33**, 665.
35. T. Bandiera, J. Lansen, C. Post and M. Varasi, *Curr. Med. Chem.*, 1997, **4**, 159.
36. R. Kisilevsky and P. E. Fraser, *Crit. Rev Biochem. Mol. Biol.*, 1997, **32**, 361.
37. N. N. Dewji and S. J. Singer, *Proc. Natl. Acad. Sci.*, 1996, **93**, 12575.
38. N. N. Dewji and S. J. Singer, *Proc. Natl. Acad. Sci.*, 1997, **94**, 9926.
39. K. Duff, C. Eckman, C. Zehr, X. Yu, C-M. Prada, J. Perez-tur, M. Hutton, L. Buee, Y. Harigaya, D. Yager, D. Morgan, M. N. Gordon, L. Holcomb, L. Refolo. B. Zenk, J. Hardy and S. Younkin, *Nature*, 1996, **384**, 710.
40. Y. Chong, *Life Sci.*, 1997, **23**, 2323.
41. T. Itoh, T. Ikeda, H. Gomi, S. Nakao, T. Suzuki and S. Itohara, *J. Biol. Chem.*, 1997, **272**, 22389.
42. L. Choo-Smith, W. Garzonrodriguez, C. G. Glabe and W. K. Surewicz, *J. Biol. Chem.*, 1997, **272**, 22987.
43. S. S. Mok, G. Sberna, D. Heffernan, R. Cappai, D. Galatis, H. J. Clarris, W. H. Sawyer, K. Beyreuther, C. L. Masters and D. H. Small, *FEBS Letters*, 1997, **415**, 303.
44. F. M. Laferla, J. C. Troncoso, D. K. Strickland, C. H. Kawas and G. Jay, *J. Clin. Invest.*, 1997, **100**, 310.

45. H. Naiki, F. Gejyo and K. Nakakuki, *Biochemistry*, 1997, **36**, 6243.
46. S. Aleshkov, C. R. Abraham and V. I. Zannis, *Biochemistry*, 1997, **36**, 10571.
47. M. H. Hammad, S. Ranganathan, E. Loukinova, W. O. Twal and W. S. Argraves, *J. Biol. Chem*., 1997, **272**, 18644.
48. M. Yaar, S. Zhai, P. F. Pilch, S. M. Doyle, P. B. Eisenhauer, R. E. Fine and B. A. Gilchrest, *J. Clin. Invest.*, 1997, **100**, 2333.
49. M. R. Hileman, B. S. Chapman, S. Rabizadeh, V. V. Krishnan, D. Dredesen, N. Assa-Munt and L. A. Plesniak, *FEBS Lett.*, 1997, **415**, 145.
50. E. Liepinsh, L. L. Ilag, G. Otting and C. F. Ibanez, *EMBO J.,* 1997, **17**, 4999.
51. R. E. W. Hancock, *Lancet*, 1997, **349**, 418.
52. T. Ravi Rajagopalan, A. Datta and D. Pei, *Biochemistry*, 1997, **36**, 13910.
53. C. B. Park, J. H. Lee, I. Y. Park, M. S. Kim and S. C. Kim, *FEBS Letters*, 1997, **411**, 173.
54. D. A. Steinberg, M. A. Hurst, C. A. Fuji, A. H. Kung, J. F. Ho, F-C. Cheng, D. J. Loury and J. C. Fiddes, *Antimicrobial Agents Chemotherapy*, 1997, **41**, 1738.
55. I. H. Lee, Y. Cho and R. I. Lehrer, *Comp. Biochem. Physiol.*, 1997, **118B**, 515.
56. S. B. Tencza, J. P. Douglass, D. J. Creighton, Jr., R. C. Montelaro and T. A. Mietzner, *Antimicrobial Agents Chemotherapy*, 1997, **41**, 2394.
57. J. Rees, M. Moniatte, *et al.*, *Insect Biochem. M*, 1997, **27**, 413.
58. D. J. Mason, R. Dybowski, J. W. Larrick and V. A. Gant, *Antimicrobial Agents Chemotherapy, 1997*, **41**, 624.
59. K. M. Huttner, C. A. Kozak and C. L. Bevins, *FEBS Letters,* 1997, **413**, 45.
60. K. A. Brogden, M. Ackermann and K. M. Huttner, *Antimicrobial Agents Chemotherapy*, 1997, **41**, 1615.
61. A. Tossi, C. Tarantino, and D. Romeo, *Eur. J. Biochem.*, 1997, **250**, 549.
62. T. Wieprecht, M. Dathe, M. Beyermann, E. Krause, W. L. Maloy, D. L. McDonald and M. Bienert, *Biochemistry*, 1997, **36**, 6124.
63. T. Wieprecht, M. Dathe, E. Krause, M. Beyermann, W. L. Maloy, D. L. McDonald and M. Bienert, *FEBS Letters*, 1997, **417**, 135.
64. T. J. Falla and R. E. W. Hancock, *Antimicrobial Agents Chemotherapy*, 1997, **41**, 771.
65. Y. Nakajima, J. Alvarez-Bravo, J-H. Cho, K-I. Homma, S. Kanegasaki and S. Natori, *FEBS Letters*, 1997, **415**, 64.
66. K. Matsuzaki, S. Yoneyama, N. Fujii, K. Miyajima, K. Yamada, Y. Kirino and K. Anzai, *Biochemistry*, 1997, **36**, 9799.
67. M. Javadpour, and M. Barkley, *Biochemistry*, 1997, **36**, 9540.
68. H. Tsai and L. A. Bobek, *Antimicrobial Agents Chemotherapy, 1997*, **41**, 2224.
69. A, A. J. DeLucca, J. M. Bland, T. J. Jacks, C. Grimm, T. E. Cleveland and T. J. Walsh, *Antimicrobial Agents Chemotherapy*, 1997, **41**, 481.
70. D. G. Lee, J-H. Park, S. Y. Shin, S. G. Lee, M. K. Lee, K. L. Kim and K-S. Hahm, *Biochem. Mol. Biol. Int.*, 1997, **43**, 489.
71. R. A. Zambias, C. James, G. K. Abruzzo, K. F. Bartizal, R. Hajdu, R. Thompson, K. H. Nollstadt, J. Marrinan and J. M. Balkovec, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2021.
72. B. Devadas, M. E. Zupec, S. K. Freeman, D. L. Brown, S. Nagarajan, J. A. Sikorski, C. A. McWherter, D. P. Getman and J. I. Gordon, *J. Med. Chem.*, 1995, **38**, 1837.
73. B. Devadas, S. K. Freeman, C. A. McWherter, D. W. Kuneman, D. V. Vinjamoori, and J. A. Sikorski, *Bioorg. Med. Chem. Lett*., 1996, **6**, 1977.
74. S. R. Nagarajan, B. Devadas, M. E. Zupec, S. K. Freeman, D. L. Brown, H-F. Lu, P. P. Mehta, N. S. Kishore, C. A. McWherter, D. P. Getman, J. I. Gordon and J. A. Sikorski, *J. Med. Chem.*, 1997, **40**, 1422.

75. B. Devadas, S. K. Freeman, M. E. Zupec, H-F. Lu, S. R. Nagarajan, N. S. Kishore, J. F. Lodge, D. W. Kuneman, C. A. McWherter, D. V. Vinjamoori, D. P. Getman, J. I. Gordon and J. A. Sikorski, *J. Med. Chem*., 1997, **40**, 2609.
76. D. L. Brown, B. Devadas, H-F. Lu, S. Nagarajan, M. E. Zupec, S. K. Freeman, C. A. McWherter, D. P. Getman and J. A. Sikorski, *Bioorg. Med. Chem. Lett*., 1997, **7**, 379.
77. M. Schafer, S. A. Mousa and C. Stein, *Eur. J. Pharmacol.*, 1997, **323**, 1.
78. C. W. Liaw, D. E. Grigoriadis, T. W. Lovenberg, E. B. De Souza and R. A. Maki, *Molecular Endocrinology,* 1997, **11**, 980.
79. C. W. Liaw, D. E. Grigoriadis, M. T. Lorang, E. B. De Souza and R. A. Maki, *Molecular Endocrinology,* 1997, **11**, 2048.
80. Y. L. Chen, R. S. Mansbach, S. M. Winter, E. Brooks, J. Collins, M. L. Corman, A. R. Dunaiskis, W. S. Faraci, R. J. Gallaschun, A. Schmidt and D. W. Schulz, *J. Med. Chem.*, 1997, **40**, 1749.
81. V. Martinez, J. Rivier, L. Wang and Y. Tache, *J. Pharmacol. Exp. Therap.*, 1997, **280**, 754.
82. R. D. Smith and P. B. M. W. M. Timmermans, *Pharmaceutical News*, 1997, **4**, 16.
83. C. I. Johnston, M. Naitoh and L. M. Burrell, *J. Hypertension*, 1997, **15** (suppl. 7), S3.
84. L. Morgan, F. Pipkin, *et al.*, *Int. J. Biol. Chem.*, 1996, **28**, 1211.
85. T. Unger, O. Chung, T. Csikos, J. Culman, S. Gallinat, P. Gohlke, S. Hohle, S. Meffert, M. Stoll, U. Stroth and Y-Z. Zhu, *J. Hypertension*, 1996, **14**, S 95.
86. S. L. Chambers, R. Ronald, J. M. Hanesworth, D. H. Kinder and J. W. Harding, *Peptides*, 1997, **18**, 505.
87. L. Maletinska, W. Neugebauer, J. Perodin, M. Lefebre and E. Escher, *J. Med. Chem.*, 1997, **40**, 3271.
88. B. Schmidt, S. Lindman, W. Tong, G. Lindeberg, A. Gogoll, Z. Lai, M. Thornwall, B. Synnergren, A. Nilsson, C. J. Welch, M. Sohtell, C. Westerlund, F. Nyberg, A. Karlen and A. Hallberg, *J. Med. Chem.*, 1997, **40**, 903.
89. C. Almansa, L. A. Gomez, F. L. Cavalcanti, A. F. de Arriba, J. Garcia-Rafanell and J. Forn, *J. Med. Chem.*, 1997, **40**,547.
90. S. Yoo, S-H. Lee, S-K. Kim and S-H. Lee, *Bioorg. Med. Chem.*, 1997, **5**, 445.
91. Y. Hashimoto, Y. Harada, H. Narita, K. Naito and S. Murata, *J. Cardiovasc. Pharmacol.*, 1997, **29**, 284.
92. S. Perlman, C.M. Costa-Neto, A.A. Miyakawa, H.T. Schambye, S.A. Hjorth, A.C.M. Paiva, R.A. Rivero, W.J. Greenlee and T.W. Schwartz, *Mol. Pharmacol.*, 1997, **51**, 301.
93. A. F. De Arriba, L. A. Gomez-Casajus, F. Cavalcanti, C. Almansa, J. Garcia-Rafanell and J. Forn, *Eur. J. Pharmacol*., 1996, **318**, 341.
94. M. Hagmann, J. Nussberger, R. B. Naudin, T. S. Burns, A. Karim, B. Waeber and R. H. Brunner, *J. Cardiovasc. Pharmacol.*, 1997, **29**, 444.
95. D. Lyons, J. Webster, A. Nixon, M. M. R. Young, J. Smith and N. Benjamin, *Br. J. Clin. Pharmacol.*, 1997, **43**, 323.
96. W.W. K. R. Mederski, D. Dorsch, M. Osswald, H. Schwartz, N. Beier, M. Christadler, K. O. Minck, P. Schelling and C. J. Schmidt, *Eur. J. Med. Chem*., 1997, **32**, 479.
97. M. Shibasaki, A. Fujimori, T. Kusayama, T. Tokioka, Y. Satoh, T. Okazaki, W. Uchida, O. Inagaki and I. Yanagisawa, *Eur. J. Pharmacol.*, 1997, **335**, 175.
98. M. Shibasaki, A. Fujimori, M. Takanashi, T. Kusayama, T. Tokioka, Y. Satoh, T. Okazaki, W. Uchida, O. Inagaki and I. Yanagisawa, *Eur. J. Pharmacol*., 1997, **335**, 167.

99. K. Tamura, M. Okuhira, H. Amano, K. Inokuma, T. Hirata, I. Mikoshiba and K. Hashimoto, *J. Cardiovasc. Pharmacol*., 1997, **30**, 607.
100. E. Villa, A. Rabano, M. Cazes, A. Cloarec, L. M. Ruilope and R. Garcia-Robles, *Am. J. Hypertension*, 1997, **10**, 275.
101. A. Virone-Oddos, V. Desangle, D. Provost, M. Cazes, F. Caussade and A. Cloarec, *Br. J. Pharmacol.*, 1997, **120**, 488.
102. A. Pansky, F. Peng, M. Eberhard, L. Baselgia, W. Siegrist, J. B. Baumann, A. N. Eberle, C. Beglinger and P. Hildebrand, *Eur. J. Clin. Invest.*, 1997, **27**, 69.
103. G. Halmos and A. V. Schally, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 956.
104. K. Szepeshazi, A. V. Schally, G. Halmos, N. Lamharzi, K. Groot and J. E. Horvath, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 10913.
105. H. Ohki-Hamazaki, K. Watase, K. Yamamoto, H. Ogura, M. Yamano, K. Yamada, H. Maeno, J. Imaki, S. Kikuyama, E. Wada and K. Wada, *Nature,* 1997*,* **390**, 165.
106. R. I. Feldman, M. F. Bartholdi and J. M. Wu, *Molecular Pharmacol*., 1996, **50**, 1346.
107. M. Akeson, E. Sainz, S. A. Mantey, R. T. Jensen and J. F. Battey, *J. Biol. Chem.*, 1997, **272**, 17405.
108. J. M. Wu, D. E. Nitecki, S. Biancalana and R. I. Feldman, *Molecular Pharmacol*., 1996, **50**, 1355.
109. S. A. Mantey, H. C. Weber, E. Sainz, M. Akeson, R. R. Ryan, T. K. Pradhan, R. P. Searles, E. R. Spindel, J. F. Battey, D. H. Coy and R. T. Jensen, *J. Biol. Chem.*, 1997, **272**, 26062.
110. M. Llinares, C. Devin, J. Azay, G. Berge, J. A. Fegrentz and J. Martinez, *Eur. J. Med. Chem.*, 1997, **32**, 767.
111. J. M. Eden, M. D. Hall, M. Higginbottom, D. C. Horwell, W. Howson, J. Hughes, R. E. Jordan, R. A. Lewthwaite, K. Martin, A. T. McKnight, J. C. O'Toole, D. R. Pinnock, M. C. Pritchard, N. Suman-Chauhan and S. C. Williams, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 2617.
112. A. Nagy, P. Armatis, R-Z. Cai, K. Szepeshazi, G. Halmos and A. V. Schally, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 652.
113. M. Pellegrini, S. Mammi, E. Peggion and D. F. Mierke, *J. Med. Chem*., 1997, **40**, 92.
114. M. Pellegrini and D. F. Mierke, *J. Med. Chem*., 1997, **40**, 99.
115. J. M. Conlon and U. Aronsson, *Peptides*, 1997, **18**, 361.
116. N. Murayama, M. A. F. Hayashi, H. Ohi, L. A. F. Ferreira, V. V. Hermann, H. Saito, Y. Fujita, S. Higuchi, B. L. Fernandes, T. Yamane and A. C. M. de Camargo, *Proc. Natl. Acad. Sci.*, 1997, **94**, 1189.
117. M. Tancredi, C. Galoppini, S. Meini, L. Quartara, C. A. Maggi and P. Rovero, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2661.
118. M. Asano, N. Inamura, C. Hatori, H. Sawai, T. Fujiwara, A. Katayama, H. Kayakiri, S. Satoh, Y. Abe, T. Inoue, Y. Sawada, K. Nakahara, T. Oku and M. Okuhara, *Br. J. Pharmacol*., 1997, **120**, 617.
119. M. Majima, N. Kawashima, I. Hiroshi and M. Katori, *Br. J. Pharmacol*., 1997, **121**, 723.
120. T. Griesbacher and F. J. Legat, *Br. J. Pharmacol*., 1997, **120**, 933.
121. A. Rizzi, F. Gobeil, G. Calo, N. Inamura and D. Regoli, *Hypertension*, 1997, **29**, 951.
122. I. Aramori, J. Zenkoh, N. Morikawa, N. O'Donnell, M. Asano, K. Nakamura, M. Iwami, H. Kojo and Y. Notsu, *Mol. Pharmacol*., 1997, **51**, 171.
123. S. M. Dankwardt, S. Ferla, J. L. Krstenansky, S. Bhakta, H. Ostrelich and K. Jarnagin, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1921.

124. I. Aramori, J. Zenkoh, N. Morikawa, M. Asano, C. Hatori, H. Sawai, H. Kayakiri, S. Satoh, T. Inoue, Y. Abe, Y. Sawada, T. Mizutani, N. Inamura, K. Nakahara, H. Kojo, T. Oku and Y. Notsu, *Mol. Pharmacol*., 1997, **52**, 16.
125. V. S. Goodfellow, C. P. Laudeman, J. I. Gerrity, M. Burkard, E. Strobel, J. S. Zuzack and D. A. McLeod, *Molecular Diversity*, 1997, **2**, 97.
126. S. Herrero, M. L. Suarez-Gea, R. Gonzalez-Muniz, M. T. Garcia-Lopez, R. Herranz, S. Ballaz, A. Barber, A. Furtuno and J. D. Rio, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 855.
127. K. Shiosaki, C. W. Lin, H. Kopecka, B. Bianchi, T. Miller, M. Stashko and D. Witte, *J. Med. Chem.*, 1997, **40**, 1169.
128. A. G. S. Blommaert, H. Dhotel, B. Ducos, C. Durieux, N. Goudreau, A. Bado, C. Garbay and B. P. Roques, *J. Med. Chem.*, 1997, **40**, 647.
129. M. E. Pierson, J. M. Comstock, R. D. Simmons, F. Kaiser, R. Julien, J. Zongrone and J. D. Rosamond, *J. Med. Chem.*, 1997, **40**, 4302.
130. S. Hagishita, K. Seno, S. Kamata, N. Haga, Y. Ishihara, M. Ishikawa and M. Shimamura, *Bioorg. Med. Chem*., 1997, **5**, 1433.
131. N. Bailey, P. C. Box, R. A. E. Carr, J. W. B. Cooke, B. Evans, H. Finch, J. E. Head, M. Pass, P. Shah and J. R. Wheatcroft, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 281.
132. G. Semple, H. Ryder, D. P. Rooker, A. R. Batt, D. A. Kendrick, M. Szelke, M. Ohta, M. Satoh, A. Nishida, S. Akuzawa and K. Miyata, *J. Med. Chem.*, 1997, **40**, 331.
133. G. C. Hirst, K. L. Queen, E. E. Sugg and T. M. Willson, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 511.
134. J. L. Castro, H. B. Broughton, M. G. N. Russell, D. Rathbone, A. P. Watt, R. G. Ball, K. L. Chapman, S. Patel, A. J. Smith, G. R. Marshall and V. G. Matassa, *J. Med. Chem.*, 1997, **40**, 2491.
135. S. Tabuchi, H. Ito, H. Sogabe, M. Kuno, I. Katsumi, N. Yamamoto, H. Mitsui and Y. Satoh, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 169.
136. B. R. Henke, C. J. Aquino, L. S. Birkemo, D. K. Croom, R. W. Dougherty, Jr., G. N. Ervin, M. K. Grizzle, G. C. Hirst, M. K. James, M. F. Johnson, K. L. Queen, R. G. Sherrill, E. E. Sugg, E. M. Suh, J. W. Szewczyk, R. J. Unwalla, J. Yingling and T. M. Willson, *J. Med. Chem*., 1997, **40**, 2706.
137. J. K. Padia, H. Cholvers, P. Daum, R. Pinnock, N. Suman-Chauhan, L. Webdale and B. K. Trivedi, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 805.
138. S. Hagishita, Y. Murakami, K. Seno, S. Kamata, N. Haga, T. Konoike, Y. Kanda, R. Kiyama, T. Shiota, Y. Ishihara, M. Ishikawa, M. Shimamura, K. Abe and K. Yoshimura, *Bioorg. Med. Chem*., 1997, **5**, 1695.
139. B. Bellier, I. McCort-Tranchepain, B. Ducos, S. Danascimento, H. Meudal, F. Noble, C. Garbay and B. P. Roques, *J. Med. Chem.*, 1997, **40**, 3947.
140. C. E. Augelli-Szafran, T. S. Purchase, B. D. Roth, B. Tait, B. K. Trivedi, M. Wilson, N. Suman-Chauhan and L. Webdale, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2009.
141. S. E. Gibson, N. Guillo, S. B. Kalindjian and M. J. Tozer, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1289.
142. Y. Kawanishi, S. Ishihara, T. Tsushima, S. Hagishita, M. Ishikawa and Y. Ishihara, *Bioorg. Med. Chem.*, 1997, **5**, 1401.
143. Y. Kawanishi, S. Ishihara, T. Tsushima, K. Seno, S. Hagishita, M. Ishikawa and Y. Ishihara, *Bioorg. Med. Chem.*, 1997, **5**, 1411.
144. Y. Kawanishi, S. Ishihara, R. Kiyama, S. Hagishita, T. Tsushima, M. Ishikawa and Y. Ishihara, *Bioorg. Med. Chem.*, 1997, **5**, 1425.
145. R. S. Ames, Yi. Li, H. M. Sarau, P. Nuthulaganti, J. J. Foley, C. Ellis, Z. Zeng, K.

Su, A. J. Jurewicz, R. P. Hertzberg, D. J. Bergsma and C. Kumar, *J. Biol. Chem.*, 1996, **271**, 20231.
146. M. Takahashi, S. Moriguchi, H. Suganuma, A. Shiota, F. Tani, H. Osui, K. Kurahashi, R. Sasaki and M. Yoshikawa, *Peptides*, 1997, **18**, 329.
147. A. M. Finch, S. M. Vogen, S. A. Sherman, L. Kirnarsky, S. M. Taylor and S. D. Sanderson, *J. Med. Chem.*, 1997, **40**, 877.
148. S. E. De Laszlo, E. E. Allen, B. Li, D. Ondeyka, R. Rivero, L. Malkowitz, C. Molineaux, S. J. Siciliano, M. S. Springer, W. J. Greenlee and N. Mantlo, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 213.
149. P. C. Astles, T. J. Brown, P.Cox, F. Halley, P. M. Lockey, C. McCarthy, I. M. McLay, T. N. Majid, A. D. Morley, B. Porter, A. J. Ratcliffe and R. J. A. Walsh, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 907.
150. P. Nambi, M. Pullen, J. Kincaid, P. Nuthulaganti, N. Aiyar, D. P. Brooks, M. Gellai and C. Kumar, *Mol. Pharmacol.*, 1997, **52**, 582.
151. Y. Masuda, T. Sugo, T. Kikuchi, M. Satoh, Y. Fujisawa, Y. Itoh, M. Wakimasu and T. Ohtaki, *Eur. J. Pharmacol.*, 1997, **325**, 263.
152. T. Suzawa, K. Shibata, T. Tanaka, Y. Matsuda and M. Yamasaki, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1715.
153. W. L. Cody, J. X. He, M. D. Reily, S. J. Haleen, D. M. Walker, E. L. Reyner, B. H. Stewart and A. M. Doherty, *J. Med. Chem.*, 1997, **40**, 2228.
154. B. Raju, I. Okun, F. Stavros and M. F. Chan, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 933.
155. B. Raju, I. Okun, F. Stavros and M. F. Chan, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 939.
156. B. Raju, C. Wu, R. Castillo, I. Okun, F. Stavros and M. F. Chan, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2093.
157. C. Wu, M. F. Chan, F. Stavros, B. Raju, I. Okun, S. Mong, K. M. Keller, T. Brock, T. P. Kogan and R. A. F. Dixon, *J. Med. Chem.*, 1997, **40**, 1690.
158. C. Wu, M. F. Chan, F. Stavros, B. Raju, I. Okun and R. S. Castillo, *J. Med. Chem.*, 1997, **40**, 1682.
159. R. H. Bradbury, C. Bath, R. J. Butlin, M. Dennis, C. Heys, S. J. Hunt, R. James, A. A. Mortlock, N. F. Sumner, E. K. Tang, B. Telford, E. Whiting and C. Wilson, *J. Med. Chem.*, 1997, **40**, 996.
160. A. M. Mortlock, C. Bath, R. J. Butlin, C. Heys, S. J. Hunt, A. C. Reid, N. F. Sumner, E. K. Tang, E. Whiting, C. Wilson and N. D. Wright, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1399.
161. D. Dorsch, W. K. R. Mederski, M. Osswald, R. M. Devant, C-J. Schmitges, M. Christadler and C. Wilm, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 275.
162. W. Neidhart, V. Breu, K. Burri, M. Clozel, G. Hirth, U. Klinkhammer, T. Giller and H. Ramuz, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2223.
163. S. Roux, V. Breu, T. Giller, W. Neidhart, H. Ramuz, P. Coassolo, J. P. Clozel and M. Clozel, *J. Pharmacol. Exp. Therap.*, 1997, **283**, 1110.
164. W. C. Patt, J. J. Edmunds, J. T. Repine, K. A, berryman, B. R. Reisdorph, C. Lee, M. S. Plummer, A. Shahripour, S. J. Haleen, J. A. Keiser, M. A. Flynn, K. M. Welch, E. E. Reynolds, R. Rubin, B. Tobias, H. Hallak and A. M. Doherty, *J. Med. Chem.*, 1997, **40**, 1063.
165. W. C. Patt, B. R. Reisdorph, J. T. Repine, A. M. Doherty, S. J. Haleen, D. M. Walker, K. M. Welch, M. A. Flynn, H. Hallak, E. L. Reyner and B. H. Stewart, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 297.
166. W. W. K. R. Mederski, M. Osswald, D. Dorsch, M. Christadler, C-J. Schmitges and C. Wilm, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1883.

167. D. C. Underwood, S. Bochnowicz, R. R. Osborn, M. A. Luttmann and D. W. P. Hay, *J. Pharmacol. Exp. Therap.*, 1997, **283**, 1130.
168. A. S. Tasker, B. K. Sorensen, H-S. Jae, M. Winn, T. W. von Geldern, D. B. Dixon, W. J. Chiou, B. D. Dayton, S. Calzadila, L. Hernandez, K. C. Marsh, J. R. Wu Wong and T. J. Opgenorth, *J. Med. Chem.*, 1997, **40**, 322.
169. H-S. Jae, M. Winn, D. B. Dixon, K. C. Marsh, B. Nguyen, T. J. Opgenorth and T. W. von Geldern, *J. Med. Chem.*, 1997, **40**, 3217.
170. E. Ghigo, E. Arvat, G. Muccioli and F. Camanni, *Eur. J. Endocrinol.*, 1997, **136**, 445.
171. J. Cheng, T-J. Wu, B. Butler and K. Cheng, *Life Sci.*, 1997, **60**, 1385.
172. G. Tolis, I. Karydis, V. Markousis, M. Karagiorga, T. Mesimeris, V. Lenaerts and R. Deghenghi, *J. Pediatric Endocrinol. Metab.*, 1997, **10**, 35.
173. B. Peschke, K. Madsen, B. S. Hansen and N. L. Johansen, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1969.
174. M. Zarandi, M. Kovacs, J. E. Horvath, K. Toth, G. Halmos, K. Groot, A. Nagy, Z. Kele and A. V. Schally, *Peptides*, 1997, **18**, 423.
175. M. Kovacs, A. V. Schally, M. Zarandi and K. Groot, *Peptides*, 1997, **18**, 431.
176. A. Jungwirth, A. V. Schally, J. Pinsky, K. Groot, P. Armatus and G. Halmos *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 5810.
177. J. R. Tata, R. P. Nargund, M. M. Murphy, D. B. R. Johnston, A. A. Patchett, K. Cheng, L. Wei, W. W-S. Chan, B. Butler, T. M. Jacks, G. Hickey and R. Smith, *Bioorg. Med. Chem. Lett.*,1997, **7**, 663.
178. J. R. Tata, Z. Lu, T. M. Jacks, K. D. Schleim, K. Cheng, L. Wei, W. W-S. Chan, B. Butler, T. Tsou, K. Leung, H-S. L. Chiu, G. Hickey, R. G. Smith and A. A. Patchett, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2319.
179. M. Ankersen, B. Peschke, B. S. Hansen and T. K. Hansen, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1293.
180. T. K. Hansen, H. Thogersen and B. S. Hansen, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2951.
181. H. Mohri, *Peptides*, 1997, **18**, 899.
182. C. G. Gahmberg, M. Tolvanen and P. Kotovuori, *Eur. J. Biochem.*, 1997, **245**, 215.
183. S. Rodan and G. Rodan, *J. Endocrinol.*, 1997, **154**(S), S 47.
184. R. K. Andrews, J. A. Lopez and M. C. Berndt, *Int. J. Biochem. Cell Biol.*, 1997, **29**, 91.
185. M. A. Gimbrone, Jr., T. Nagel and J. N. Topper, *J. Clin. Invest.*, 1997, **99**, 1809.
186. J. C. Loftus and R. C. Liddington, *J. Clin. Invest.*, 1997, **99**, 2302.
187. B. S. Coller, *J. Clin. Invest.*, 1997, **99**, 1467.
188. E. Ruoslahti and E. Engvall, *J. Clin. Invest.*, 1997, **99**, 1149.
189. J. Bischoff, *J. Clin. Invest.*, 1997, **99**, 373.
190. C. Li, J. B. McCarthy, L. T. Furcht and G. B. Fields, *Biochemistry*, 1997, **36**, 15404.
191. P. Vanderslice, K. Ren, J. K. Revelle, D. C. Kim, D. Scott, R. J. Bjercke, E. T. H. Yeh, P. J. Beck and T. P. Kogan, *J. Immunol.*, 1997, **158**, 1710.
192. B. W. McIntyre, D. G. Woodside, D. A. Caruso, D. K. Wooten, S. I. Simon, S. Neelamegham, J. K. Revelle and P. Vanderslice, *J. Immunol.*, 1997, **158**, 4180.
193. J. V. Moyano, B. Carnemolla, C. Dominguez-Jimenez, M. Garcia-Gila, J. P. Albar, P. Sanchez-Aparicio, A. Leprini, G. Querze, L. Zardi and A. Garcia-Pardo, *J. Biol. Chem.*, 1997, **272**, 24832.
194. K. C. Carson, C. F. Schwender, H. N. Shroff, N. A. Cochran, D. L. Gallant and M. J. Briskin, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 711.

195. J. E. Mogford, G. E. Davis and G. A. Meininger, *J. Clin. Invest*., 1997, **100**, 1647.
196. K. Suehiro, J. Gailit and E. F. Plow, *J. Biol. Chem.*, 1997, **272**, 5360.
197. D. Panda, G. C. Kundu, B. I. Lee, A. Peri, D. Fohl, I. Chackalaparampil, B. B. Mukherjee, X. D. Li, D. C. Mukherjee, S. Seides, J. Rosenberg, K. Stark and A. B. Mukherjee, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 9308.
198. J. Wermuth, S. L. Goodman, A. Jonczyk, and H. Kessler, *J. Am. Chem. Soc*., 1997, **119**, 1328.
199. T-A. Tran, R-H. Mattern, Q. Zhu and M. Goodman, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 997.
200. T. J. Kunicki, D. S. Annis and B. Felding-Habermann, *J. Biol. Chem.*, 1997, **272**, 4103.
201. A. Mousa and W. F. DeGrado, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1371.
202. V. W. Engelman, G. A. Nickols, F. P. Ross, M. A. Horton, D. W. Griggs, S. L. Settle, P. G. Ruminski and S. L. Teitelbaum, *J. Clin. Invest*., 1997, **99**, 2284.
203. R. M. Keenan, W. H. Miller, C. Kwon, F. E. Ali, J. F. Callahan, R. R. Calvo, S-M. Hwang, K. D. Kopple, C. E. Peishoff, J. M. Samanen, A. S. Wong, C-K. Yuan and W. F. Huffman, *J. Med. Chem.*, 1997, **40**, 2289.
204. C-B. Xue, J. Roderick, S. Jackson, M. Rafalski, A. Rockwell, S. Mousa, R. E. Olson and W. F. DeGrado, *Bioorg. Med. Chem*., 1997, **5**, 693.
205. C. B. Xue, J. Wityak, T. M. Sielecki, D. J. Pinto, D. G. Batt, G. A. Cain, M. Sworin, A. L. Rockwell, J. J. Roderick, S. Wang, M. J. Orwat, W. E. Frietze, L. L. Bostrom, J. Liu, C. A. Higley, F. W. Rankin, E. Tobin, G. Emmett, G. K. Lalka, J. Y. Sze, S. V. Di Meo, S. A. Mousa, M. J. Thoolen, A. L. Racanelli, E. A. Hausner, T. M. Reilly, W. F. DeGrado, R. R. Wexler and R. E. Olson, *J. Med. Chem.*, 1997, **40**, 2064.
206. J. D. Prugh, R. J. Gould, R. J. Lynch, G. Zhang, J. J. Cook, M. A. Holahan, M. T. Stranieri, G. R. Sitko, L. Gaul, R. A. Bednar, B. Bednar and G. D. Hartman, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 865.
207. B. C. Askew, C. J. McIntyre, C. A. Hunt, D. A. Claremon, J. J. Baldwin, P. S. Anderson, R. J. Gould, R. J. Lynch, C. C-T. Chang, J. J. Cook, J. J. Lynch, M. A. Holahan, G. R. Sitko and M. T. Stranien, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1531.
208. J. J. Cook, G. R. Sitko, M. A. Holahan, M. T. Stranieri, J. D. Glass, B. C. Askew, C. J. McIntyre, D. A. Claremon, J. J. Baldwin,G. D. Hartman, R. J. Gould and J. J. Lynch Jr., *J. Pharmacol. Exp. Therap.*, 1997, **281**, 677.
209. B. A. Askew, R. A. Bednar, B. Bednar, D. A. Claremon, J. J. Cook, C. J. McIntyre, C. A. Hunt, R. J. Gould, R. J. Lynch, J. J. Lynch Jr., S. L. Gaul, M. T. Stranieri, G. R. Sitko, M. A. Holahan, J. D. Glass, T. Hamill, L. M. Gorham, T. Prueksaritanont, J. J. Baldwin and G. D. Hartman, *J. Med. Chem.*, 1997, **40**, 1779.
210. K. M. Brashear, J. A. Cook, B. Bednar, R. A. Bednar, R. J. Gould, W. Halczenko, M. A. Holahan, R. J. Lynch, G. D. Hartman and J. H. Hutchinson, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2793.
211. G. Kottirsch, H-G. Zerwes, N. S. Cook and C. Tapparelli, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 727.
212. T. Harada, J. Katada, A. Tachiki, T. Asari, K. Iijima, I. Uno, I. Ojima and Y. Hayashi, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 209.
213. T. Asari, S. Ishikawa, T. Sasaki, J. Katada, Y. Hayashi, T. Harada, M. Yano, E. Yasuda, I. Uno and I. Ojima, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2099.
214. B. K. Blackburn, A. Lee, M. Baier, B. Kohl, A. G. Olivero, R. Matamoros, K. D. Robarge and R. S. McDowell, *J. Med. Chem.*, 1997, **40**, 717.
215. J. Wityak, T. M. Sielecki, D. J. Pinto, G. Emmett, J. Y. Sze, J. Liu, A. E. Tobin, S.

Wang, B. Jiang, P. Ma, S. A. Mousa, R. R. Wexler, and R. E. Olson, *J. Med. Chem.*, 1997, **40**, 50.
216. M. J. Fisher, B. Gunn, C. S. Harms, A. D. Kline, J. T. Mullaney, A. Nunes, R. M. Scarborough, A. E. Arfsten, M. A. Skelton, S. L. Um, B. G. Utterback and J. A. Jakubowski, *J. Med. Chem.*, 1997, **40**, 2085.
217. M. J. Fisher, B. P. Gunn, C. S. Harms, A. D. Kline, J. T. Mullaney, R. M. Scarborough, M. A. Skelton, S. L. Um, B. G. Utterback and J. A. Jakubowski, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2537.
218. D. J. Sall, A. E. Arfsten, J. A. Bastian, M. L. Denney, C. S. Harms, J. R. McCowan, J. M. Morin, Jr., J. W. Rose, R. M. Scarborough, M. S. Smyth, S. L. Um, B. G. Utterback, R. T. Vasileff, J. H. Wikel, V. L. Wyss and J. A. Jakubowski, *J. Med. Chem.*, 1997, **40**, 2843.
219. T. Su, M. A. H. Naughton, M. S. Smyth, J. W. Rose, A. F. Arfsten, J. R. McCowan, J. A. Jakubowski, V. L. Wyss, K. J. Ruterbories, D. J. Sall and R. M. Scarborough, *J. Med. Chem.*, 1997, **40**, 4308.
220. A. Tanaka, H. Sakai, T. Ishikawa, T. Aoki, Y. Motoyama and H. Takasugi, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 521.
221. S. I. Klein, M. Czekaj, B. F. Molino and V. Chu, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1773.
222. A. V. Schally and A. M. Comaru-Schally, *Adv. Drug Delivery Rev.*, 1997, **28**, 157.
223. A. G. Craig, W. H. Fischer, M. Park, J. E. Rivier, B. D. Musselman, J. F. F. Powell, S. M. Reska-Skinner, M. O. Prakash, G. O. Mackie and N. M. Sherwood, *FEBS Letters*, 1997, **413**, 215.
224. W.H. Yu, S. Karanth, A. Walczewska, S.A. Sower and S.M. McCann, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 9499.
225. I. Mezo, S. Lovas, I. Palyi, B. Vincze, A. Kalnay, G. Turi, Z. Vadasz, J. Seprodi, M. Idei, G. Toth, E. Gulyas, F. Otvos, M. Mak, J. E. Horvath, I. Teplan and R. F. Murphy, *J. Med. Chem.*, 1997, **40**, 3353.
226. G. Jiang, C. Miller, S. C. Koerber, J. Porter, A. G. Craig, S. Bhattacharjee, P. Craft, T. P. Burris, C. A. Campen, C. L. Rivier and J. E. Rivier, *J. Med. Chem.*, 1997, **40**, 3739.
227. M. Keramida, J. M. Matsoukas, D. Panagiotopoulos, K. Alexopoulos, E. Matsoukas, J. Cladas, V. A. Tzingounis, E. Cardamakis, H. L. S. Maia, D. Pati and H. R. Habibi, *Lett. Peptide Sci.*, 1996, **3**, 257.
228. K. Mountjoy and J. Wong, *Mol. Cell. Endocrinol.*, 1997, **128**, 171.
229. W. Fan, B. A. Boston, R. A. Kesterson, V. J. Hruby and R. D. Cone, *Nature*, 1997, **385**, 165.
230. M. M. Ollmann, B. D. Wilson, Y-K. Yang, J. A. Kerns, Y. Vhen, I. Gantz and G. S. Barsh, *Science*, 1997, **278**, 135.
231. L. L. Kiefer, O. R. R. Ittoop, K. Bunce, A. T. Truesdale, D. H. Willard, J. S. Nichols, S. G. Blanchard, K. Mountjoy, W-J. Chen and W. O. Wilkison, *Biochemistry*, 1997, **36**, 2084.
232. D. Huszar, C. A. Lynch, V. Fairchild-Huntress, J. H. Dunmore, Q. Fang, L. R. Berkemeier, W. Gu, R. A. Kesterson, B. A. Boston, R. D. Cone, F. J. Smith, L. A. Campfield, P. Burn and F. Lee, *Cell*, 1997, **88**, 131.
233. R. A. H. Adan and W. H. Gispen, *Peptides*, 1997, **18**, 1279.
234. L. L. Kiefer, O. R. R. Ittoop, K. Bunce, A. T. Truesdale, D. H. Willard, J. S. Nichols, S. G. Blanchard, K. Mountjoy, W-J. Chen and W. O. Wilkison, *Biochemistry*, 1997, **36**, 2084.
235. C. Haskell-Luevano, G. Nikiforovich, S. D. Sharma, Y-K. Yang, C. Dickinson, V. J. Hruby, and I. Gantz, *J. Med. Chem.*, 1997, **40**, 1738.

236. P-J. Peng, U. G. Sahm, R. V. M. Doherty, R. G. Kinsman, S. H. Moss and C. W. Pouton, *Peptides*, 1997, **18**, 1001.
237. V. J. Hruby, D. Lu, S. D. Sharma, A. De L. Castrucci, R. A. Kesterson, F. A. Al-Obeidi, M. E. Hadley and R. D. Cone, *J. Med. Chem.*, 1995, **38**, 3454.
238. H. B. Schioth, R. Muceniece, F. Mutulis, P. Prusis, G. Lindeberg, S. D. Sharma, V. J. Hruby and J. E. S. Wikberg, *Peptides*, 1997, **18**, 1009.
239. C. Haskell-Luevano, S. Hendrata, C. North, T. K. Sawyer, M. E. Hadley, V. J. Hruby, C. Dickinson and I. Gantz, *J. Med. Chem.*, 1997, **40**, 2133.
240. H. B. Schioth, R. Muceniece, M. Larsson, F. Mutulis, M. Szardenings, P. Prusis, G. Lindeberg and J. E. S. Wikberg, *Eur. J. Pharmacol.*, 1997, **319**, 369.
241. M. Szardenings, S. Tornroth, F. Mutulis, R. Muceniece, K. Keinanen, A. Kuusinen and J. E. S. Wikberg, *J. Biol. Chem.*, 1997, **272**, 27943-27948.
242. R. A. H. Adan, J. Oosterom, G. Ludvigsdottir, J. H. Brakkee, J. P. H. Burbach and W. H. Gispen, *Eur. J. Pharmacol.*, 1994, **269**, 331.
243. H. B. Schioth, R. Muceniece and J. E. S. Wikberg, *Peptides*, 1997, **18**, 761.
244. W. Fan, B. A. Boston, R. A. Kesterson, V. J. Hruby and R. D. Cone, *Nature*, 1997, **385**, 165.
245. C. Haskell-Luevano, K. Toth, L. Boteju, C. Job, A. M. de L. Castrucci, M. E. Hadley and V. J. Hruby, *J. Med. Chem.*, 1997, **40**, 2740.
246. A. A. Balasubramaniam, *Peptides*, 1997, **18**, 445.
247. S. D. Samarasinghe, A. Balasubramaniam and M. E. Johnson, *Curr. Med. Chem.*, 1997, **4**, 151.
248. D. O'Shea, D. G. A. Morgan, K. Meeran, C. M. B. Edwards, M. D. Turton, S. J. Choi, M. M. Heath, I. Gunn, G. M. Taylor, J. K. Howard, C. I. Bloom, C. J. Small, O. Haddo, J. J. Ma, W. Callinan, D. M. Smith, M. A. Ghatei and S. R. Bloom, *Endocrinology*, 1997, **138**, 196.
249. A. Bischoff, P. Avramidis, W. Erdbrugger, K. Munter and M. C. Michel, *Br. J. Pharmacol.*, 1997, **120**, 1335.
250. C. J. Small, D. J. A. Morgan, K. Meeran, M. M. Heath, I. Gunn, C. M. B. Edwards, J. Gardiner, G. M. Taylor, J. D. Hurley, M. Rossi, A. P. Goldstone, D. O'Shea, D. M. Smith, M. A. Ghatei and S. R. Bloom, *Proc. Natl. Acad. Sci.*, 1997, **94**, 11686.
251. M. Bitran, A. J. Daniels and M. P. Boric, *Eur. J. Pharmacol.*, 1997, **319**, 43.
252. M. J. D. McCloskey, M. J. Moriarty, A. Tseng, J. Shine and E. K. Potter, *Neuropeptides*, 1997, **31**, 193.
253. D. A. Kirby, K. T. Britton, M. L. Aubert and J. E. Rivier, *J. Med. Chem.*, 1997, **40**, 210.
254. E. Grouzmann, T. Buclins, M. Martire, C. Cannizzaro, B. Dorner, A. Razaname and M. Mutter, *J. Biol. Chem.*, 1997, **272**, 7699.
255. P. A. Hipskind, K. L. Lobb, J. A. Nixon, T. C. Britton, R. F. Bruns, J. Catlow, D. K. Dieckman-McGinty, S. L. Gackenheimer, B. D. Gitter, S. Iyengar, D. A. Shober, R. M. A. Simmons, S. Swanson, H. Zarrinmayeh, D. M. Zimmerman and D. R. Gehlert, *J. Med. Chem.*, 1997, **40**, 3712.
256. F. Hong, B. Cusack, A. Fauq and E. Richelson, *Current Med. Chem.*, 1997, **4**, 421.
257. S. Ushiro, K. Mizoguchi, S. Yoshida, S. Jimi, T. Fujiwara, M. Yoshida, E. T. Wei, P. Kitabgi, S. Amagaya, M. Ono and M. Kuwano, *FEBS Letters*, 1997, **418**, 341.
258. D. Gully, F. Jeanjean, M. Poncelet, R. Steinberg, P. Soubrie, G. Le Fur, and J. P. Maffrand, *Fundamental & Clinical Pharmacology*, 1996, **9**, 513.
259. D. J. Smith, A. A. Hawranko, P. J. Monroe, D. Gully, M. O. Urban, C. R. Craig, J. P. Smith and D. L. Smith, *J. Pharmacol. Exp. Therap.*, 1997, **282**, 899.

260. Y-P. Pang, B. Cusack, K. Groshan, and E. Richelson, *J. Biol. Chem.*, 1996, **271**, 15060.
261. S. J. Johnson, H. C. Akunne, T. G. Heffner, S. R. Kesten, T. A. Pugsley, L. D. Wise and D. J. Wustrow, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 561.
262. D. Gully, B. Labeeuw, R. Boigegrain, F. Oury-Donat, A. Bachy, M. Poncelet, R. Steinberg, M. F. Suaud-Chagny, V. Santucci, N. Vita, F. Pecceu, C. Labbe-Jullie, P. Kitabji, P. Soubrie, G. Le Fur and J. P. Maffrand, *J. Pharmacol. Exp. Therap.*, 1997, **280**, 802.
263. S. J. Johnson, H. C. Akunne, T. G. Heffner, S. R. Kesten, T. A. Pugsley, L. D. Wise and D. J. Wustrow, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 561.
264. D. Gully, B. Labeeuw, R. Boigegrain, F. Oury-Donat, A. Bachy, M. Poncelet, R. Steinberg, M. F. Suaud-Chagny, V. Santucci, N. Vita, F. Pecceu, C. Labbe-Jullie, P. Kitabji, P. Soubrie, G. Le Fur and J. P. Maffrand, *J. Pharmacol. Exp. Therap.*, 1997, **280**, 802.
265. D. L. Heyl and S. E. Schullery, *Curr. Med. Chem.*, 1997, **4**, 117.
266. J-C. Meunier, *Eur. J. Pharmacol.*, 1997, **340**, 1.
267. H. Tollemer, J. Leprince, L. Galas, F. Vandesande, J-C. Le Mevel, M-C. Tonon, J. M. Conlon and H. Vaudry, *Biochem. Biophys. Res. Commun.*, 1997, **238**, 653.
268. S-J. Chen, Y-Z. Chen and J-Y. Wang, *Biochem. Mol. Biol. Int.*, 1997, **43**, 583.
269. T. Hiranuma, K. Iwao, K. Kitamura, T. Matsumiya and T. Oka, *J. Pharmacol. Exp. Therap.*, 1997, **281**, 769.
270. C. Chen and G. M. Pollack, *J. Pharmacol. Exp. Therap.*, 1997, **283**, 1151.
271. V. B. Lang, P. Langguth, C. Ottiger, H. Wunderli-Allenspach, D. Rognan, B. Rothen-Rutishauser, J-C. Perriard, S. Lang, J. Biber and H. P. Merkle, *J. Pharm. Sci.*, 1997, **86**, 846.
272. T. Yamashita, E. Tsuru, E. Banjyo, M. Doe, K. Shibata, M. Yasuda and M. Gemba, *Chem. Pharm. Bull.*, 1997, **45**, 1940.
273. K. Burgess, W. Li, D. S. Linthicum, Q. Ni, D. Pledger, R. B. Rothman and A. Shitangkoon, *Bioorg. Med. Chem.*, 1997, **5**, 1867.
274. K. Kitagawa, N. Mizobuchi, T. Hama, T. Hibi, R. Konishi and S. Futaki, *Chem. Pharm. Bull.*, 1997, **45**, 1782.
275. B. Drouillat, B. Kellam, G. Dekany, M. S. Starr and I. Toth, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2247.
276. A. Capasso, P. Amodeo, G. Balboni, R. Guerrini, L. H. Lazarus, P. A. Temussi and S. Salvadori, *FEBS Letters*, 1997, **417**, 141.
277. S. Salvadori, G. Balboni, R. Guerrini, R. Tomatis, C. Bianchi, S. D. Bryant, P. S. Cooper and L. H. Lazarus, *J. Med. Chem.*, 1997, **40**, 3100.
278. S. Liao, J. Lin, M. D. Shenderovich, Y. Han, K. Hasohata, P. Davis, W. Qiu, F. Porreca, H. I. Yamamura and V. J. Hruby, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 3049.
279. S. A. Thomas, T. J. Abbruscato, V. S. Hau, T. J. Gillespie, J. Zsigo, V. J. Hruby and T. P. Davis, *J. Pharmacol. Exp. Therap.*, 1997, **281**, 817.
280. A. Fiori, P. Cardelli, L. Negri, M. R. Savi, R. Strom and V. Erspamer, *Proc. Natl. Acad. Sci.*, 1997, **94**, 9469.
281. S. D. Bryant, R. Guerrini, S. Salvadori, C. Bianchi, R. Tomatis, M. Attila and L. H. Lazarus, *J. Med. Chem.*, 1997, **40**, 2579.
282. S. E. Schullery, T. Mohammedshah, H. Makhlouf, E. L. Marks, B. S. Wilenkin, S. Escobar, C. Mousigian and D. L. Heyl, *Bioorg. Med. Chem.*, 1997, **5**, 2221.
283. G. Toth, Z. Darula, A. Peter, F. Fulop, D. Tourwe, H. Jaspers, P. Verheyden, Z. Bocskey, Z. Toth and A. Borsodi, *J. Med. Chem.*, 1997, **40**, 990.
284. R. Tomatis, M. Marastoni, G. Balboni, R. Guerrini, A. Capasso, L. Sorrentino, V.

Santagada, G. Caliendo, L. H. Lazarus and S. Salvadori, *J. Med. Chem.*, 1997, **40**, 2948.
285. R. Raghubir, T. Naqvi, S. Varma, W. Haq, K. B. Mathur and G. K. Patnaik, *Med. Chem. Res.*, 1997, **7**, 16.
286. B. Bozu, F. Fulop, G. K. Toth and M. Szucs, *Neuropeptides*, 1997, **31**, 367.
287. M. K. Greenwald, M. L. Stitzer and K. A. Haberny, *J. Pharmacol. Exp. Therap.*, 1997, **281**, 1154.
288. M. R. Tessmer, J-P. Meyer, V. J. Hruby and D. A. Kallick, *J. Med. Chem.*, 1997, **40**, 2148.
289. G. Paterlini, P. Portoghese and D. M. Ferguson, *J. Med. Chem.*, 1997, **40**, 3254.
290. J. Y. Eduardo, R. Butelman, J. H. Woods, B. T. Chait and M. J. Kreek, *J. Pharmacol. Exp. Therap.*, 1997, **280**, 1147.
291. J. Y. Eduardo, R. Butelman, J. H. Woods, B. T. Chait and M. J. Kreek, *J. Pharmacol. Exp. Therap.*, 1997, **282**, 633.
292. H. Choi, T. F. Murray, G. E. DeLander, W. K. Schmidt and J. V. Aldrich, *J. Med. Chem.*, 1997, **40**, 2733.
293. K. Soderstrom, H. Choi, F. W. Berman, J. V. Aldrich and T. F. Murray, *Eur. J. Pharmacol.*, 1997, **338**, 191.
294. S. Arttamangkul, J. Ishmael, T. F. Murray, D. K. Grandy, G. E. DeLander, B. L. Kieffer and J. V. Aldrich, *J. Med. Chem.*, 1997, **40**, 1211.
295. H-P. Nothacker, R. R. Reinscheid, A. Mansour, R. A. Henningsen, A. Ardati, F. J. Monsma Jr., S. J. Watson and O. Civelli, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 8677.
296. F. Genck, J-L. Moreau, J. R. Martin, G. J. Kilpatrick, R. K. Reinscheid, F. J. Monsma, Jr., H-P. Nothacker and O. Civelli, *Proc. Natl. Acad. Sci.*, 1997, **94**, 14854.
297. D. R. Kapusta, S. F. Sezen, J-K. Chang, H. Lippton and V. A. Kenigs, *Life Sci.*, 1997, **60**, PL15.
298. B. Gumusel, Q. Hao, A. Hyman, J-K. Chang, D. R. Kapusta and H. Lippton, *Life Sci.*, 1997, **60**, 141.
299. G. C. Rossi, L. Leventhal, E. Bolan and G. W. Pasternak, *J. Pharmacol. Exp. Therap.*, 1997, **282**, 858.
300. J-L. Botour, C. Moisand, H. Mazarguil, C. Mollerau and J-C. Meunier, *Eur. J. Pharmacol.*, 1997, **321**, 97.
301. C. T. Dooley, C. G. Spaeth, I. P. Berzetei-Gurske, K. Craymer, I. D. Adapa, S. R. Brandt, R. A. Houghten and L. Toll, *J. Pharmacol. Exp. Therap.*, 1997, **283**, 735.
302. R. Guerrini, G. Calo, A. Rizzi, C. Bianchi, L.H. Lazarus, S. Salvadori, P.A. Temussi and D. Regoli, *J. Med. Chem.*, 1997, **40**, 1789.
303. S. Lapalu, C. Moisand, H. Mazarguil, G. Cambois, C. Mollereau and J-C. Meunier, *FEBS Lett.*, 1997, **417**, 333.
304. Y.C. Patel, and C.B. Srikant, *Trends Endocrinol. Metab.*, 1997, **8**, 398.
305. L. J. Hofland and S. W. J. Lamberts, *Bailliere's Clin. Endocrinol. Metab.*, 1996, **10**, 163.
306. J. A. Koenig, J. M. Edwardson and P. P. A. Humphrey, *Br. J. Pharmacol.*, 1997, **120**, 45.
307. J. A. Koenig, J. M. Edwardson and P. P. A. Humphrey, *Br. J. Pharmacol.*, 1997, **120**, 52.
308. I. Shimon and S. Melmed, *J. Endocrinol.*, 1997, **155**(S1): S 3.
309. K. Aurang and K. C. K. Lloyd, *J. Pharmacol. Exp. Therap.*, 1997, **281**, 245.
310. G. Vainas, V. Pasaitou *et al.*, *J. Exp. Clin. Chem.*, 1997, **16**, 119.
311. R. Von Essen, R. Ostermaier, E. Grube, W. Maurer, U. Tebbe, R. Erbel, M. Roth, W. Oel, J. Brom and G. Weidinger, *Circulation*, 1997, **96**, 1482.

312. P. Caron, I. Morange-Ramos, M. Cogne and P. Jaquet, *J. Clin. Endocrinol. Metab*., 1997, **82**, 18.
313. A. K. Flogstad, J. Halse, S. Bakke, I. Lancranjan, P. Marbach, C. H. Burns and J. Jervell, *J. Clin. Endocrinol. Metab*., 1997, **82**, 23.
314. G. Osapay, L. Prokai, H-S. Kim, K. F. Medzihradszky, D. H. Coy, G. Liapakis, T. Reisine, G. Melacini, Q. Zhu, S. H. H. Wang, R-H. Mattern and M. Goodman, *J. Med. Chem*., 1997, **40**, 2241.
315. G. Melacini, Q. Zhu, G. Osapay and M. Goodman, *J. Med. Chem*., 1997, **40**, 2252.
316. P. Leroux, C. Bucharles, E. Bologna and H. Vaudry, *Eur. J. Pharmacol.*, 1997, **337**, 333.
317. R. T. Bass, B. L. Buckwalter, B. P. Patel, M. H. Pausch, L. A. Price, J. Strnad and J. R. Hadcock, *Mol. Pharmacol*., 1996, **50**, 709.
318. G. F. Wilkinson, R. J. Thurlow, L. A. Sellers, J. E. Coote, W. Feniuk and P. P. A. Humphrey, *Br. J. Pharmacol.*, 1996, **118**, 445.
319. L. Quartara and C. A. Maggi, *Neuropeptides*, 1997, **31**, 537.
320. J. E. Krause, P. T. Staveteig, J. N. Mentzer, S. K. Schmidt, J. B. Tucker, R. M. Brodbeck, J-Y. Bu and V. V. Karpitskiy, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 310.
321. G. Caliendo, P. Grieco, E. Perissutti, V. Santagada, G. Saviano, T. Tancredi and P. A. Temussi, *J. Med. Chem.*, 1997, **40**, 594.
322. G. J. Boks, J. P. Tollenaere and J. Kroon, *Bioorg. Med. Chem.*, 1997, **5**, 535.
323. M. A. Cascieri, E. Ber, T. M. Fong, J. J. Hale, F. Tang, L-L. Shiao, S. G. Mills, M. MacCoss, S. Sadowski, M. R. Tota and C. D. Strader, *Eur. J. Pharmacol.*, 1997, **325**, 253.
324. L. Singh, M. J. Field, J. Hughes, B-S. Kuo, N. Suman-Chauhan, B. R. Tuladhar, D. S. Wright and R. J. Naylor, *Eur. J. Pharmacol.*, 1997, **321**, 209.
325. S. Iyengar, P. A. Hipskind, D. R. Gehlert, D. Schober, K. L. Lobb, J. A. Nixon, D. R. Helton, M. J. Kallman, S. Boucher, R. Couture, D. L. Li and R. M. A. Simmons, *J. Pharmacol. Exp. Therap.*, 1997, **280**, 774.
326. L. A. Phebus, K. W. Johnson, P. W. Stengel, K. L. Lobb, J. A. Nixon and P. A. Hipskind, *Life Sci.*, 1997, **60**, 1553.
327. G. Improta, M. Broccardo, A. Tabacco and S. Evangelista, *Neuropeptides*, 1997, **31**, 399.
328. C. Polidori, I. Panocka, R. Ciccocioppo, M. Broccardo, G. Improta, D. Regoli and M. Massi, *Peptides*, 1997, **18**, 825.
329. G. Mignogna, C. Severini, G. F. Erspamer, R. Sicliano, G. Kreil and D. Barra, *Peptides*, 1997, **18**, 367.
330. J. E. Muren and D. R. Nassel, *Peptides*, 1997, **18**, 7.
331. M. J. Seckl, T. Higgins, F. Widmer and E. Rozengurt, *Cancer Res*., 1997, **57**, 51.
332. D. A. Jones, A. J. MacLellan, J. Cummings, A. A. Ritchie, S. P. Langdon and J. F. Smyth, *Peptides*, 1997, **18**, 1073.
333. R. Patacchini, S. Giuliani, M. Lazzeri, A. Turini, L. Quartara and C. A. Maggi, *Neuropeptides*, 1997, **31**, 71.
334. J-L. Fauchere, N. Kucharczyk, E. Jacoby, M. Lonchampt, P. Robineau, C. Dacquet, D. Regoli and E. Canet, *Bioorg. Med. Chem. Lett*., 1997, **7**, 203.
335. M. Astolfi, R. Patacchini, M. Maggi and S. Manzini, *Neuropeptides*, 1997, **31**, 373.
336. D. C. Horwell, D. Naylor and H. M. G. Willems, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 31.
337. S. J. Veenstra, K. Hauser, and C. Betschart, *Bioorg. Med. Chem. Lett*., 1997, **7**, 347.
338. S. J. Veenstra, K. Hauser and P. Felber, *Bioorg. Med. Chem. Lett*., 1997, **7**, 351.
339. Y. Ikeura, T. Tanaka, Y. Kiyota, S. Morimoto, M. Ogino, T. Ishimaru, I. Kamo, T. Doi and H. Natsugari, *Chem. Pharm. Bull.*, 1997, **45**, 1642.

340. G. Remond, B. Portevin, J. Bonnet, E. Canet, D. Regoli and G. De Nanteuil, *Eur. J. Med. Chem.*, 1997, **32**, 843.
341. D. R. Armour, N. M. Aston, K. M. L. Morriss, M. S. Congreve, A. B. Hawcock, D. Marquess, J. E. Mordaunt, S. A. Richards and P. Ward, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2037.
342. T. P. Burkholder, E. M. Kudlacz, G. D. Maynard, X-G. Liu, T-B. Le, M. E. Webster, S. W. Horgan, D. L. Wenstrup, D. W. Freund, F. Boyer, L. Bratton, R. S. Gross, R. W. Knippenberg, D. E. Logan, B. K. Jones and T-M. Chen, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2531.
343. C. J. Swain, B. J. Williams, R. Baker, M. A. Cascieri, G. Chicchi, M. Forrest, R. Herbert, L. Keown, T. Ludduwahetty, S. Luell, D. E. MacIntyre, J. Metzger, S. Morton, A. P. Owens, S. Sadowski and A. P. Watt, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2959.
344. X. Emonds-Alt, C. Advenier, C. Cognon, T. Croci, S. Daoui, J. P. Ducoux, M. Landi, E. Naline, G. Neliat, M. Poncelet, V. Proietto, D. Van Broeck, P. Vilain, P. Soubrie, G. Le Fur, J. P. Maffrand and J. C. Breliere, *Neuropeptides*, 1997, **31**, 449.
345. H. G. Chen, F-Z. Chung, O. P. Goel, D. Johnson, S. Kesten, J. Knobelsdorf, H. T. Lee and J. R. Rubin, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 555.
346. G. A. M. Giardina, H. M. Sarau, C. Farina, A. D. Medhurst, M. Grugni, L. F. Raveglia, D. B. Schmidt, R. Rigolio, M. Luttmann, V. Vecchietti and D. W. Hay, *J. Med. Chem.,* 1997, **40**, 1794.
347. H. M. Sarau, D. E. Griswold, W. Potts, J. J. Foley, D. B. Schmidt, E. F. Webb, L. D. Martin, M. E. Brawner, N. A. Elshourbagy, A. D. Medhurst, G. A. M. Giardina and D. W. P. Hay, *J. Pharmacol. Exp. Therap.*, 1997, **281**, 1303.
348. G. D. Maynard, L. D. Bratton, J. M. Kane, T. P. Burkholder, B. Santiago, K. T. Stewart, E. M. Kudlacz, S. A. Shatzer, R. W. Knippenberg, A. M. Ferrell and D. E. Logan, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2819.
349. A. A. Mazurov, S. A. Andronati, T. I. Korotenko, N. I. Sokolenko, A. I. Dyadenko, Y. E. Shapiro, V. Y. Gorbatyuk and T. A. Voronina, *Bioorg. Med. Chem.*, 1997, **5**, 2029.
350. Y. Ala, D. Morin, E. Mahe, N. Cotte, B. Mouillac, S. Jard, C. Barberis, E. Tribollet, J-J. Dreifuss, W. H. Sawer, N. C. Wo, W. Y. Chan, A. S. Kalodziejczyk, L. L. Cheng and M. Manning, *Eur. J. Pharmacol.*, 1997, **331**, 285.
351. Z. Strakova, A. Kumar, A. J. Watson and M. S. Soloff, *Mol. Pharmacol.*, 1997, **51**, 217.
352. M. Thibonnier, J. A. Preston, N. Dulin, P. L. Wilkins, L. N. Berti-Mattera and R. Mattera, *Endocrinology*, 1997, **138**, 4109.
353. M. Moutiez, G. Lippens, C. Sergheraert and A. Tartar, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 719.
354. L. Chen, I. Zoulikova, J. Slaninova and G. Barany, *J. Med. Chem.*, 1997, **40**, 864.
355. A. Matsuhisa, A. Tanaka, K. Kikuchi, Y. Shimada, T. Yatsu and I. Yanagisawa, *Chem. Pharm. Bull.*, 1997, **45**, 1870.
356. A. Tahara, Y. Tomura, K-I. Wada, T. Kusayama, J. Tsukada, M. Takanashi, T. Yatsu, W. Uchida and A. Tanaka, *J. Pharmacol. Exp. Therap.*, 1997, **282**, 301.
357. T. Yatsu, Y. Tomura, A. Tahara, K. Wada, J. Tsukada, W. Uchida, A. Tanaka and T. Takenaka, *Eur. J. Pharmacol.*, 1997, **321**, 225.
358. A. Tahara, Y. Tomura, K. Wada, T. Kusayama, J. Tsukada, N. Ishii, T. Yatsu, W. Uchida and A. Tanaka, *J. Cardiovasc. Pharmacol.*, 1997, **30**, 759.
359. M. Iqbal, P. A. Messina, B. Freed, M. Das, S. Chatterjee, R. Tripathy, M. Tao, K. A. Josef, B. Dembofsky, D. Dunn, E. Griffith, R. Siman, S. E. Senadhi, W. Biazzo,

D. Bozyczko-Coyne, S. L. Meyer, M. A. Ator and R. Bihovsky, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 539.
360. S. Chatterjee, S. Senadhi, D. Bozyczko-Coyne, R. Siman and J. P. Mallamo, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 287.
361. S. Chatterjee, M. Iqbal, J. C. Kauer, J. P. Mallamo, S. Senadhi, S. Mallya, D. Bozyczko-Coyne and R. Siman, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 1619.
362. S. Chatterjee, K. Josef, G. Wells, M. Iqbal, R. Bihovsky, J. P. Mallamo, M. A. Ator, D. Bozyczko-Coyne, S. Mallya, S. Senadhi and R. Siman, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 1237.
363. S. Chatterjee, M. A. Ator, D. Bozyczko-Coyne, K. Josef, G. Wells, R. Tripathy, M. Iqbal, R. Bihovsky, S. E. Senadhi, S. Mallya, T. M. O'Kane, B. A. McKenna, R. Siman and J. P. Mallamo, *J. Med. Chem.*, 1997 **40**, 3820.
364. H. Li, L. Bergeron, V. Cryns, M. S. Pasternack, H. Zhu, L. Shi, A. Greenberg and J. Yuan, *J. Biol. Chem.*, 1997, **272**, 21010.
365. K-C. Chou, D. Jones and R. L. Heinrikson, *FEBS Letters*, 1997, **419**, 49.
366. M. D. Jacobson, *Trends Cell Biol.*, 1997, **7**, 467.
367. W. R. MacLellan and M. D. Schneider, *Circulation Res.*, 1997, **81**, 137.
368. G. M. Cohen, *Biochem. J.*, 1997, **326**, 1.
369. N. Inohara, T. Koseki, Y. Hu, S. Chen and G. Nunez, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 10717.
370. S. V. Gulnik, L. I. Suvorov, P. Majer, J. Collins, B. P. Kane, D. G. Johnson and J. W. Erickson, *FEBS Letters*, 1997, **413**, 379.
371. D. Arnold, W. Keilholz, H. Schild, T. Dumrese, S. Stevanovic and H-G. Rammensee, *Eur. J. Biochem.*, 1997, **249**, 171.
372. D. S. Yamashita, W. W. Smith, B. Zhao, C. A. Janson, T. A. Tomaszek, M. J. Bossard, M. A. Levy, H-J. Oh, T. J. Carr, S. K. Thompson, C. F. Ijames, S. A. Carr, M. McQueney, K. J. D'Alessio, B. Y. Amegadzie, C. R. Hanning, S. Abdel-Meguid, R. L. DesJarlais, J. G. Gleason and D. F. Veber, *J. Am. Chem. Soc.*, 1997, **119**, 11351.
373. S. K. Thompson, S. M. Halbert, M. J. Bossard, T. A. Tomaszek, M. A. Levy, B. Zhao, W. W. Smith, S. S. Abdel-Meguid, C. A. Janson, K. J. D'Alessio, M. S. McQueney, B. Y. Amegadzie, C. R. Hanning, R. L. DesJarlais, J. Briand, S. K. Sarkar, M. J. Huddleston, C. F. Ijames, S. A. Carr, K. T. Garnes, A. Shu, J. R. Heys, J. Bradbeer, D. Zembryki, L. L. Rykaczewski, I. E. James, M. W. Lark, F. D. Drake, M. Gowen, J. G. Gleason and D. F. Veber, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 14249.
374. C. Linnevers, S. P. Smeekens and D. Bromme, *FEBS Letters*, 1997, **405**, 253.
375. L. Tong, C. Qian, M-J. Massariol, P. R. Bonneau, M. G. Cordingley and L. Lagace, *Nature*, 1996, **383**, 272.
376. X. Qiu, J. S. Culp, A. G. DiLella, B. Hellmig, S. S. Hoog, C. A. Janson, W. W. Smith and S. S. Abdel-Meguid, *Nature*, 1996, **383**, 275.
377. H-S. Shieh, R. G. Kurumbail, A. M. Stevens, R. A. Stageman, E. J. Sturman, J. Y. Pak, A. J. Wittwer, M. O. Palmier, R. C. Wiegand, B. C. Holwerda and W. C. Stallings, *Nature*, 1996, **383**, 279.
378. N. A. Abood, L. A. Schretzman, D. L. Flynn, K. A. Houseman, A. J. Wittwer, V. M. Dilworth, P. J. Hippenmeyer and B. C. Holwerda, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2105.
379. W. Ogilvie, M. Bailey, M-A. Poupart, A. Abraham, A. Bhavsar, P. Bonneau, J. Bordeleau, Y. Bousquet, C. Chabot, J-S. Duceppe, G. Fazal, S. Goulet, C. Grand-Maitre, I. Guse, T. Halmos, P. Lavallee, M. Leach, E. Malenfant, J. O'Meara, R.

Plante, C. Plouffe, M. Poirier, F. Soucy, C. Yoakim and R. Deziel, *J. Med. Chem.*, 1997, **40**, 4113.
380. C. Blackburn, A. Pingali, T. Kehoe, L. W. Herman, H. Wang and S. A. Kates, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 823.
381. J. A. Robl, C-Q. Sun, J. Stevenson, D. E. Ryono, L. M. Simpkins, M. P. Cimarusti, T. Dejneka, W. A. Slusarchyk, S. Chao, L. Stratton, R. N. Misra, M. S. Bednarz, M. M. Asaad, H. S. Cheung, B. F. Abboa-Offei, P. L. Smith, P. D. Mathers, M. Fox, T. R. Schaeffer, A. A. Seymour and N. C. Trippodo, *J. Med. Chem.*, 1997, **40**, 1570.
382. H. Ishizuka, K. Konno, H. Naganuma, K. Sasahara, Y. Kawahara, K. Niinuma, H. Suzuki and Y. Sugiyama, *J. Pharmacol. Exp. Therap.*, 1997, **280**, 1304.
383. G. M. Ksander, R. de Jesus, A. Yuan, R. D. Ghai, A. Trapani, C. McMartin and R. Bohacek, *J. Med. Chem.*, 1997, **40**, 495.
384. G. M. Ksander, R. de Jesus, A. Yuan, R. D. Ghai, A. Trapani, C. McMartin and R. Bohacek, *J. Med. Chem.*, 1997, **40**, 506.
385. S. M. Gardiner, P. A. Kemp, F. Brunner-Ferber and T. Bennett, *Br. J. Pharmacol.*, 1997, **122**, 1687.
386. S. Gardiner, P. Kemp, *et al.*, *Br. J. Pharmacol.*, 1997, **122**, 1694.
387. S. De Lombaert, L. B. Stamford, L. Blanchard, J. Tan, D. Hoyer, C. G. Diefenbacher, D. Wei, E. M. Wallace, M. A. Moskal, P. Savage and A. Y. Jeng, *Bioorg. Med. Chem. Lett.*, 1997, **8**, 1059.
388. W. Liu, R. Takayanagi, T. Ito, K. Oba and H. Nawata, *FEBS Letters*, 1997, **420**, 103.
389. D. J. Livingston, *J. Cellular Biochem.*, 1997, **64**, 19.
390. T. A. Rano, T. Timkey, E. P. Peterson, J. Rotonda, D. W. Nicholson, J. W. Becker, K. T. Chapman and N. A. Thornberry, *Chem. Biol.*, 1997, **4**, 149.
391. R. E. Dolle, C. V. C. Prasad, C. P. Prouty, J. M. Salvino, M. M. A. Awad, S. J. Schmidt, D. Hoyer, T. M. Ross, T. L. Graybill, G. J. Speier, J. Uhl, B. E. Miller, C. T. Helaszek and M. A. Ator, *J. Med. Chem.*, 1997, **40**, 1941.
392. J. M. C. Golec, M. D. Mullican, M. A. Murcko, K. P. Wilson, D. P. Kay, S. D. Jones, R. Murdoch, G. W. Bemis, S. A. Raybuck, Y-P. Luong and D. J. Livingston, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2181.
393. G. Semple, D. M. Ashworth, G. R. Baker, A. R. Batt, A. J. Baxter, D. W. M. Benzies, L. H. Elliot, D. M. Evans, R. J. Franklin, P. Hudson, P. D. Jenkins, G. R. Pitt, D. P. Rooker, A. Sheppard, M. Szelke, S. Yamamoto and Y. Isomura, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1337.
394. C. P. Prouty, G. J. Speier, D. Hoyer, R. E. Dolle, C. T. Helaszek, M. A. Ator, J. Uhl and J. Strasters, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 41.
395. R. E. Dolle, D. Hoyer, J. M. Rinker, T. M. Ross, S. J. Schmidt, C. T. Helaszek and M. A. Ator, *Bioorg. Med. Chem. Lett.*, 1997, 1003.
396. P. D. Edwards, D. W. Andisik, C. A. Bryant, B. Ewing, B. Gomes, J. J. Lewis, D. Rakiewicz, G. Steelman, A. Strimpler, D. A. Trainor, P. A. Tuthill, R. C. Mauger, C. A. Veale, R. A. Wildonger, J. C. Williams, D. J. Wolanin and M. Zottola, *J. Med. Chem.*, 1997, **40**, 1876.
397. C. A. Veale, P. R. Burnstein, C. M. Bohnert, F. J. Brown, C. Bryant, J. R. Damewood, Jr., R. Earley, S. W. Feeney, P. D. Edwards, B. Gomes, J. M. Hulsizer, B. J. Kosmider, R. D. Krell, G. Moore, T. W. Salcedo, A. Shaw, D. S. Silberstein, G. B. Steelman, M. Stein, A. Strimpler, R. M. Thomas, E. P. Vacek, J. C. Williams, D. J. Wolanin and S. Woolson, *J. Med. Chem.*, 1997, **40**, 3173.
398. H. Tokumitsu, Y. Fukumori, Y. Tsuda, Y. Okada, F. Kasuo, K. Igarashi and M. Fukui, *Chem. Pharm. Bull.*, 1997, **45**, 1845.

399. W. Yang, K. D. Villar, J. Urano, H. Mitsuzawa and F. Tamanoi, *J. Cellular Biochem. Suppl.*, 1997, **27**, 12.
400. C.A. Omer and N.E. Kohl, *Trends Pharmacol. Sci.*, 1997, **18**, 437.
401. D. M. Leonard, *J. Med. Chem.*, 1997, **40**, 2971.
402. K. Del Villar, H. Mitsuzawa, W. Yang, I. Sattler and F. Tamanoi, *J. Biol. Chem.*, 1997, **272**, 680.
403. G. Byk, Y. Lelievre, M. Duchesne, F. F. Clerc, D. Sherman and J. D. Guitton, *Bioorg. Med. Chem.*, 1997, **5**, 115.
404. H. Reuveni, A. Gitler, E. Poradosu, C. Gilon and A. Levitzki, *Bioorg. Med. Chem.*, 1997, **5**, 85-92
405. C. J. Burns, J-D. Guitton, B. Baudoin, Y. Lelievre, M. Duchesne, F. Parker, N. Fromage and A. Commercon, *J. Med. Chem.*, 1997, **40**, 1763.
406. C. J. Dinsmore, T. M. Williams, K. Hamilton, T. J. O'Neill, E. Rands, K. S. Koblan, N. E. Kohl, J. B. Gibbs, S. L. Graham, G. D. Hartman and A. I. Oliff, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1345.
407. D. M. Leonard, K. R. Shuler, C. J. Poulter, S. R. Eaton, T. K. Sawyer, J. C. Hodges, T-Z. Su, J. D. Scholten, R. C. Gowan, J. S. Sebolt-Leopold and A. M. Doherty, *J. Med. Chem.*, 1997, **40**, 192.
408. D. J. McNamara, E. Dobrusin, D. M. Leonard, K. R. Shuler, J. S. Kaltenbronn, J. Quin III, S. Bur, C. E. Thomas, A. M. Doherty, J. D. Scholten, K. K. Zimmerman, B. S. Gibbs, R. C. Gowan, M. P Latash, W. R. Leopold, S. A. Przybranowski and J. S. Sebolt-Leopold, *J. Med. Chem.*, 1997, **40**, 3319.
409. A. K. Mallams, F. G. Njoroge, R. J. Doll, M. E. Snow, J. J. Kaminski, R. R. Rossman, B. Vibulbhan, W. R. Bishop, P. Kirschmeier, M. Liu, M. S. Bryant, C. Alvarez, D. Carr, L. James, I. King, Z. Li, C-C. Lin, C. Nardo, J. Petrin, S. W. Remiszewski, A. G. Taveras, S. Wang, J. Wong, J. Catino, V. Girijavallabhan and A. K. Ganguly, *Bioorg. Med. Chem.*, 1997, **5**, 93.
410. F. G. Njoroge, B. Vibulbhan, D. F. Rane, W. R. Bishop, J. Petrin, R. Patton, M. S. Bryant, K-J. Chen, A. A. Nomeir, C-C. Lin, M. Liu, I. King, J. Chen, S. Lee, B. Yaremko, J. Dell, P. Lipari, M. Malkowski, Z. Li, J. Catino, R. J. Doll, V. Girijavallabhan and A. K. Ganguli, *J. Med. Chem.*, 1997, **40**, 4290.
411. F. G. Njoroge, R. J. Doll, B. Vibulbhan, C. S. Alvarez, W. R. Bishop, J. Petrin, P. Kirschmeier, N. I. Carruthers, J. K. Wong, M. M. Albanase, J. J. Piwinski, J. Catino, V. Girijavallabhan and A. K. Ganguly, *Bioorg. Med. Chem.*, 1997, **5**, 101.
412. J. K. Kaminski, D. F. Rane, M. E. Snow, L. Weber, M. L. Rothofsky, S. D. Anderson and S. L. Lin, *J. Med. Chem.*, 1997, **40**, 4103.
413. P. Stober, M. Schelhaas, E. Nagele, P. Hagenbuch, J. Retey and H. Waldmann, *Bioorg. Med. Chem.*, 1997, **5**, 75.
414. M. Chu, R. Mierzwa, E. Barrabee, A. King, M. Hallade, J. Terracciano, M. G. Patel, R. Patton, W. R. Bishop and M. S. Puar, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2547.
415. T. T. Dabrah, T. Kaneko, W. Massefski, Jr. and E. B. Whipple, *J. Am. Chem. Soc.*, 1997, **119**, 1594.
416. D. R. Burton, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 10018.
417. J. D. Ahlers, T. Takeshita, C. D. Pendleton and J. A. Berzofsky, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 10856.
418. D. C. Chan, D. Fass, J. M. Berger and P. S. Kim, *Cell*, 1997, **88**, 263.
419. J. B. Ghiara, D. C. Ferguson, A. C. Satterthwait, H. J. Dyson and I. A. Wilson, *J. Mol. Biol.*, 1997, **266**, 31.
420. J. Lescar, R. Stouracova, M-M. Riottot, V. Chitarra, J. Brynda, M. Fabry, M. Horejsi, J. Sedlacek and G. A. Bentley, *J. Mol. Biol.*, 1997, **267**, 1207.

421. J. K. Judice, J. Y. K. Tom, W. Huang, T. Wrin, J. Vennari, C. J. Petropoulos and R. S. McDowell, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 13426.
422. S. Misumi, A. Kudo, R. Azuma, M. Tomonaga, K. Furuishi and S. Shoji, *Biochem. Biophys. Res. Commun.*, 1997, **241**, 275.
423. D. Quasmi, E. de Rosny, L. Rene, B. Badet, I. Vergely, N. Boggetto and M. Reboud-Ravaux, *Bioorg. Med. Chem.*, 1997, **5**, 707.
424. E. E. Rutenber, J. J. De Voss, L. Hoffman, R. M. Stroud, K. H. Lee, J. Alvarez, F. McPhee, C. Craik and P. R. Ortiz de Montellano, *Bioorg. Med. Chem.*, 1997, **5**, 1311.
425. J. Weber, P. Majer, J. Litera, J. Urban, M. Soucek, J. Vondrasek, J. Konvalinka, P. Novek, J. Sedlacek, P. Strop, H-G. Krausslich and I. Pichova, *Arch. Biochem. Biophys.*, 1997, **341**, 62.
426. P. L. Beaulieu, D. Wernic, A. Abraham, P. C. Anderson, T. Bogri, Y. Bousquet, G. Croteau, I. Guse, D. Lamarre, F. Liard, W. Paris, D. Thibeault, S. Pav and L. Tong, *J. Med. Chem.*, 1997, **40**, 2164.
427. A. D. Abell and G. J. Foulds, *J. Chem. Soc, Perkin Trans. 1*, 1997, 2475.
428. N. Choy, H. Choi, W. H. Jung, C. R. Kim, H. Yoon, S. C. Kim, T. G. Lee and J. S. Koh, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2635.
429. A. B. Smith, III, R. Hirschmann, A. Pasternak, W. Yao and P. A. Sprengeler, *J Med. Chem.*, 1997, **40**, 2440.
430. D. J. Kempf, A. Molla, K. C. Marsh, C. Park, A. D. Rodrigues, M. Korneyeva, S. Vasavanonda, E. McDonald, C. A. Flentge, S. Muchmore, N. E. Wideburg, A. Saldivar, A. Cooper, W. M. Kati, K. D. Stewart and D. W. Norbeck, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 699.
431. S. W. Kaldor, V. J. Kalish, J. F. Davies, II, B. V. Shetty, J. E. Fritz, K. Appelt, J. A. Burgess, K. M. Campanale, N. Y. Chirgadze, D. K. Clawson, B. A. Dressman, S. D. Hatch, D. A. Khalil, M. B. Kosa, P. P. Lubbenhusen, M. A. Muesing, A. K. Patick, S. H. Reich, K. S. Su and J. H. Tatlock, *J. Med. Chem.* 1997, **40**, 3979.
432. D. Lamarre, G. Croteau, E. Wardrop, L. Bourgon, D. Thibeault, C. Clouette, M. Vaillancourt, E. Cohen, C. Pargellis, C. Yoakim and P. C. Anderson, *Antimicrobial Agents Chemotherapy*, 1997, **41**, 965.
433. M. L. Smidt, K. E. Potts, S. P. Tucker, L. Blystone, T. R. Stiebel, Jr., W. C. Stallings, J. J. McDonald, D. Pillay, D. D. Richman and M. L. Bryant, *Antimicrobial Agents Chemotherapy*, 1997, **41**, 515.
434. S. E. Hagen, J. V. N. Vara Prasad, F. E. Boyer, J. M. Domagala, E. L. Ellsworth, C. Gajda, H. W. Hamilton, L. J. Markoski, B. A. Steinbaugh, B. D. Tait, E. A. Lunney, P. J. Tummino, D. Ferguson, D. Hupe, C. Nouhan, S. J. Gracheck, J. M. Saunders and S. VanderRoest, *J. Med. Chem.* 1997, **40**, 3707.
435. B. D. Tait, S. Hagen, J. Domagala, E. L. Ellsworth, C. Gajda, H. W. Hamilton, J. V. N. Vara Prasad, D. Ferguson, N. Graham, D. Hupe, C. Nouhan, P. J. Tummino, C. Humblet, E. A. Lunney, A. Pavlovsky, J. Rubin, S. J. Gracheck, E. T. Baldwin, T. N. Bhat, J. W. Erickson, S. V. Gulnik and B. Liu, *J. Med. Chem.* 1997, **40**, 3781.
436. S. M. Poppe, D. E. Slade, K-T. Chong, R. R. Hinshaw, P. J. Pagano, M. Markowitz, D. D. Ho, H. Mo, R. R. Gorman III, T. J. Dueweke, S. Thaisrivongs and W. G. Tarpley, *Antimicrob. Agents Chemotherap.*, 1997, **41**, 1058.
437. H. I. Skulnick, P. D. Johnson, P. A. Arristof, J. K. Morris, K. D. Lovasz, W. Jeffrey Howe, K. D. Watenpaugh, M. N. Janakiraman, D. J. Anderson, R. J. Reischer, T. M. Schwartz, L. S. Banitt, P. K. Tomich, J. C. Lynn, M-M. Horng, K-T. Chong, R. R. Hinshaw, L. A. Dolak, E. P. Seest, F. J. Schwende, B. D. Rush, G. M. Howard, L. N. Toth, K. R. Wilkinson, T. J. Kakuk, C. W. Johnson, S. L. Cole, R. M. Zaya,

G. L. Zipp, P. L. Possert, R. J. Dalga, W-Z. Zhong, M. G. Williams and K. R. Romines, *J. Med. Chem.* 1997, **40**, 1149.
438. T. M. Schwartz, G. L. Bundy, J. W. Strohbach, S. Thaisrivongs, P. D. Johnson, H. I. Skulnick, P. K. Tomich, J. C. Lynn, T. Chong, R. R. Hinshaw, T. J. Raub, G. E. Padbury and L. N. Toth, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 399.
439. J. M. Smallheer, R. J. McHugh, C-H. Chang, R. F. Kaltenbach III, T. V. Worley, R. M. Klabe, L. T. Bacheler, M. M. Rayner, S. Erickson-Viitanen and S. P. Seitz, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1365.
440. D. A. Nugiel, K. Jacobs, L. Cornelius, C-H. Chang, P. K. Jadhav, E. R. Holler, R. M. Klabe, L. T. Bacheler, B. Cordova, S. Garber, C. Reid, K. A. Logue, L. J. Gorey-Feret, G. N. Lam, S. Erickson-Viitanen and S. P. Seitz, *J. Med. Chem.*, 1997, **40**, 1465.
441. G. Delucca, *Bioorg. Med. Chem. Lett.*, 1997, **7**,501.
442. G. Delucca, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 495.
443. G. V. De Lucca, J. Liang, P. E. Aldrich, J. Calabrese, B. Cordova, R. M. Klabe, M. M. Rayner and C-H. Chang, *J. Med. Chem.*, 1997, **40**, 1707.
444. W. W. Wilkerson, S. Dax and W. W. Cheatham, *J Med. Chem.*, 1997 **40**, 4079.
445. P. K. Jadhav, P. Ala, F. J. Woerner, C-H. Chang, S. S. Garber, E. D. Anton and L. T. Bachler, *J Med. Chem*., 1997 **40**, 181.
446. P. J. Ala, E. E. Huston, R. M. Klabe, D. D. McCabe, J. L. Duke, C. J. Rizzo, B. D. Korant, R. J. DeLoskey, P. Y. S. Lam, C. N. Hodge and C-H. Chang, *Biochemistry*, 1997, **36**, 1573.
447. J. Hulten, N. M. Bonham, U. Nillroth, T. Hansson, G. Zuccarrello, A. Bouzide, J. Aqvist, B. Classon, U. Helena Danielson, A. Karlen, I. Kvarnstrom, B. Samuelsson and A. Hallberg, *J. Med. Chem.*, 1997, **40**, 885.
448. K. Backbro, S. Lowgren, K. Osterlund, J. Atepo, T. Unge, J. Hulten, N. M. Bonham, W. Schaal, A. Karlen and A. Hallberg, *J. Med. Chem.*, 1997, **40**, 898.
449. P. K. Jadhav, F. J. Woerner and H-W. Man, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2145.
450. J. W. Janetka, P. Raman, K. Satyshur, G. Flentke, D. H. Rich, *J. Am. Chem. Soc*., 1997, **119**, 441.
451. T. Komai, R. Yagi, H. Suzuki-Sunagawa, Y. Ishikawa, A. Kasuya, S. Miyamoto, H. Handa and T. Nishigaki, *Biochem. Biophys. Res. Commun.*, 1997, **230**, 557.
452. U. Nillroth, L. Vrang, P-O. Markgren, J. Hulten, A. Hallberg and U. H. Danielson, *Antimicrobial Agents Chemotherapy*, 1997, **41**, 2383.
453. A. D. Baxter, J. Bird, R. Bhogal, T. Massil, K. J. Minton, J. Montana and D. A. Owen, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 897.
454. A. D. Baxter, R. Bhogal, J. M. Bird, G. M. Buckley, D. S. Gregory, P. C. Hedger, D. T. Manallack, T. Massil, K. J. Minton, J. G. Montana, S. Neidle, D. A. Owen and R. J. Watson, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2765.
455. C. K. Esser, N. J. Kevin, N. A. Yates and K. T. Chapman, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2639.
456. C. K. Esser, R. L. Bugianesi, C. G. Caldwell, K. T. Chapman, P. L. Durette, N. N. Girotra, I. E. Kopka, T. J. Lanza, D. A. Levorse, M. MacCoss, K. A. Owens, M. M. Ponpipom, J. P. Simeone, R. K. Harrison, L. Niedzwiecki, J. W. Becker, A. I. Marcy, M. G. Axel, A. J. Christen, J. McDonnell, V. L. Moore, J. M. Olszewski, C. Saphos, D. M. Visco, F. Shen, A. Colletti, P. A. Krieter and W. K. Hagmann, *J. Med. Chem.*, 1997, **40**, 1226.
457. R. J. Cherney, C. P. Decicco, D. J. Nelson, L. Wang, D. T. Meyer, K. D. Hardman, R. A. Copeland and E. C. Amer, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1757.

458. C. P. Decicco, J. L. Seng, K. E. Kennedy, M. B. Covington, P. K. Welch, E. C. Arner, R. L. Magolda and D. J. Nelson, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2331.
459. A. Miller, M. Askew, R. P. Beckett, C. L. Bellamy, E. A. Bone, R. E. Coates, A. H. Davidson, A. H. Drummond, P. Huxley, F. M. Martin, L. Saroglou, A. J. Thompson, S. E. van Dijk and M. Whittaker, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 193.
460. R. Hirayama, M. Yamamoto, T. Tsukida, K. Matsuo, Y. Obata, F. Sakamoto and S. Ikeda, *Bioorg. Med. Chem.*, 1997, **5**, 765.
461. L. J. MacPherson, E. K. Bayburt, M. P. Capparelli, B. J. Carroll, R. Goldstein, M. R. Justice, L. Zhu, S. Hu, R. A. melton, L. Fryer, R. L. Goldberg, J. R. Doughty, S. Spirito, V. Blancuzzi, D. Wilson, E. M. O'Bryne, V. Ganu and D. T. Parker, *J. Med. Chem.*, 1997, **40**, 2525.
462. S. Hanessian, A. Griffin and P. V. Devasthale, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 3119.
463. J. E. Sheppeck, II, C-M. Gauss and A. R. Chamberlin, *Bioorg. Med. Chem*., 1997, **5**, 1739.
464. A. R. Saltiel and T. K. Sawyer, *Chem. Biol.*, 1996, **3**, 87.
465. N. K. Tonks and B. G. Neel, *Cell*, 1996, **87**, 365.
466. J. M. Denu, J. A. Stuckey, M. A. Saper and J. E. Dixon, *Cell*, 1996, **87**, 361.
467. Y. A. Puius, Y. Zhao, M. Sullivan, D. S. Lawrence, S. C. Almo and Z-Y. Zhang, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 13420.
468. M. Akamatsu, P. P. Roller, L. Chen, Z-Y. Zhang, B. Ye and T. R. Burke, Jr., *Bioorg. Med. Chem*., 1997, **5**, 157.
469. R. L. Rice, J. M. Rusnak, F. Yokokawa, S. Yokokawa, D. J. Messner, A. L. Boynton, P. Wipf and J. S. Lazo, *Biochem*istry, 1997, **36**, 15965.
470. P. Wipf, A. Cunningham, R. L. Rice and J. S. Lazo, *Bioorg. Med. Chem*., 1997, **5**, 165.
471. Y. Yamada, K. Ando, Y. Ikemoto, H. Tada, E. Shirakawa, E. Inagaki, S. Shibata, I. Nakamura, Y. Hayashi, K. Ikegami and I. Uchida, *Chem. Pharm. Bull.*, 1997, **45**, 1631.
472. Y. Yamada, K. Ando, K. Komiyama, S. Shibata, I. Nakamura, Y. Hayashi, K. Ikegami and I. Uchida, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1863.
473. R. Goschke, N. C. Cohen, J. M. Wood and J. Maibaum, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2735.
474. A. Vindigni, Q. D. Dang and E. Di Cera, *Nature Biotechnol.*, 1997, **15**, 891.
475. G. De Simone, G. Balliano, P. Milla, C. Gallina, C. Giordano, C. Tarricone, M. Rizzi, M. Bolognesi and P. Ascenzi, *J. Mol. Biol.*, 1997, **269**, 558.
476. S. Y. Tamura, B. M. Shamblin, T. K. Brunck and W. C. Ripka, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1359.
477. Y. Akiyama, S. Tsutsumi, E. Hatsushiba, S. Ohuchi and T. Okonogi, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 533.
478. C. Dominguez, D. J. Carini, P. C. Weber, R. M. Knabb, R. S. Alexander, C. A. Kettner and R. R. Wexler, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 79.
479. M. L. Quan, J. Wityak, C. Dominguez, J. V. Duncia, C. A. Kettner, C. D. Ellis, A. Y. Liauw, J. M. Park, J. B. Santella, R. M. Knab, M. J. Thoolen, P. C. Weber and R. R. Wexler, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1595.
480. J. E. Semple, D. C. Rowley, T. K. Brunck and W. C. Ripka, *Bioorg Med. Chem. Lett.*, 1997, **7**, 315.
481. P. E. J. Sanderson, D. L. Dyer, A. M. Naylor-Olsen, J. P. Vacca, S. J. Gardell, S. D. Lewis, B. J. Lucas Jr., E. A. Lyle, J. J. Lynch Jr. and A. M. Mulichak, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1497.

482. S. Y. Tamura, J. E. Semple, J. E. Reiner, E. A. Goldman, T. K. Brunck, M. S. Lim-Wilby, S. H. Carpenter, W. E. Rote, G. L. Oldeshulte, B. M. Richard, R. F. Nutt and W. C. Ripka, , *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1543.
483. S. Y. Tamura, E. A. Goldman, T. K. Brunck, W. C. Ripka and J. E. Semple, *Bioorg Med. Chem. Lett.*, 1997, **7**, 331.
484. A. Salimbeni, F. Paleari, R. Canevotti, M. Criscuoli, A. Lippi, M. Angiolini, L. Belvisi, C. Scolastico and L. Colombo, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2205.
485. T. J. Tucker, W. C. Lumma, S. D. Lewis, S. J. Dardell, B. J. Lucas, E. P. Baskin, R. Woltman, J. J. Lynch, E. A. Lyle, S. D. Appleby, I-W. Chen, K. B. Dancheck and J. P. Vacca, *J. Med. Chem.*, 1997, **40**, 1565.
486. T. J. Tucker, W. C. Lumma, A. M. Mulichak, Z. Chen, A. M. Naylor-Olsen, S. D. Lewis, R. Lucas, R. M. Freidinger and L.C. Kuo, *J. Med. Chem.*, 1997, **40**, 830.
487. T. J. Tucker, W. C. Lumma, S. D. Lewis, S. J. Gardell, B. J. Lucas, J. T. Sisko, J. J. Lynch, E. A. Lyle, E. P. Baskin, R. F. Woltmann, S. D. Appleby, I-W. Chen, K. B. Dancheck, A. M. Naylor-Olsen, J. A. Krueger, C. M. Cooper and J. P. Vacca, *J. Med. Chem.*, 1997, **40**, 3687.
488. W. von der Saal, R. Kucznierz, H. Leinert and R. A. Engh, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1283.
489. D-M. Feng, S. J. Gardell, S. D. Lewis, M. G. Bock, Z. Chen, R. M. Freidringer, A. M. Naylor-Olsen, H. G. Ramjit, R. Woltmann, E. P. Baskin, J. J. Lynch, R. Lucas, J. A. Shafer, K. B. Dancheck, I-W. Chen, S-H. Mao, J. A. Krueger, T. R. Hare, A. M. Mulichak and J. P. Vacca, *J. Med. Chem.*, 1997, **40**, 3726.
490. W. Von der Saal, R. Kucznierz, H. Leinert and R. A. Engh, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1283.
491. J. E. Semple, N. K. Minami, S. Y. Tamura, T. K. Brunck, R. F. Nutt and W. C. Ripka, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2421.
492. A-C. Teger-Nilsson, R. Bylund, D. Gustafsson, E. Gyzander and U. Eriksson, *Thrombosis Res.*, 1997, **85**, 133.
493. S. Kim, S. Y. Hwang, Y. K. Kim, M. Yun and Y. S. Oh, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 769.
494. T. A. Lyle, Z. Chen, S. D. Appleby, R. M. Freidringer, S. J. Gardell, S. D. Lewis, Y. Li, E. A. Lyle, J. J. Lynch, Jr., A. M. Mulichak, A. S. Ng, A. M. Naylor-Olsen and W. M. Sanders, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 67.
495. J. Sturzebecher, D. Prasa, J. Hauptmann, H. Vieweg and P. Wikstrom, *J. Med. Chem.*, 1997, **40**, 3091.
496. D. K. Jones-Hertzog and W. L. Jorgensen, *J. Med. Chem.*, 1997, **40**, 1539.
497. D. J. Sall, J. A. Bastian, S. L. Briggs, J. A. Buben, N. Y. Chiradze, D. K. Clawson, M. L. Denney, D. D. Giera, D. S. Gifford-Moore, R. W. Harper, K. L. Hauser, V. J. Klimkowski, T. J. Kohn, H-S. Lin, J. R. McCowen, A. D. Palkowitz, G. F. Smith, K. Takeuchi, K. J. Thrasher, J. M. Tinsley, B. G. Utterback, S-C. B. Yan and M. Zhang, *J. Med. Chem.*, 1997, **40**, 3489.
498. Q. Lou, M. E. Leftwich, R. T. McKay, S. E. Salmon, L. Rychetsky and K. S. Lam, *Cancer Res.*, 1997, **57**, 1877.
499. W. Lu, I. Apostol, M. A. Qasim, N. Warne, R. Wynn, W. L. Zhang, S. Anderson, Y. W. Chiang, E. Ogin, I. Rothberg, K. Ryan and M. Laskowski, Jr., *J. Mol. Biol.*, 1997, **266**, 441.
500. J. D. McBride, N. Freeman, G. J. Domingo and R. J. Leatherbarrow, *J. Mol. Biol.*, 1996, **259**, 819.
501. J. F. Lynas and B. Walker, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1133.
502. J. F. Lynas and B. Walker, *Biochem. J.*, 1997, **325**, 609.

503. A. Jaskiewicz, A. Lesner, J. Rozycki, S. Rodziewicz, K. Rolka, U. Ragnarsson and G. Kupryszewski, *Biochem. Biophys. Res. Commun.*, 1997, **240**, 869.
504. T. S. Morris, S. FrorMann, S. Shechosky, C. Lowe, M. S. Lall, V. Gauss-Muller, R. H. Purcell, S. U. Emerson, J. C. Vederas and B. A. Malcolm, *Bioorg. Med. Chem.*, 1997, **5**, 797.
505. V. Martichonok and J. B. Jones, *Bioorg. Med. Chem.*, 1997, **5**, 679.
506. K. Umemura, K. Kondo, Y. Ikeda, T. Kobayashi, Y. Urata and M. Nakashima, *Br. J. Chem. Pharmacol.*, 1997, **43**, 613.
507. A. Kato, A. Fukunari, Y. Sakai and T. Nakajima, *J. Pharmacol. Exp. Therap.*, 1997, **283**, 328.
508. E. T. Boder and K. D. Wittrup, *Nature Biotechnology*, 1997, **15**, 553.
509. T. Clackson and J. A. Wells, *Trends Biotechnology*, 1994, **12**, 173.
510. R. Cortese, F. Felici, G. Galfre, A. Luzzago, P. Monaci and A. Nicosia, *Trends Biotechnology*, 1994, **12**, 262.
511. H. R. Hoogenboom, *Trends Biotechnology*, 1997, **15**, 62.
512. N. C. Wrighton, F. X. Farrell, R. Chang, A. K. Kashyap, F. P. Barbone, L. S. Mulcahy, D. L. Johnson, R. W. Barrett, L. K. Jolliffe and W. J. Dower, *Science*, 1996, **273**, 458.
513. O. Livnah, E. A. Stura, D. L. Johnson, S. A. Middleton, L. S. Mulcahy, N. C. Wrighton, W. J. Dower, L. K. Jolliffe and I. A. Wilson, *Science*, 1996, **273**, 464.
514. N. C. Wrighton, P. Balasubramanian, F. P. Barbone, A. K. Kashyap, F. X. Farrell, L. K. Jolliffe, R. W. Barrett and W. J. Dower, *Nature Biotechnology,* 1997, **15**, 1261.
515. S. E. Cwirla, P. Balasubramanian, D. J. Duffin, C. R. Wagstrom, C. M. Gates, S. C. Singer, A. M. Davis, R. L. Tansik, L. C. Mattheakis, C. M. Boytos, P. J. Schatz, D. P. Baccanari, N. C. Wrighton, R. W. Barrett and W. J. Dower, *Science*, 1997, **276**, 1696.
516. T. Kimura, H. Kaburaki, S. Miyamoto, J. Katayama and Y. Watanabe, *J. Biochem.*, 1997, **122**, 1046.
517. Y. S. Gho, J. E. Lee, K. S. Oh, D. G. Bae and C-B. Chae, *Cancer Res.*, 1997, **57**, 3733.
518. S. D. Yanofsky, D. N. Baldwin, J. H. Butler, F. R. Holden, J. W. Jacobs, P. Balasubramanian, J. P. Chinn, S. E. Cwirla, E. Peters-Bhatt, E. A. Whitehorn, E. H. Tate, A. Akeson, T. L. Bowlin,W. J. Dower and R. W. Barrett, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 7381.
519. A. L. Akeson, C. W. Woods, L. C. Hsieh, R. A. Bohnke, B. L. Ackermann, K. Y. Chan, J. L. Robinson, S. D. Yanofsky, J. W. Jacobs, R. W. Barrett and T. L. Bowlin, *J. Biol. Chem.*, 1996, **271**, 30517-30523.
520. C. Souriau, P. Fort, P. Roux, O. Hartley, M-P. Lefranc and M. Weill, *Nucleic Acids Research*, 1997, **25**, 1585.
521. M. van Meijer, Y. Roelofs, J. Neels, A. J. G. Horrevoets, A-J. van Zonneveld and H. Pannekoek, *J. Biol. Chem.*, 1996, **271**, 7423.
522. L. Dente, C. Vetriani, A. Zucconi, G. Pelicci, L. Lanfrancone, P. G. Pelicci and G. Cesareni, *J. Mol. Biol.*, 1997, **269**, 694.
523. T. Nishi, R. J. A. Budde, J. S. McMurray, N. U. Obeyesekere, N. Safdar, V. A. Levin and H. Saya, *FEBS Letter*, 1996, **399**, 237.
524. L. T. Nevalainen, T. Aoyama, M. Ikura, A. Crivic, H. Yan, N-H. Chua and A. C. Nairn, *Biochem. J.*, 1997, **321**, 107.
525. N. Adey, and B. Kay, *Biochem. J.*, 1997, **324**, 523.
526. A. Bottger, V. Bottger, C. Garcia-Echeverria, P. Chene, H-K. Hochkeppel, W. Sampson, K. Ang, S. F. Howard, S. M. Picksley and D. P. Lane, *J. Mol. Biol.*, 1997, **269**, 744.

527. P. Chene, P. Mittl and M. Grutter, *J. Mol. Biol.*, 1997, **269**, 873.
528. V. Bottger, A. Bottger, S. F. Howard, S. M. Picksley, P. Chene, C. Garcia-Echeverria, H-K. Hochkeppel and D. P. Lane, *Oncogene*, 1996, **13**, 2141.
529. R. Pasqualini, and E. Ruoslahti, *Nature*, 1996, **380**, 364.
530. R. Pasqualini, E. Koivunen and E. Ruoslahti, *Nature Biotechnology*, 1997, **15**, 542.
531. Z. Cai and A. Garen, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 9261.
532. I. Kuwabara, H. Maruyama, Y. Gi Mikawa, R. I. Zuberi, F-T. Liu and I. N. Maruyama, *Nature Biotechnology*, 1997, **15**, 74.
533. B. Parhami-Seren, T. Keel and G. L. Reed, *J. Mol. Biol.*, 1997, **271**, 331.
534. M. Li, W. Yu, C-H. Chen, S. Swirla, E. Whitehorn, E. Tate, R. Raab, M. Bremer and B. Dower, *Nature Biotechnology*, 1996, **14**, 986.
535. M. Li, *Nature Biotechnology*, 1997, **15**, 559.
536. R. Jelinek, T. D. Terry, J. J. Gesell, P. Malik, R. N. Perham and S. J. Opella, *J. Mol. Biol.*, 1997, **266**, 649.
537. J. Yanagisawa, M. Takahashi, H. Kanki, H. Yano-Yanagisawa, T. Tazunoki, E. Sawa, T. Nishitoba, M. Kamishohara, E. Kobayashi, S. Kataoka and T. Sato, *J. Biol. Chem.*, 1997, **272**, 8539.
538. G. Blandino and S. Strano, *J. Exp. Clin. Chem.*, 1997, **16**, 3.
539. Y. Furuya, S. Krajewski, J. I. Epstein, J. C. Reed and J. T. Isaacs, *Clin. Cancer Res.*, 1996, **2**, 389.
540. O. Gallo, V. Boddi, A. Calzolari, L. Simonetti, M. Trovati and S. Bianchi, *Clin. Cancer Res.*, 1996, **2**, 261.
541. M. Simonen, H. Keller and J. Heim, *Eur. J. Biochem.*, 1997, **249**, 85.
542. H. Zha and J. Reed, *J. Biol. Chem.*, 1997, **272**, 31482.
543. J. Zha, H. Harada, K. Osipov, J. Jockel, G. Waksman and S. J. Korsmeyer, *J. Biol. Chem.*, 1997 **272**, 24101.
544. M. Aritomi, N. Kunishima, N. Inohara, Y. Ishibashi, S. Ohta and K, Morikawa, *J. Biol. Chem.*, 1997, **272**, 27886.
545. M. Sattler, H. Liang, D. Nettesheim, R. P. Meadows, J. E. Harlan, M. Eberstadt, H. S. Yoon, S. B. Shuker, B. S. Chang, A. J. Minn, C. B. Thompson and S. W. Fesik, *Science*, 1997, **275**, 983.
546. P. S. Charifson, L. M. Shewchuk, W. Rocque, C. W. Hummel, S. R. Jordan, C. Mohr, G. J. Pacofsky, M. R. Peel, M. Rodriguez, D. D. Sternbach and T. G. Consler, *Biochemistry*, 1997, **36**, 6283.
547. D. N. Burshtyn, W. Yang, T. Yi and E. O. Long, *J. Biol. Chem.*, 1997, **272**, 13066.
548. B. Gay, P. Furet, C. Garcia-Echeverria, J. Rahuel, P. Chene, H. Fretz, J. Schoepfer and G. Caravatti, *Biochemistry*, 1997, **36**, 5712.
549. P. Furet, B. Gay, C. Garcia-Echeverria, J. Rahuel, H. Fretz, J. Schoepfer and G. Caravatti, *J. Med. Chem.*, 1997, **40**, 3551.
550. M. S. Plummer, E. A. Lunney, K. S. Para, A. Shahripour, C. J. Stankovic, C. Humblet, J. H. Fergus, J. S. Marks, R. Herrera, S. Hubbell, A. Saltiel and T. K. Sawyer, *Bioorg. Med. Chem.*, 1997, **5**, 41.
551. M. S. Plummer, D. R. Holland, A Shahripour, E. A. Lunney, J. H. Fergus, J. S. Marks, P. McDonnell, W. T. Mueller and T. K. Sawyer, *J. Med. Chem.*, 1997, **40**, 3719.
552. C. J. Stankovic, N. Surendran, E. A. Lunney, M. S. Plummer, K. S. Para, A Shahripour, J. H. Fergus, J. S. Marks, R. Herrera, S. E. Hubbell, C. Humblet, A. R. Saltiel, B. H. Stewart and T. K. Sawyer, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1909.
553. X. Zhang, D. Piatier-Tonneau, C. Auffray, R. Murali, A. Mahapatra, F. Zhang, C. C. Maier, H. Saragovi and M. I. Greene, *Nature Biotechnology,* 1996, **14**, 472.

554. Z. Zhang, M. Gaubin, L. Briant, V. Srikantan, R. Murali, U. Saragovi, D. Weiner, C. Devaux, M. Autiero, D. Piatier-Tonneau and M. I. Greene, *Nature Biotechnol.*, 1997, **15**, 150.
555. T. Satoh, S. Li, T. M. Friedman, R. Wiaderkiewicz, R. Korngold and Z. Huang, *Biochem. Biophys. Res. Commun*., 1996, **224**, 438.
556. T. Satoh, J. M. Aramini, S. Li, T. M. Friedman, J. Gao, A. E. Edling, R. M. Townsend, U. Koch, S. Choksi, M. W. Germann, R. Korngold and Z. Huang, *J. Biol. Chem*., 1997, **272**, 12175.
557. S. Li, J. Gao, T. Satoh, T. M. Friedman, A. E. Edling, U. Koch, S. Choksi, X. Han, R. Korngold and Z. Huang, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 73.
558. S. Gangwar, G. M. Pauletti, B. Wang, T. J. Siahaan, V. J. Stella and R. T. Borchardt, *DDT*, 1997, **2**, 148.
559. S. Gangwar, G. M. Pauletti, T. J. Siahaan, V. J. Stella and R. T. Borchardt, *J. Org. Chem.*, 1997, **62**, 1356.
560. M. Shimizu, M. Tsunogai and S. Arai, *Peptides*, 1997, **18**, 681.
561. K. Tamura, K. A. Agrios, D. V. Velde, J. Aube and R. T. Borchardt, *Bioorg. Med. Chem.*, 1997, **5**, 1859.
562. T. Uchida, N. Nagareya, S. Sakakibara, Y. Konishi, A. Nakai, M. Nishikata, K. Matsuyama and K. Yoshida, *Chem. Pharm. Bull.*, 1997, **45**, 1539.
563. H. J. Lee, G. Riley, O. Johnson, J. L. Cleland, N. Kim, M. Charnis, L. Bailey, E. Duenas, A. Shahzamani, M. Marian, A. J. S. Jones and S. D. Putney, *J. Pharmacol. Exp. Therap.*, 1997, **281**, 1431.
564. J. K. Li, N. Wang and X. S. Wu, *J. Pharmaceutical Sci.*, 1997, **86**, 891.
565. B. Jeong, Y. H. Bae, D. S. Lee and S. W. Kim, *Nature*, 1997, **388**, 860.
566. L. Schoofs, D. Veelaert, J. Vanden Broeck and A. De Loof, *Peptides*, 1997, **18**, 145.
567. D. Veelaert, L. Schoofs, P. Verhaert and A. De Loof, *Biochem. Biophys. Res. Commun.*, 1997, **241**, 530.
568. F. Morishita, Y. Nakanishi, S. Kaku, Y. Furukawa, S. Ohta, T. Hirata, M. Ohtani, Y. Fujisawa, Y. Muneoka and O. Matsushima, *Biochem. Biophys. Res. Commun.*, 1997, **240**, 354.
569. T. A. Day, A. G. Maule, C. Shaw and R. A. Pax, *Peptides*, 1997, **18**, 917.
570. T. M. Kubiak, A. G. Maule, N. J. Marks, R. A. Martin and J. R. Wiest, *Peptides*, 1996, **17**, 1267.
571. R. N. Johnston, C. Shaw, D. W. Halton, P. Verhaert, K. L. Blair, G. P. Brennan, D. A. Price, P. A. V. Anderson, *J. Neurochemistry*, 1996, **67**, 814.
572. J. W. Bowman, A. R. Friedman, D. P. Thompson, A. K. Ichhpurani, M. F. Kellman, N. Makis, A. G. Maule and T. G. Geary, *Peptides*, 1996, **17**, 381.
573. A. B. Lange, Z. Wang, I. Orchard and A. N. Starratt, *Peptides*, 1996, **17**, 375.
574. J. M. Hinton, R. H. Osborne, H. Bartosz-Bechowski and D. Konopinska, *J. Insect Physiology*, 1996, **42**, 449.
575. M. Kuczer, G. Rosinski, M. Lisowski, B. Picur and D. Konopinska, *Int. J. Peptide Protein Res*., 1996, **48**, 286-291.

4
Cyclic, Modified and Conjugated Peptides

BY JOHN S. DAVIES

1 Introduction

Thirty volumes ago, at the commencement[1] of this series of Reports, this coverage was referred to as 'Peptides of Abnormal Structure'. However, the continuity in the sub-divisions of this Chapter over the years implies that Nature's wealth of cyclic peptide structures, although varied, are not 'abnormal' but follow a 'norm', which on average are annually written up in approximately 250 publications. In 1997, the greatest activity was seen in cyclopentapeptides and glycopeptides, and although specialist peptide Journals are increasing their share of publications, traditional organic and biochemical Journals represent the major source of the research reports.

The major source of retrieval was again CA Selects[2] on Amino Acids, Peptides and Proteins (up to Issue 11, June 1998). A dedicated volume[3] to solid phase peptide synthesis in the *Methods in Enzymology* series contains authoritative accounts of interest to the cyclohomodetic synthesis sections of this Chapter and to the sections on glycopeptides. Again the major part of this report has been compiled from reading original Journal articles, with a few inaccessible ones being interpreted from their Abstracts. Conference proceedings have not been used as a source, although the arrival of many short papers in the 24th European Peptide Symposium,[4] during the writing of this Report deserves acknowledgement. Of direct interest to this Chapter is the publication in this conference proceedings of Prof. R. Hirschmann's, Josef Rudinger Award Lecture on peptidomimetic research.[5]

2 Cyclic Peptides

2.1 General Considerations – Structural elucidation of complicated naturally-occurring cyclopeptides is now routinely carried out by the combined use of modern physical methods on small quantities of material. With the development of hplc techniques combined with ion-spray and tandem mass spectrometry it is also possible to 'zoom' in on the details of the products and side-products derived from cyclisation reactions. In addition to the desired cyclic peptide[6] formed by BOP cyclisations, evidence has been obtained for cyclic dimeric products and products from aspartimide rearrangement. Using HATU and HAPyU coupling

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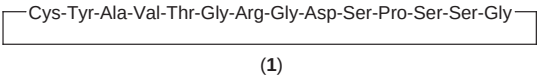
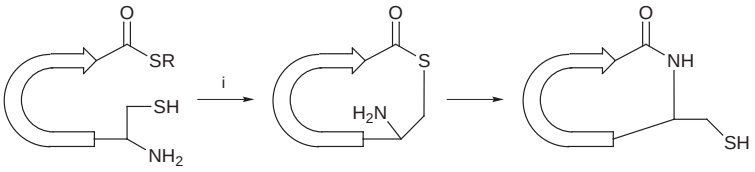
reagents instead of BOP, the products were mainly linear tetramethylguanidinium and dipyrrolidinylguanidinium derivatives resulting from alkylation of the amino group by the reagents.

Cyclisation on solid phase support is now an acknowledged protocol for the synthesis of cyclic homodetic peptides and justifies a review,[7] together with the emergence of cyclic peptide libraries[8] with five, six and seven amino acid residues in the ring. The synthesis of cyclopeptides as conformational restrictors has been the subject of a mini-review,[9] and although the use of the disulfide bond is not comprehensively covered in this Chapter, the current protocols in its synthesis have undergone detailed comparisons[10] and have been the subject of review.[11]

Peptide backbone cyclisation of unprotected peptides is experiencing successful breakthroughs. Thus the cyclopeptide (1) was cleanly synthesised[12] from its unprotected linear precursor using activation by a 3-carboxy-4-nitrophenylthioester. Tam's group have further expanded their orthogonal coupling strategy as summarised in Scheme 1 to synthesise the disulfide rich cyclopsychotride cyclopeptide[13] and have found[14] that Ag^+ ion-assisted lactamisation or lactonisation can take place using thioesters in aqueous buffered solutions. The same group have also applied[15] the principles of Scheme 1 to make a whole range of cyclic peptides suitable for dendrimer formation as in (2).

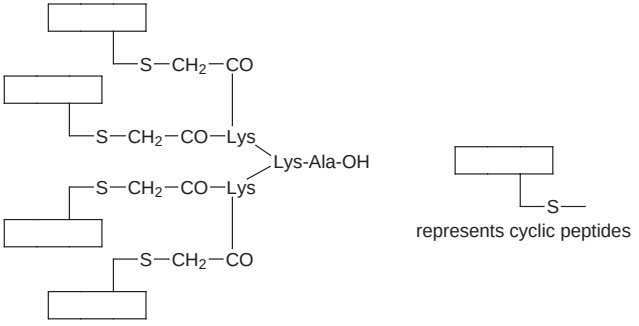


(**1**)



Reagent: i, $(HOOCCH_2CH_2)_3P$, pH 7·4

Scheme 1

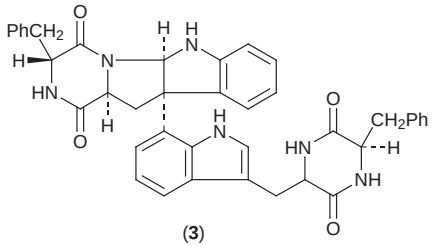


(**2**)

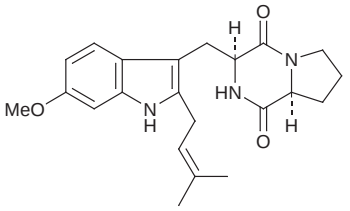
A number of marine cyclopeptides contain bromine atoms, but their efficient synthesis has been hampered by low-yielding syntheses of bromo(or chloro)tryptophans. A regiospecific bromination using N-bromosuccinimide has now improved[16] the situation.

2.2 Dioxopiperazines (Cyclic Dipeptides*)* – Marine-derived fungi, such as a saltwater culture of *Aspergillus niger* derived from the Caribbean *Hyrtios* sponge, has yielded[17] an interesting unsymmetrical dioxopiperazine alkaloid, asperazine, (3) which has cytotoxic properties. Tryprostatin (A) (4) also from a marine fungal strain BM939 has been enantiospecifically synthesised[18] for the first time. All four stereoisomers of bis(methylthio)silvatin (5), from *Gliocladium virens*, have been synthesised.[19] After cyclisation using NH_3/MeOH, the resulting dioxopiperazine when treated with LDA/Me_2S_2 gave a *cis*/*trans* mixture which could be purified by HPLC. Demethoxyfumitremorgin C (6) from *Aspergillus fumigatus* BM939 has been synthesised[20] in 20% overall yield, while the South China Sea sponge *Dysidea fragilis* has yielded[21] a new multichloro-substituted dioxopiperazine, dysamide D (7).

Asymmetric catalysis of the hydrocyanation of aldehydes by cyclo[-Phe-His] continues to be of interest. Two enantiomers of α-methylphenylalanine have been incorporated[22] into dioxopiperazine (8). Only the dioxopiperazine with an (*S*)-α-MePhe residue gave good enantiomeric excess of (*R*)-mandelonitrile. A suggestion that the aldehyde carbonyl H-bonds to the NH of α-MePhe is given as an



(3)



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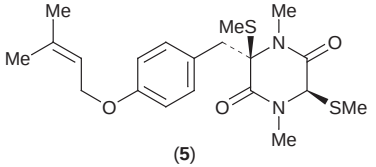

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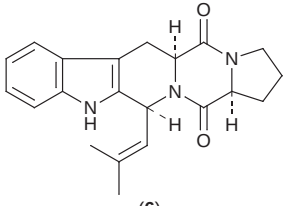

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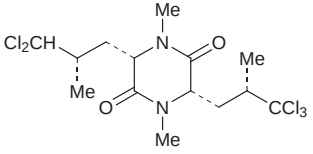
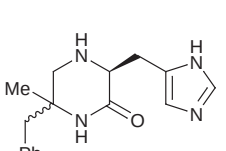
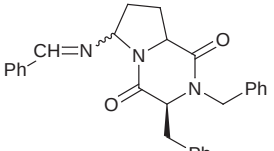


(7)

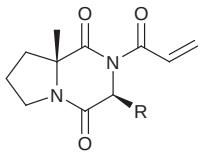
explanation, and has received support[23] in the observation that N-methylation of the dioxopiperazine amide NHs reduces catalytic efficiency. Methylated modifications[24] of cyclo-[-Phe-His] also provide evidence to support the view that a highly ordered supramolecular complex of dipeptide acts as catalyst. There is increasing interest in dioxopiperazines as chiral auxiliaries. Hence (9) can be directly subjected[25] to a Mannich reaction to give, on hydrolysis, an (*S*) β-amino acid, and (10) was a promising auxiliary[26] for an asymmetric Diels-Alder reaction between its side chain alkene bond and a substituted diene.



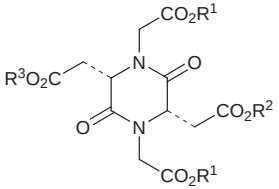
(**8**)



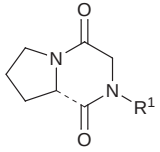
(**9**)



(**10**)

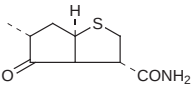


(**11**)

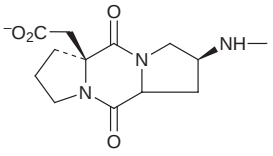


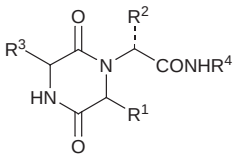
(**12**) R^1 = Leu-Gly-NH_2

(**13**) R^1 =



The new dioxopiperazine tetra-carboxylic acid template (11) has been employed[27] to prepare libraries of small/medium size organic molecules. Dioxopiperazines (12) and (13) have been synthesised[28] as analogues of L-Pro-Leu-Gly-NH_2 to investigate changes in the modulation at the dopamine D_2 receptor. Both (12) and (13) increased the affinity of the receptor for agonists and increased the % of D_2 receptors in the high affinity state. The template (14) has been synthesised[29] in a form suitable for solid phase protocols, and incorporated into cyclo[-Ala-Asn-Pro-Asn-Ala-Ala-(14)] containing the NPNA motif. The *trans* form of (14) tended to epimerise under basic/hydrolytic conditions. Dioxopiperazines such as (15) have been produced[30] on solid supports. The cyclisation step involved cyclisation and simultaneous cleavage from the resin. Strategies for getting amino acids into the brain have included[31] synthesising (16). Synthesis[32] of a series of N-substituted dioxopiperazines has been effected by auto-condensation promoted by triethylborane or triphenylsilane. Cyclic dipeptides (-Acc-X) based[33] on 1-aminocyclopropane-1-carboxylic acid (Acc) where X = Gly, Ala, Val, Leu, Phe or Tyr showed no proliferative or antiproliferative activity on normal human cells or HL60 leukemia cells. The anthelmintic activity[34] of cyclo(-Phe-Phe) has been published, and a stereoselective synthesis[35] of (17), a potential antihypertensive agent, together with its X-ray crystallographic analysis has been carried out.


(14)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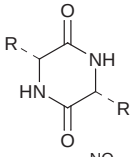
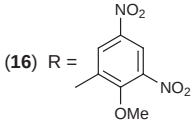
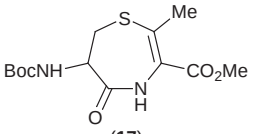
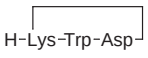



(16) R =


(17)

Synthesis and conformational analysis[36] of cyclo(-D-Phe-Sar), using molecular modelling, has revealed several low energy conformers with Z-peptide bonds in linear precursors, readily amenable to cyclisation. Strongly acidic conditions favoured cyclisation to cyclo-(-D-Phe-Sar). Cyclic dipeptides, cyclo(H-Lys-Asp-OH) and cyclo(H-Glu-Lys-OH) where a lactam bond exists between Lys/Asp(Glu) side chains have been subject[37] to theoretical analysis, CD, and NMR studies. Only one conformer was found for the Asp analogue, while the Glu containing dipeptide existed in minor conformations as well.

2.3 Cyclotripeptides – Cyclic tripeptides remain a minority species, and head to tail homodetic examples are rare. However, head to side-chain cyclic tripeptides such as (18) have been synthesised[38] as potential endothelin receptor antagonists. Automated allylic cleavage procedures with $Pd[P(Ph)_3]_4$ on solid phase resin gave free side chains for cyclisation, but the D-Asp analogue was produced in the solution phase. A 12-membered cyclic tripeptide (19) has been synthesised[39] in eight steps [two sequential Horner-Wittig couplings and enantioselective hydrogenation with Rh(MeDuphos)]. Peptidomimetic (19) was designed as a helix-turn-helix motif of DNA-binding proteins, while the synthesis[40] of the novel cyclotrihydrazine peptide (20) opens the way to other cyclopseudo peptides.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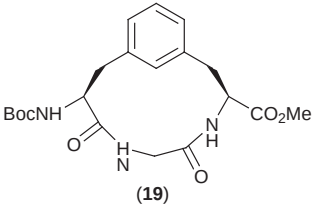
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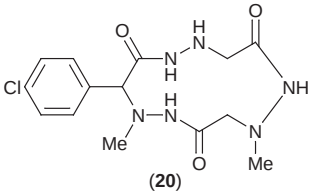
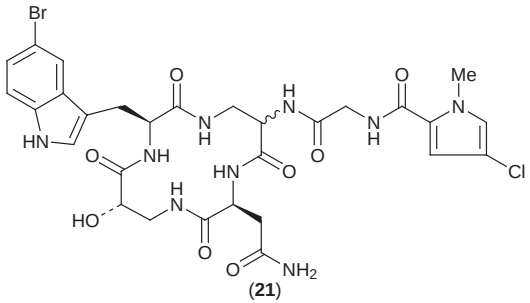
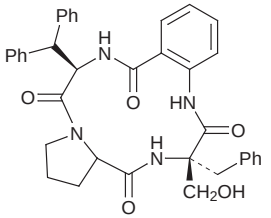

(20)

2.4 Cyclotetrapeptides – A cyclotetrapeptide core with a dipeptide side-chain provide[41] many of the novel features of cyclocinamide (A) (21) from the marine sponge *Psammocinia*. Cyclocinamide A exhibited *in vitro* selective activity against colon-38 tumour cells.

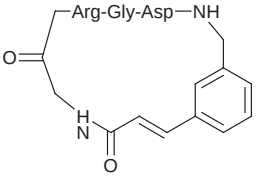
Diazaethylene glycol (Deg, $NH_2(CH_2)_2O(CH_2)_2OH$) has been incorporated[42] with the RGD integrin ligand to form cyclo(-Arg-Gly-Asp-Deg). A solution phase approach was used and cyclisation was carried out using the BOP reagent at the carboxyl of glycine. Moderate inhibitor activity towards fibrinogen and fibronectin was displayed and a conformational study showed that only Asp-NH was shielded for the solvent. Cyclic analogues typified by (22) have been synthesised[43] as type II and II′ β-turn analogues of the tripeptide LY301621 which potentiates the activity of methicillin against methicillin resistant *S. aureus*. Not much enhancement in biological activity was obtained by cyclisation, which was carried out via the PyBOP reagent. Previously reported structures (*Tetrahedon Lett*. 1991, **32**, 2609) for three cancerostatic, all L-cyclotetrapeptides, cyclo(Pro-Leu)$_2$, cyclo(Pro-Val)$_2$ and cyclo(Pro-Phe)$_2$ from tunicate, have been questioned.[44] A synthetic study showed that attempts to synthesise cyclo(Pro-Val-Pro-Tyr) gave cyclo(Pro-Val-Pro-D-Tyr) in 31% yield, and the synthesis of all L-analogues predominately gave cyclooctapeptides. A Heck coupling between an acrylic acid amide and 3-iodobenzylamine has been achieved[45] in 2 hr on solid phase (8 hr in solution phase), to yield the RGD analogue (23).



(**21**)



(**22**)



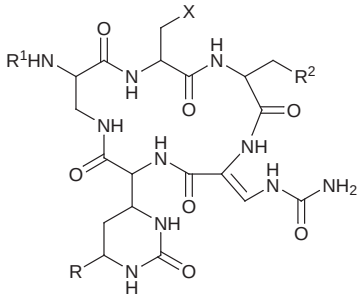
(**23**)

The conformation of cyclo(-γ-Abu-Pro)$_2$ has been shown[46] by NMR, IR and CD techniques to be highly solvent-dependent. It has a *cis-trans-cis-trans* amide bond arrangement (two cis-γ-Abu-Pro bonds) in all solvents, but changing the polarity of the solvent induces an inversion of the *cis* and *trans* conformations.

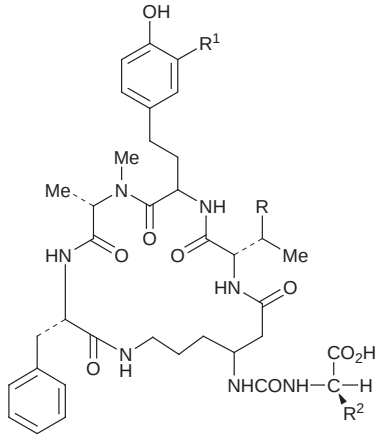
2.5 Cyclopentapeptides – A 231-reference review[47] places in context the central role of cyclopentapeptides containing the Arg-Gly-Asp motif as selective inhibitors of the $\alpha_v\beta_3$ integrin. Many of these cyclopeptidomimetics exhibit antagonist activity in the nanomolar range and suppress tumour induced angiogenesis. Cyclo(Arg-Gly-Asp-D-Phe-Val) turns out to be one of the best and selective inhibitors for $\alpha_v\beta_3$ integrin so a structure-activity assessment[48] of 18 analogues has been carried out. Detailed NMR studies have accompanied these studies in order to highlight the reasons for changes in activity. Retro-inverso cyclic analogues show both a dramatic decrease in activity and a drastic change in their spatial structures. Yet a retro-inverso analogue of a poor antagonist cyclo(D-Arg-Gly-Asp-Phe-Val) was found to be highly active. In the synthesis of the analogues, Fmoc-protocol on an o-chlorotritylchloride resin was used, with Gly always C-terminal. Cyclisation was carried out using DPPA and the solid base ($NaHCO_3$) method. Substitution[49] of unnatural lipophilic amino acids into cyclic Arg-Gly-Asp motifs based on cyclo-(Arg-Gly-Asp-Phe-D-Xaa) and cyclo(Arg-Gly-Asp-D-Phe-Xaa) where Xaa and D-Xaa are 2(*S*)-2-aminohexadecanoic acid or N-hexadecylglycine demonstrates the possibility of developing selective interaction for $\alpha_v\beta_3$ over $\alpha_{IIb}\beta_3$ integrins. The lipid modification had no impact on the conformation of the template structures, but the biological activity is dependent on spatial requirements of the lipid anchor in the receptor binding pocket. Cyclopeptide analogues, cyclo[Arg-Gly-Asp-D-Phe-Lys(or Tyr)], have also been synthesised[50] using Fmoc-protocols followed by cyclisation using water soluble carbodiimide (EDCI). Further derivatisation of the side-chain of lysine with diethylenetriamine pentaacetic anhydride, and the iodination of the tyrosyl analogue gave carrier molecules capable of transporting radioisotopes (^{111}In and ^{125}I) into blood platelets as prototypes for medical imaging. Three cyclic -Arg-Gly-Asp-pentapeptides and two cyclic hexapeptide analogues have been synthesised[51] by solid phase techniques to study the influence of substituting β-amino acids into the cyclopeptide backbone. Changing, e.g., cyclo(Arg-Gly-Asp-Phe-Val) to cyclo(-Arg-Gly-Asp-β-Phe-Val) gave better antagonists of the $GP_{IIb/IIIa}$ receptors. Cyclisations in the solution phase were carried out by DPPA under high dilution, while on-resin cyclisation took place with TBTU, with the side chain of Asp linked to the resin.

The battle against methicillin-resistant *S. aureus* and vancomycin-resistant enterococci has spurred on the search for new antibiotics. Thus extensive modification of tuberactinomycin B (viomycin) (24) and capreomycin (25), already used as tuberculostatic agents, has been reported.[52–54] Modifications at the dehydroureido (6a) position,[52] with substitutions such as 6a-(3′4′-dichlorophenylamino) in viomycin and 4′-cyclohexylphenylamino in capreomycin have yielded compounds with activity against *Pasteurella* spp, methicillin-resistant *S. aureus* and vancomycin-resistant enterococci. Benzylcarbamate substitution[53] at position R in (24) gave more potent derivatives than the parent antibiotic, while modification of the β-lysine side-chain of capreomycin (25) gave compounds[54] with antibacterial potency against multidrug-resistant pathogens.

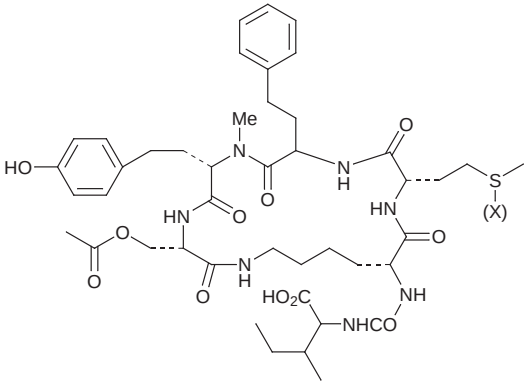
Further members of the anabaenopeptins family, E (26) and F (27) have been characterised[55] from the cyanobacterium *Oscillatoria agardhii* (NIES-204). Oscil-



(**24**) Viomycin, R = OH, X = OH, $R^1 = NH_2(CH_2)_3CH(NH_2)CH_2CO–$, R^2 = OH
(**25**) Capreomycin, R = R^1 = H, X = NH_2, $R^2 = NHCOCH(NH_2)(CH_2)_3NH_2$



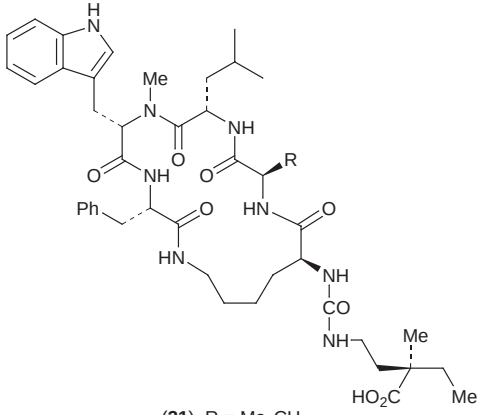
(**26**) R = R^1 = Me, $R^2 = (CH_2)_3NH(=NH)NH_2$
(**27**) R = Et, R^1 = H, $R^2 = (CH_2)_2NH(=NH)NH_2$
(**28**) R = Et, R^1 = H, $R^2 = CH_2Ph(OH)$



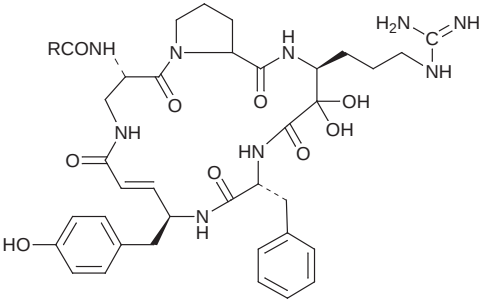
(**29**) X = O_2
(**30**) X = O

lamide Y (28), and three stereochemical analogues, produced by the same cyanobacterium have been synthesised[56] on solid phase using a combination of Fmoc/allyl protocols on an acid labile Wang type linker. The urea function was incorporated using D-lysine, and cyclisations were carried out using PyBroP. Contrary to literature reports none of the four analogues exhibited inhibition of chymotrypsin. *Nodularia spumigena* AV1 has yielded[57] the nodulapeptins A (29) and B (30), which are similar to the anabaenopeptins. This core cyclic structure was also discovered[58] in mozamides A (31) and B (32) from a theonellid sponge, which bear similarities to keranamide and konbamide, although the latter lack D-residues.

Estrogen-like activity has been associated[59] with cyclo(-Ala-Gly-Val-Lys-Tyr) (segetalin G) and cyclo(-Arg-Phe-Ser-Gly-Tyr) (segetalin H) from the seeds of *Vaccaria segetalis*. Their backbone structure,[60] determined by distance geometry calculation and restrained energy minimisation from NMR data, showed segetalin G to contain a βII-turn at Tyr^4-Ala^5, and segetalin H to contain a βII′ turn at Gly-Tyr with a γ-turn at Arg-Phe-Ser. Thionation[61] of segetalins A and B with Lawesson's reagent gave a number of thioamide analogues, but thiosegetalin A_2



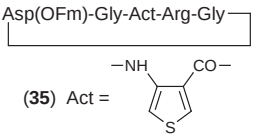
(**31**) R = Me_2CH
(**32**) R = Me(Et)CH



(**33**) R = H
(**34**) R = Me

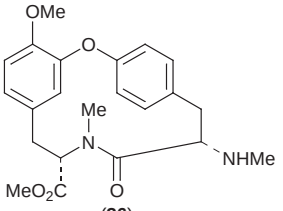
(Gly^{1}ψ[CSNH]Val^2,Ala^{6}ψ[CSNH]Gly^1) segetalin A was the only one to show estrogen-like activity against ovariectomised rats.

To complement the solid state structure of cyclotheonamide A (33) already published in 1993, an NMR study[62] revealed that the conformation of residues important for active site binding (D-Phe, h-Arg and Pro) is nearly identical in the solution and solid states, so it appears to be pre-ordered prior to binding to a serine protease. A total synthesis[63] of cyclotheonamide B (34) uses a convergent approach utilising precursors forming each half of the molecule around points (a) and (b) in (34). Final cyclisation was carried out at point (a) using TBTU/HOBt/DMAP. Release of the oxo-amide moiety was achieved via a Dess-Martin periodinane oxidation of a hydroxyl group. Incorporation[64] of the α-ethylthio group into the cyclic pentapeptide cyclo[-Val-D-Gly(SEt)-Pro-Phe-D-Ala], directly via a Kaiser resin attached to D-Ala, has provided a convenient electrophilic centre which can be stereospecifically displaced by a range of nucleophiles. A restrained Pro-containing analogue[65] of the nodularin macrocycle cyclo[β-Ala-2*R*Glu(α-OMe)-γ-2*S*-Pro-2*R*-Asp(α-OMe)-β-2*S*-Phe] has been synthesised both on- and off-resin. When the α-carboxyl of Asp was linked to the Wang resin and its β-carboxyl protected by an allyl group, the subsequent peptide assembly when treated with PyBOP/HOBt for 7 days gave 30% yield of pure cyclic material. Cyclisation of a linear precursor via a pentafluorophenyl ester off-resin gave a 54% yield, but the authors felt that the on-resin method offered advantages. The cyclic endothelin receptor antagonist BQ123, cyclo(-D-Trp-D-Asp(OBz)-Pro-D-Val-Leu) has been synthesised[66] in 50% yield by heating a thioester-resin linked linear precursor in DMF or dioxan at 75 °C for 24 hrs. α-Aspartyl-containing cyclic peptides such as (35) have been formed[67] via cyclisation at the Gly carboxyl using BOP reagent. Removal of the fluorenylmethyl group with tetra-n-butylammonium fluoride favoured formation of the β-aspartyl peptide, but piperidine gave the α-form preferentially (α:β, 9:1). Cyclic mixtures containing 4 and 256 cyclic pseudopeptide components with a single bond [CH_2NH] surrogate such as cyclo(D-Pheψ[CH_2NH]Xxx-Arg-Gly-Asp) have been synthesised[68] via an on-resin approach featuring side chain attachment of Boc-Asp-OFm to the solid resin. HATU provided an efficient cyclisation reagent for the pseudopeptide libraries.

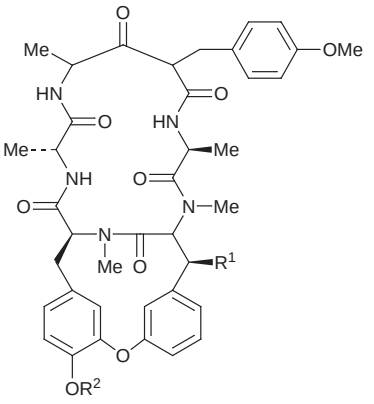


2.6 Cyclohexapeptides – Higher plants such as *Stellaria delavayi* provide, in their roots, sources[69] of cyclohexapeptides, delavayins A, [cyclo(-Gly-Ser-HOIle-Phe-Phe-Ala)], and B, [cyclo(-Gly-Ser-Ile-Phe-Phe-Ala)] and a cycloheptapeptide delavayin C [cyclo(-Gly-Tyr-Tyr-Tyr-Pro-Val-Pro)]. As noted in last year's Report, epimerisation at C-9 has been a confusing aspect of the total synthesis of

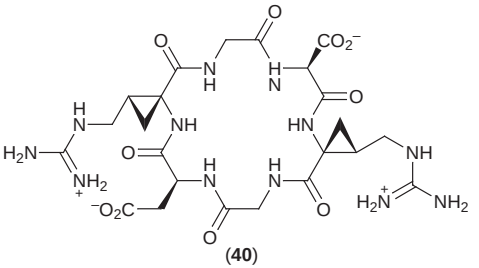
deoxybouvardin (37) and related compounds. A full report[70] of a remarkably facile epimerisation that went undetected has appeared. Another approach,[71] based on the formation of the diaryl bridge in (36) via an intramolecular S_NAr reaction from an ortho-nitro-fluoro substituted linear precursor, also suffers from C-9 epimerisation, but some of the correct isomer is produced. The scheme is considered therefore to offer a formal total synthesis of deoxybouvardin (37). Although RA-VII (38) has been evaluated clinically, one drawback is its low water solubility. Derivative (39) of deoxybouvardin has been synthesised,[72] and is 250 times more soluble in water and is more potent than (38).



(**36**)



(**37**) $R^1 = R^2 = H$
(**38**) $R^1 = R^2 = Me$
(**39**) $R^1 = H$, $R^2 =$

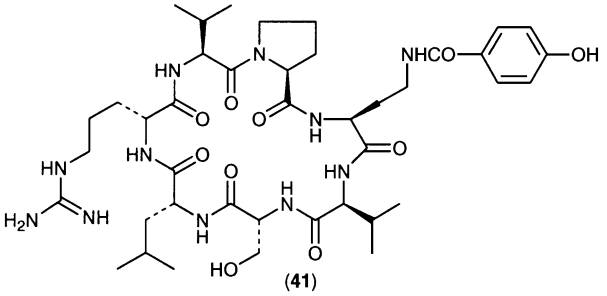


(**40**)

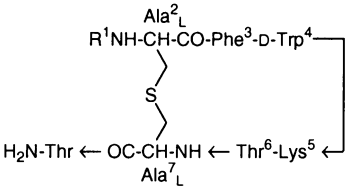
Spirocyclic analogues [40, and its cyclo[(2*S*,3*S*)cycloR,GD(2*S*,3*S*^{1})cyclo-R]GD analogue] of RGD cyclohexapeptides have been synthesised[73] by linking Gly to an HMPB-MBHA resin, followed by Fmoc-based assembly of the linear precursor, followed by cyclisation in solution using BOP/HOBt/DIPEA. Compound (40) seemed more flexible than its analogue in solution and had a greater affinity for the vitronectin $\alpha_v\beta_3$ receptor. An ensemble-averaging (EA) protocol to determine the interconverting conformations in NMR-determined structures of cyclopeptides has been described.[74] Averaging the NMR parameters over a set of

conformations that exist simultaneously has shown that cyclo(Gly-Pro-Phe-Gly-Pro-Nle) exists in solution as either a βVIII-βII/iγ-βI or a βII-βI/βI-βI equilibrium.

2.7 Cycloheptapeptides – Questions have been raised recently on the purity of extracted samples of the axinastatins, especially the discrepancy between the biological activity of natural and synthetic forms. With the availability of efficient synthetic routes to axinastatin 2, [cyclo(Asn-Pro^2-Phe-Val-Leu-Pro^6-Val)], axinastatin 3 [cyclo(Asn-Pro^2-Phe-Ile-Leu-Pro^6-Val)] and axinastatin 4 [cyclo(Thr-Pro^2-Leu-Trp-Val-Pro^6-Leu)] their conformations in d_6-DMSO have been studied.[75] In all structures Pro^2 is in the i+1 position of a βI turn and Pro^6 occupies the i+2 position of a βVIa turn about the *cis* amide between residue 5 and Pro^6. All three axinastatins can be characterised by six *trans* and one *cis* amide bonds resulting in a βI/βIIa turn motif similar to many cycloheptapeptides. All three were also inactive or of low activity. Cupolamide A (41), a cytotoxic compound (IC_{50}=7.5 μg ml^{-1} against P.388 murine leukemia cells) has been isolated[76] from the sponge *Theonella cupola*, and yunnanin A, cyclo(-Gly-Tyr-Gly-Gly-Pro-Phe-Pro) from the roots of *Stellaria yunnanensis* has been studied[77] by X-ray crystallography and NMR methods. Three intramolecular H-bonds forming a type II, a type II′β-turn and a classical β-bulge with all *trans* amide bonds was seen in solution and in the solid state. Using similar physical methods[78] segetalins D, cyclo(-Gly-Leu-Ser-Phe-Ala-Phe-Pro) and E, cyclo(-Gly-Tyr-Val-Pro-Leu-Trp-Pro) from the seeds of *Vaccaria segetalis* have shown the classical β-bulge conformation of cycloheptapeptides.



(41)



(42)

2.8 Cyclooctapeptides – Plants have this year sourced a wide variety of cyclooctapeptides. Thus the roots of *Stellara delavayi* have yielded[79] stelladelins

A-C, a cyclic undecapeptide and two cyclooctapeptides, the seeds of *Annona squamosa* have given[80] annosquamosin A, and roots of *Stellaria dichotoma* var. lanceolata have yielded[81] dichotomin H and I. The structures appear in Table 1

Table 1

Stelladin A	cyclo(-Gly-Pro-Pro-Pro-Leu-Leu-Gly-Pro-Pro-Tyr-Tyr)
Stelladin B	cyclo(-Gly-Ile-Pro-Pro-Ala-Tyr-Asp-Leu)
Stelladin C	cyclo(-Val-Pro-Tyr-Pro-Pro-Phe-Tyr-Ser)
Annosquamosin A	cyclo(-Pro-Met(O)-Thr-Ala-Ile-Val-Gly-Tyr)
Dichotomin H	cyclo(-Ala-Pro-Thr-Phe-Tyr-Pro-Leu-Ile)
Dichotomin I	cyclo(-Val-Pro-Thr-Phe-Tyr-Pro-Leu-Ile)
Cyclogossine B	cyclo(-Val-Gly-Gly-Trp-Leu-Ala-Ala-Ile)

The latex of *Jatropha gossypifolia* (Euphorbiaceae) has in addition to the known cycloheptapeptide, cyclogossine A, yielded[82] cyclogossine B (see Table 1). Cyclooctapeptide analogues (42) of sandostatin containing a lanthionine bridge instead of a disulfide link have been synthesised[83] by cyclisation on an oxime resin. The bridge modification gave analogues with significantly increased receptor binding selectivity. The compounds had higher stability towards enzyme degradation giving a 2.4 times longer half-life than sandostatin.

Amongst a series of cyclic peptide systems studied theoretically,[84] with possibilities for adaptation as bioelectronic devices, was cyclo[(-D-Ala-Glu-D-Ala-Gln)$_2$]. A wide HOMO-LUMO gap of ~5.0 eV was calculated for this molecule. An alternating D-L sequence, as in cyclo[-D-Leu-Lys(Npa)-D-Leu-Lys-D-Leu-Lys-D-Leu-Lys], was shown[85] to be more efficient in quenching the fluorescence of cyclo[-D-Leu-Lys(ABz)-D-t-Leu-Glu-D-t-Leu-Glu-D-t-Leu-Glu] than a control peptide. The formation constant of the 1:1 complex of cyclo(-Phe-Pro)$_4$ with L-Phe-OMe·HCl has been shown[86] to be 13.2 times that with D-Phe-OMe·HCl. Three intermolecular H-bonds between ProCO, PheCO and the amino group, the PheNH and the CO group were seen with L-Phe-OMe·HCl, but only one H-bond between PheCO and the NH_2 group in the D-form.

2.9 Cyclononapeptides – The cyclolinopeptide A (CLA) isolated from linseed oil in 1959 has recently been actively researched due to its distinct immunosuppressive activity. Linear and cyclic analogues,[87] in which one or both Phe residues at positions 8 and 9 were sulfonated, have been synthesised by solid phase methodology and cyclisation with the BOP reagent. All analogues retained some immunosuppressive activity. The Phe residues in CLA have also been replaced[88] by D-Phe, D-Tyr-, D-Trp or L-Trp, each analogue being synthesised by solid phase protocols. Highest biological activity was observed for the cyclic peptides modified in position 9, with [D-Phe^9]-CLA proving the best, and also showed the best conformational correlation with CLA. A new cyclolinopeptide B, cyclo-(-Pro-Pro-Phe-Phe-Val-Ile-Met-Leu-Ile), has been isolated[89] from the seeds of *Linum usitatissimum*, and shows immunosuppressive activity comparable with that of cyclosporin A. Cyclophilin B's rotamase activity can be enhanced[90] by a

new cyclic nonapeptide, curcacycline B, cyclo(-Leu-Gly-Ser-Pro-Ile-Leu-Leu-Gly-Ile) isolated and characterised from *Jatropha curcas*.

Chemical degradation, ESIMS-MS and 2D NMR methods have been used[91] to elucidate the structures of dichotomins F, cyclo(-Val-Leu-Pro-Ser-Val-Tyr-Pro-Tyr-Phe), and G, cyclo(-Ser-Pro-Leu-Pro-Ile-Pro-Pro-Phe-Tyr), from the roots of *Stellaria dichotoma L.* var. *lanceolata Bge*. Both showed moderate cyclooxygenase activity. Previously characterised and synthesised cycleonuripeptides A-C have been studied[92] using a plethora of NMR and calculation techniques. The backbone structures of A, cyclo(-Gly-Pro-Pro-Pro-Tyr-Pro-Pro-Met-Ile), B, cyclo(-Gly-Pro-Pro-Pro-Tyr-Pro-Pro-Met(O)-Ile), and its isomer C, consist of two β-turns, a βVI turn at Pro^3-Pro^4 and a βI-turn at Pro^7-Met^8. A transannular 4→1 H-bond between Tyr^5NH and Pro^2CO, two H-bonds between Gly^1NH and Pro^6CO and between Ile^9NH and Pro^6CO complete the β-bulge conformational picture.

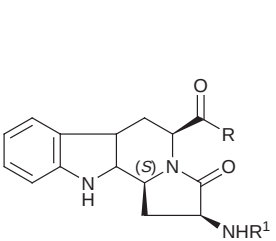
The N-terminal cyclic peptide of RES701-1, an endothelin type B receptor antagonist, has been chemically attached to the C-terminal peptide from the endothelin family. PyBOP/HOBt mixtures were used[93] to cyclise the N-terminal nine-residue RES-701-1 to give ultimately (43), which had a binding potency of IC_{50}=0.24 nM for type B receptor.

(**43**)

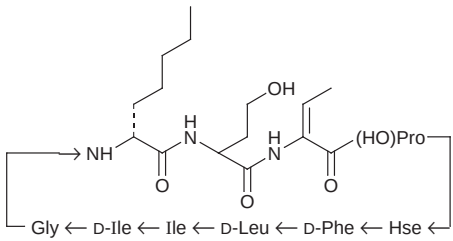
2.10 Cyclodecapeptides – The new proline-rich cycloleonuripeptide D, cyclo(-Ser-Pro-Pro-Pro-Tyr-Phe-Gln-Thr-Pro-Ile) from the fruits of *Leonurus heterophyllus*, has been subjected[94] to NMR and X-ray analysis. The backbone contains a type I β-turn at Pro-Ile and a type III β-turn at Pro-Tyr. A transannular 4→1 H-bond between PheNH and ProCO encompasses Pro-Pro-Tyr, in which the Pro-Pro bond has been judged to be *cis*. Similarities with [Phe^4,Val^6] antamanide have been noticed. A similar backbone conformation to antamanide has been revealed[95] in the crystal conformation of cyclo(-Pro^1-Pro^2-Ile^3-Phe-Val-Leu-Pro-Pro-Tyr-Ile) and includes a 5→1 transannular α-turn H-bond encompassing Pro^1-Pro^2-Ile^3, and a H-bond between Phe^4NH and Ile^{10}CO. An active synthetic analogue of cyclolinopeptide A, having the structure cyclo(Pro-Phe-Phe-Aib-Leu)$_2$ has been examined[96] by X-ray studies in the solid state and NMR spectroscopy in solution. All peptide bonds are *trans*, and the molecule is perfectly symmetrical in both solid and solution states. Six intramolecular H-bonds and the formation of four turns (three type I and one of type III) are found in the topology.

A pair of L-1-pyrenylalanines (Pya) have been substituted[97] into various positions in gramicidin S to explore the involvement of β-sheet structures with amphiphilic character. Synthesis was carried out on solid phase with subsequent cyclisation cleavage using a *p*-nitrobenzophenone oxime resin. The direct formation of gramicidin S by the dimerisation-cyclisation of pentapeptide active esters having no ornithinyl protection, has been examined.[98] Of the four linear

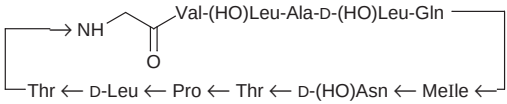
precursors, H-X-Pro-Y-Orn-Leu-ONSu (where X = L or D-Phe, Y = L- or D-Val), only H-D-Phe-Pro-Val-Orn-Leu-ONSu gave semi-gramicidin S (cyclic monomer) and gramicidin S in yields of 15 and 38% respectively. The analogue with a D-Phe-Pro-D-Val sequence gave exclusively the cyclic monomer. Gramicidin S has been chosen[99] as a test compound to assess the efficiency of the type II β-turn mimetic 2-amino-3-oxohydroindolizine[8,7-b]indole-5-carboxylate (IBTM) (44). Cyclisation of the gramicidin S analogues containing *S*-IBTM was only possible in the solution phase, while solid phase mediated cyclisations accomodated both *R* and *S*-IBTM. NMR studies clearly showed that only the *R*-diastereoisomer of IBTM is a suitable mimic of a type II β-turn and only [*R*-IBTM^{4,5}] gramicidin S and [*R*-IBTM^{4,5}, Lys$^{2,2'}$]gramicidin S retain the biological activity of the parent.



(**44**)



(**45**)

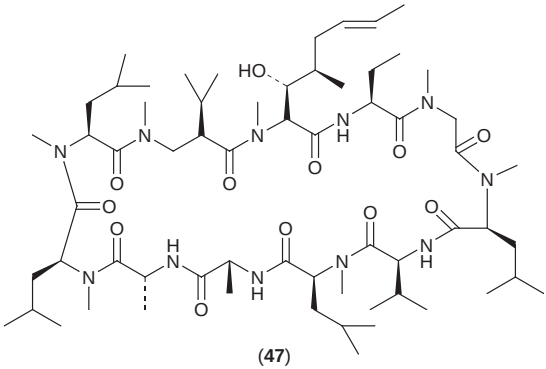


(**46**)

2.11 Cycloundecapeptides – The tropical marine cyanobacterium *Lyngbya majuscula* has yielded[100] two major cytotoxic cyclopeptides which show unusual biological synergism. Laxaphycin A has the structure (45) and B a cyclododecapeptide has structure (46). Laxaphycin A is closely related to the previously discovered homothamnin A (Gerwick *et al.* 1992). Cyanobacterium *Microcystis aeuruginosa* (NIES-88), on further investigation of its extracts, not only contains kawaguchipeptin A (see this Report, Vol. 29, 1998, p. 284) but also produces kawaguchipeptin B, cyclo(-Trp-Ser-Thr-Pro-Trp-Leu-Asn-Gly-Asp-Asn-Asn), which shows antibacterial activity.[101]

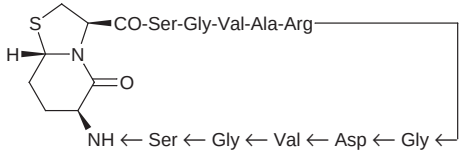
A total synthesis of cyclosporin A (47) using solid phase protocols (D.H. Rich *et al.*, see this Report Vol. 28 1997, p. 250) has already been carried out, but in order to synthesise a diversity of analogues, a detailed study[102] of the efficiency of the coupling stages has proved fruitful. With the plethora of hindered N-methylated amino acids, the molecule offers a demanding challenge. On assessment a number of links proved difficult to achieve, e.g. 10/11, 9/10, 6/5 and 11/1. The last pair, MeVal^{11} and MeBmt^{1} proved very difficult, so a strategy was devised to start with MeVal^{11} and proceed to residue 1, followed by cyclisation

with Castro's BOP reagent. Tandem mass spectrometry,[103] incorporating laser-desorption or fast ion bombardment with time of flight ion analysis, offers quantitative analyses of cyclosporins in biological samples. Hydrolytic conditions[104] for the formation of open-chain oligopeptides from cyclosporin A have been investigated, and cyclosporin A has been regiospecifically alkylated[105] at Val^5 to yield derivatives devoid of immunosuppressive activity *in vitro*, but they have binding affinity for cyclophilin A.



(**47**)

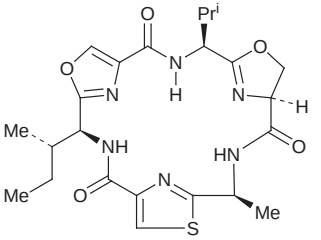
2.12 Cyclododecapeptides – The β-turn mimetic BTD has been incorporated[106] into an RGD cyclopeptide (48) which shows selective binding to the $\alpha_v\beta_3$ receptor. The analogue was synthesised on a Kaiser resin (Ala residue C-terminal), and its conformation supports the hypothesis that a γ-turn is important for specific binding to $\alpha_v\beta_3$ receptors. The conformational preferences[107] of cycloleonurinin, cyclo(-Gly-Pro-Thr-Gln-Tyr-Pro-Pro-Tyr-Tyr-Thr-Pro-Ala-), from the fruits *Leonurus heterophyllus*, has been narrowed to a uniquely determined conformation of a βVI turn at Pro^6-Pro^7 and a βI turn at Pro^{11}-Ala^{12}. Two transannular 4 →1 H-bonds to construct the two β-turns, and two H-bonds between Tyr^9-NH and Pro^7CO, and between Thr^{10}-NH and Tyr^8CO to construct γ-turns together with H-bonds between Ala^{12}-NH and Thr^{10}-OH were observed. The two Pro residues span a *cis* amide conformation.



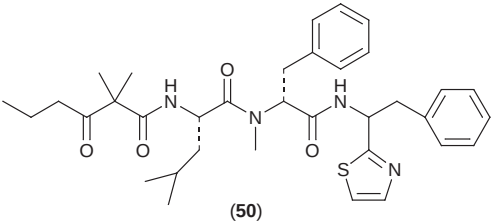
(**48**)

Under section 2.10 of this Chapter (Ref. 98), dimerisation-cyclisation studies on linear precursors of gramicidin S were reported. The same authors have extended[108] their studies to the antibiotic gratisin, cyclo(-D-Phe-Pro-D-Tyr-Val-

Orn-Leu)$_2$, and have found that in the cyclisation of six hexapeptide succinimide esters having Val, Orn, Leu, D-Phe, Pro or D-Tyr at each C-terminus, only H-D-Phe-Pro-D-Tyr-Val-Orn-Leu-ONSu gave semi-gratisin (cyclic monomer) and gratisin in 31% and 8% yields respectively. In both gramicidin S and gratisin biomimetic syntheses, having a Leu residue at the C-terminus is essential. As part of a study[109] to investigate the β-turn character of the repetitive domain of high mol. wt. (HMW) proteins from wheat, the cyclic peptides cyclo(-Pro-Gly-Gln-Gly-Gln-Gln-Pro-Gly-Gln-Gly-Gln-Gln) (peptide 1), cyclo(-Gly-Tyr-Tyr-Pro-Thr-Ser-Pro-Gln-Gln-Gly-Ala) (peptide 2), cyclo(-Pro-Gly-Gln-Gly-Gln-Gln-Gly-Tyr-Tyr-Pro-Thr-Ser-Pro-Gln-Gln) (peptide 3) have been synthesised and have undergone conformational analysis. A type II β-turn was observed in peptide 1 covering the Gln-Pro-Gly-Gln residues, while peptides 2 and 3 include a βI turn at Tyr-Pro-Thr-Ser and Ser-Pro-Gln-Gln with additional βII at Gly-Ala-Gly-Tyr (peptide 2) and Gln-Gln-Gly-Tyr (peptide 3). The Pro in Tyr-Pro-Thr-Ser was up to 50% populated in the *cis* form, but other prolines were more than 90% in the *trans* form.



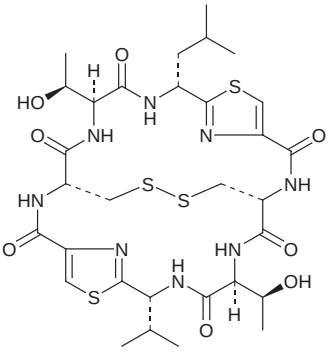
(**49**)



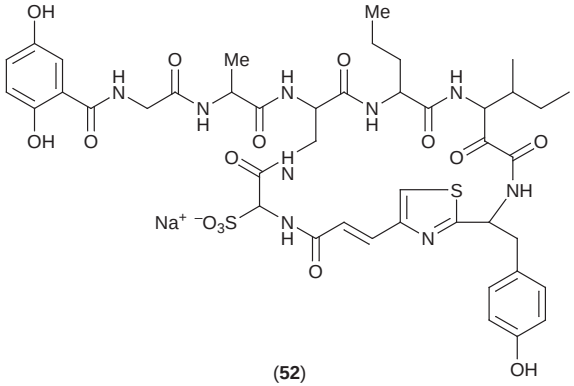
(**50**)

2.13 Peptides Containing Thiazole/Oxazole Rings – The sea hare *Dolabella auricularia* has been a rich store of cyclopeptides and this year again has yielded[110] the cyclohexapeptide dolastatin I (49). Dolastatin 18 has also been characterised[111] from the same source, and is cytotoxic (0.39 μg mL^{-1} in the nonsmall cell lung cancer assay NCI-H460), but although key-worded as a cyclic peptide it is in fact linear having structure (50). Extracts from the tunicate *Lissoclinium patella* have yielded[112] six known compounds plus preulithiacyclamide (51), while a new 4-propenoyl-2-tyrosylthiazole residue has been characterised[113] in oriamide (52) from the marine sponge *Theonella* sp. Studies aimed at total syntheses within the thiostrepton family of antibiotics have moved on as far as the pyridine-thiazole fragment[114] (53), and chemically modified thiazolyl peptide antibiotic based on MDL62,879(GE2270A) were less active[115] than the parent antibiotic.

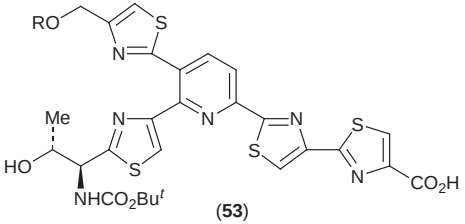
2.14 Cyclodepsipeptides – The dominant role of the sea hare *Dolabella auricularia* as a source of the dolastatins has been reviewed,[116] and the chemistry of the cytotoxic compounds isolated from both *Dolabella* and *Aplysia* species has been discussed.[117] But the source is not 'spent', as a further dolastatin 16 (54) has been



(**51**)

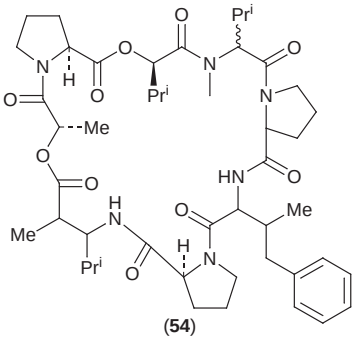


(**52**)

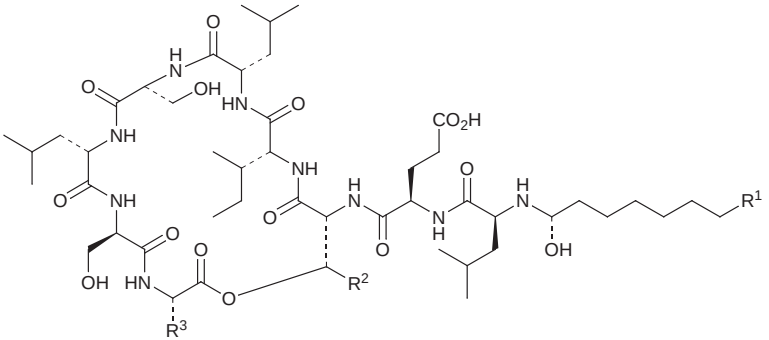


(**53**)

discovered[118] which shows strong inhibition of growth against a variety of human cancer cell lines. Massetolides A (56) - H (63), as well as the known compound viscosin (55) have been isolated[119] from cultures of two *Pseudomonas* spp. Both viscosin and massetolide A exhibit *in vitro* antimicrobial activity against *Mycobacterium tuberculosis* and *M. avium-intracellulare*. Elastase and chymotrypsin inhibitors nostopeptins A and B from the cyanobacterium *Nostoc minutum* have been characterised[120] as having the basic structure (64), with the same 3-amino-6-hydroxy-2-piperidone residue as in dolastatin and micropeptins. Further micropeptin structures have been revealed[121] in work on the cyanobacterium *Micro-*



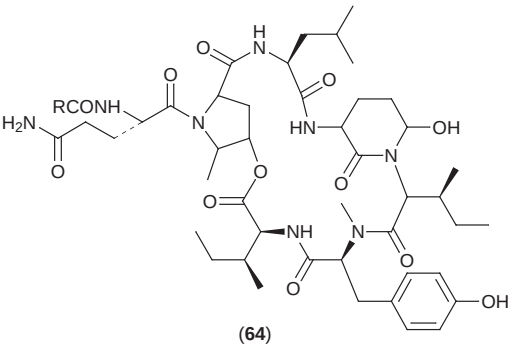
(**54**)



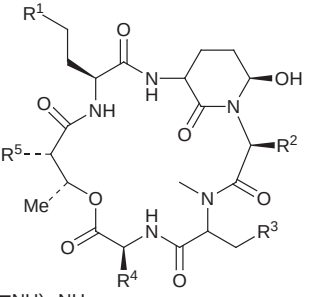
(**55**) Viscosin, R^1 = Me, R^2 = H, R^3 = CH(Me)Et
(**56**) Massetolide A, R^1 = R^2 = Me, R^3 = CH(Me)Et
(**57**) Massetolide B, R^1 = Et, R^2 = Me, R^3 = CH(Me)Et
(**58**) Massetolide C, R^1 = Pr, R^2 = Me, R^3 = CH(Me)Et
(**59**) Massetolide D, R^1 = R^2 = Me, R^3 = CH_2CHMe_2
(**60**) Massetolide E, R^1 = Me, R^2 = H, R^3 = $CHMe_2$
(**61**) Massetolide F, R^1 = Me, R^2 = H, R^3 = CH_2CHMe_2
(**62**) Massetolide G, R^1 = Et, R^2 = H, R^3 = CH(Me)Et
(**63**) Massetolide H, R^1 = Pr, R^2 = H, R^3 = CH(Me)Et

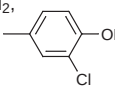
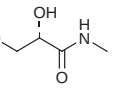
cystis aeruginosa. Thus, micropeptins 478-A and B have been identified as structures (65) and (66) respectively. *Microcystis viridis* (NIES-103), has yielded[122] micropeptin 103 (67), which inhibits chymotrypsin and thrombin and contains the novel N-methyl Trp residue. A new antitumour antibiotic, thiocorline (68), with potent cytotoxic activity, has been isolated[123] from marine *Micromonospora*.

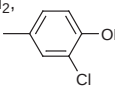
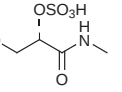
In 1996 the structure of fusaricidin A was worked out, and in the same culture broth from *Bacillus polymyxa* KT-8, fusaricidins B (69), C (70) and (71) have been isolated.[124] As defined by their name, calcium dependent peptide antibiotics from *Streptomyces coelicolor* A3(2) inhibit the growth of Gram positive bacteria in the presence of Ca^{2+} ions. The four antibiotics CDA 1 (72), 2 (73), 3 (74) and 4 (75) were identified[125] in the culture medium. From *Nostoc* sp. GSV224, cryptophycin 46 (76) and two other analogues have been identified[126] in trace amounts.

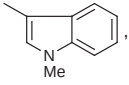
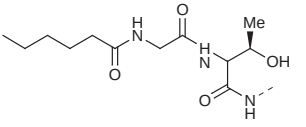


(**64**)

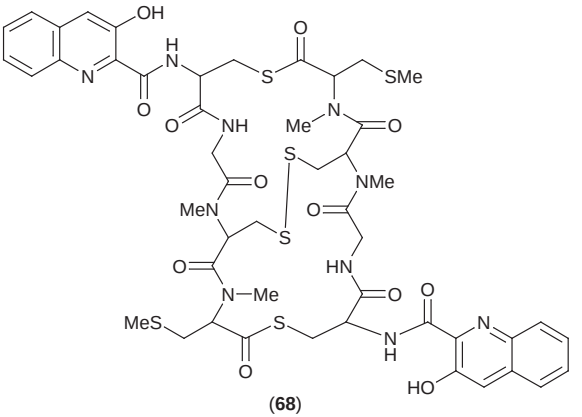


(**65**) $R^1 = CH_2NH{-}C(=NH){-}NH_2$,
$R^2 = R^4 = CH(Me)Et$, $R^3 =$  , $R^5 = HO_3SO$ 

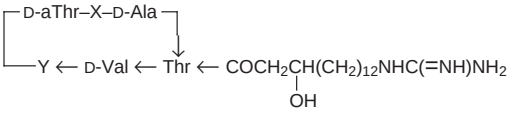
(**66**) $R^1 = CH_2NH{-}C(=NH){-}NH_2$,
$R^2 = R^4 = CH(Me)Et$, $R^3 =$  , $R^5 = HO_3SO$ 

(**67**) $R^1 = CONH_2$, $R^2 = PhCH_2$,
$R^3 =$  , $R^4 = CHMe_2$, $R^5 =$ 

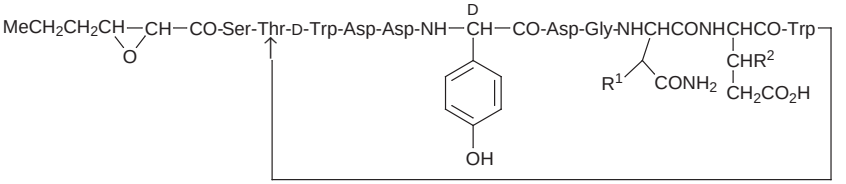
Once again, the natural cyclodepsipeptides have offered demanding synthetic challenges to a number of research groups, so what follows is a distillation of progress published in 1997. Enantiospecific total syntheses[127] of the potent antitumour macrolides cryptophycins 1 (77) and 8 (78) have been carried out. The final links were made at points (a) and (b) using 2,4,6-trichlorobenzoyl chloride and O-benzotriazol-1-yl, N,N,N^{1}N^1-bis(pentamethylene) uronium hexafluorophosphate (76% yield over 2 steps). An efficient synthesis[128] of destruxin B (79) has involved a [3+3] fragment condensation utilising final linking at points (a) and (b) in structure (79). Boc-hydrazide was used for the C-terminal MeAla residue to inhibit facile dioxopiperazine formation from MeVal-MeAla. Novel esterase-sensitive cyclic prodrugs of peptides with increased protease stability



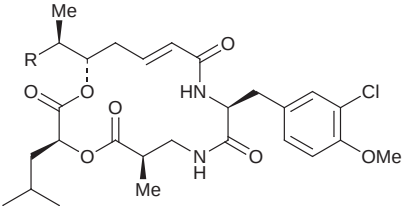
(**68**)



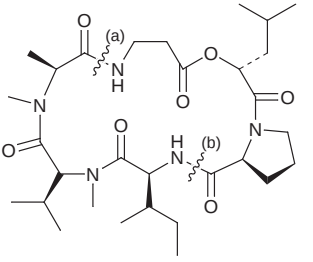
(**69**) X = D-Gln, Y = Val
(**70**) X = D-Asn, Y = Tyr
(**71**) X = D-Gln, Y = Tyr



(**72**) CDA1, $R^1 = OPO_3H_2$, $R^2 = H$
(**73**) CDA2, $R^1 = OPO_3H_2$, $R^2 = Me$
(**74**) CDA3, $R^1 = OH$, $R^2 = H$
(**75**) CDA4, $R^1 = OH$, $R^2 = Me$

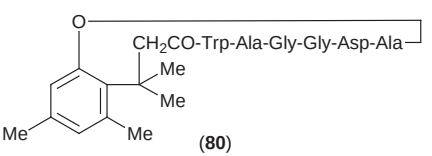


(**76**) R = PhCH=CH
(**77**) R = PhCH–CH (epoxide)
(**78**) R = PhCH(Cl)CH(Me)OH

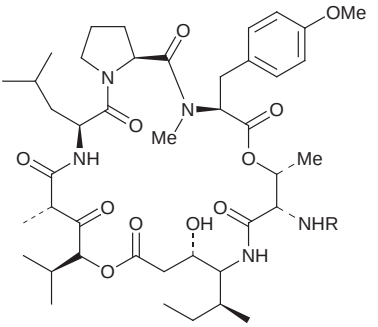


(**79**)

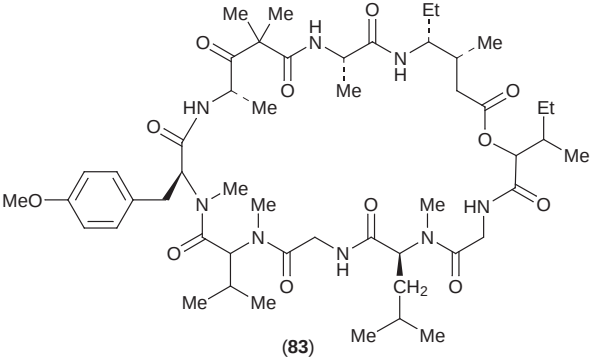
feature lactonisation stages which convert them into novel cyclodepsipeptides. Cyclo(-Ala-OCH_2O_2C-Trp-Ala-Gly-Gly-Asp) was synthesised[129] by pre-substitution of the ester links between Ala and Trp. Final cyclisation between Asp and Ala was carried out in 20% yield by BOP-Cl/DMAP under high dilution conditions. The cyclic structures proved to be 25-fold more stable than the linear hexapeptide. On the same theme[130] lactonisation using a "trimethyl lock" insert to produce compound (80) was achieved via BOP-Cl between Asp and Ala residues. Didemnin A (81) has become a prime synthetic target as other members of the family are derived from it by side chain attachment. Two approaches[131] to (81) have been researched, one of these was the elaboration of a linear heptadepsipeptide incorporating the first residue of the didemnin side chain, D-MeLeu, while the second approach was carried out without the side chain residue. Macrocyclisation was carried out between Pro-COOH and N-methylated-Tyr derivative, using HATU/HOAt in 28% yield when D-MeLeu was attached, 76% in its absence. A full range of reagents, BOP, PyBrOP, PyAOP, and HBTU were used to construct the linear precursors. Didemnin B (82) was obtained by coupling the side chain MeCOCO-Pro onto the amino group of (81). As didemnin B (82) has undergone phase II clinical trials for antitumour activity, the thrust to synthesise analogues is intense. Thus a reduced ring analogue[132] of didemnin B, where non-essential amino acids such as Leu and Pro have been



(**80**)



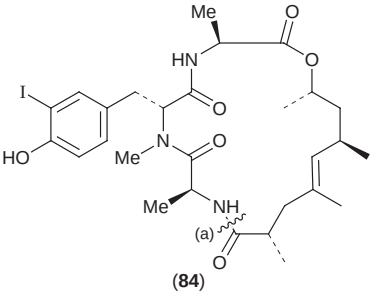
(**81**) R = H
(**82**) R = MeCOCO-Pro



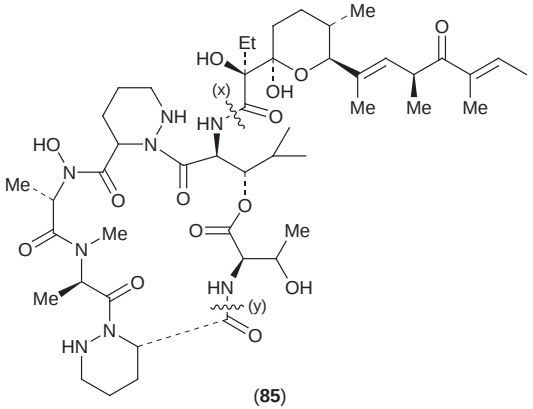
(**83**)

replaced by a covalent linker between Ala and the isostatine residue to keep the Tyr side chain and isostatine hydroxyl in bioactive conformation, has been synthesised but no biological activity was reported. Acyclic analogues of didemnin B with isostatine and ester replacements have also been synthesised.[133]

Until a total synthesis of dolastatin 11 (83) was achieved[134] stereogenic centres in dolastatin-related cyclodepsipeptides were unconfirmed. The synthesis was similar to Shiori's convergent synthesis of the didemnins in 1989, where the final cyclisation was at point (a) using HBTU (20-24% yield) or BOP-Cl (13% yield). However, Shiori *et al.*[135] have moved on to expedite a synthesis of geodiamolide A (84) from marine sponges, the final macrocyclisation occurring at point (a) in (84) using DPPA (di-phenylphosphorazidate). Esterification between polyketide and tripeptide units was effected under high pressure conditions. Another synthesis of geodiamolide A has also appeared[136] in a Journal inaccessible for reviewing. A first asymmetric total synthesis[137] of the antitumour antibiotic A83586C (85) has been based on a redesigned approach where the side chain was attached as a complete unit at point (x) in (85) after the macrocyclisation had occurred using HATU at position (y). The antiparasitic cyclo-octadepsipeptide PF1022A (86) and analogues have been assembled[138] on an oxime resin which had been attached at the lactic acid residue. The final cyclisation took place almost uniquely in ethyl acetate as solvent and provided an opportunity to

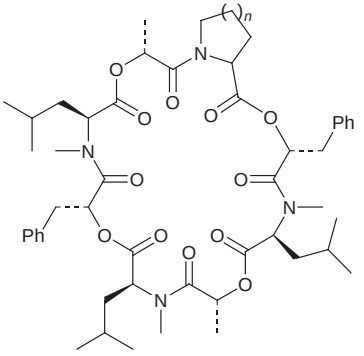


(**84**)

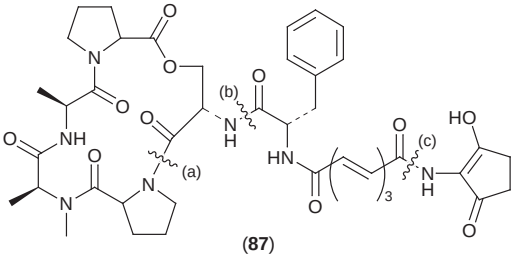


(**85**)

generate combinatorial libraries. Enopeptin B (87) from *Streptomyces* sp. RK-1051, has an interesting multi-origin biosynthetic profile and the problem with its synthesis has been the drawing together of three quite different fragments. Positions (a), (b) and (c) were chosen[139] as linkage points for a total synthesis with point (a) being "zipped up" as a pentafluorophenyl ester in 68% yield. The polyene link at (b) was effected by HATU coupling, with the final amide bond at (c) being made using i-BuOCOCl. Scarcity in natural supply of aurilide (88) from the sea hare *Dolabella auricularia* has prevented an assessment of its cytotoxicity being made, but an enantioselective synthesis[140] in 4% overall yield has given material whose cytotoxicity against $HeLaS_5$ cells was rated as IC_{50} 0.011 μg mL^{-1}. Macrocyclisation was achieved using BOP-Cl on the carboxyl of Val which was then linked by the MeAla-NH (point (a)).

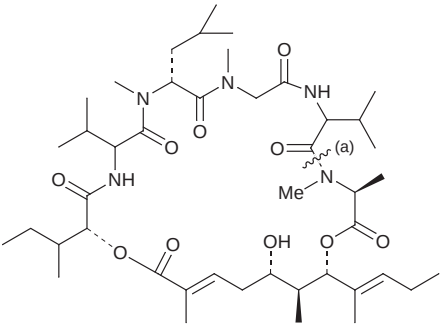


(**86**) *n* = 1 or 2

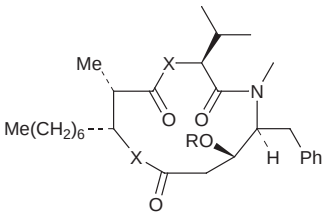


(**87**)

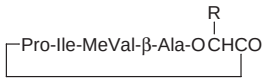
Hydroxyproline analogues of the multi-drug resistance reverser, hapalosin (89) have been synthesised[141] where the proline residue replaces MePhe. All analogues less potent than hapalosin have either two free hydroxyl groups or two aromatic groups. More potency is achieved by having only one free hydroxyl and an aromatic group. Replacing[142] the depside bonds as in (90) to form a triamide also reduced activity. Varying the side-chain (R) in destruxin E analogues (91), by making R = CH_2Ph,$CH_2C{\equiv}CH$ or $CH_2C{\equiv}CTMS$, both in D and L-forms,[143] still produces toxic effects against *Galleria mellonella* larvae, but the most potent had R = CH_2C(CH, equipotent with destruxin E.



(88)

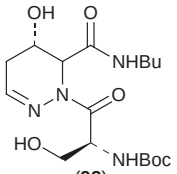


(89) X = O, R = H
(90) X = NH, R = H

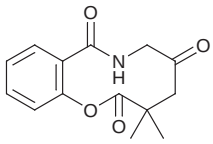


(91)

The cyclic hexadepsipeptide framework of enniatin B has been used as a template matching the β-turn tripeptide of tendamistat.[144] Progress is being made towards the eventual synthesis of the luzopeptins, with the synthesis[145] of dipeptide (92). The combination of the "azirine/oxazolone method" for the acid catalysed amide cyclisation in DMF has proved[146] an excellent route to (93). Of some interest to cyclodepsipeptide synthetic "gurus" is the fact that macrocyclic lactones can be synthesised[147] from ω-hydroxyacids using di-t-butylpyrocarbonate (Boc_2O).

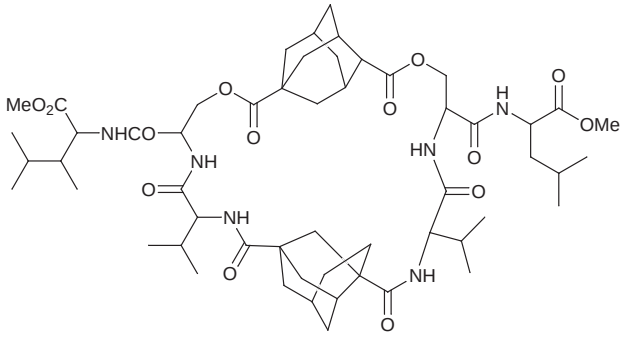


(92)



(93)

The annual interest in ionophores has still produced a trio of publications. The X-ray structure[148] of [D-Hyi^2, L-Hyi^4]meso-valinomycin from ethanol solution, i.e. cyclo[D-Val-D-Hyi-L-Val-L-Hyi-(D-Val-L-Hyi-D-Hyi)$_2$], turns out to be very similar to that obtained from acetone solution in 1991 and confirms that it is sterically incapable of forming complex ions. A less lipophilic analogue[149] of valinomycin, with each Val in the 3rd and 7th position replaced by Ala shows a crystal structure very similar to previously published isoleucinomycin. The structural features of valinomycin and nonactin in an environment resembling that of a membrane, such as monolayers at interfaces, have been investigated.[150] Grazing incidence X-ray diffraction at the interface revealed that the presence of lipids induced ordered stacking of valinomycin-KCl complexes into three or four layers, which may have a bearing on ion transport through membranes via stacking. A simple 2-step synthetic strategy[151] has provided an entry to a large variety of adamantane containing serine-based cyclodepsipeptides, such as (94), which was the most efficient ion transporter in lipid bilayer membranes.



(**94**)

3 Modified and Conjugated Peptides

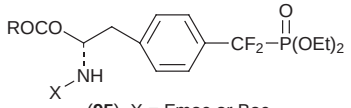
Post-translational modifications of peptides and proteins remains a very buoyant area of research, and the papers reviewed under this sub-section mainly belong to peptides bearing non-peptidic conjugates. The glycopeptide antibiotics, have been included once again, not for their interest as conjugated peptides, but because of their highly modified structures and their current importance as 'last-resort' antibiotics used in the constant battle against β-lactam resistance.

3.1 Phosphopeptides – Modern chemical approaches to the synthesis of phosphopeptides have been reviewed.[152] The 'building block' approach seems to be winning the publication stakes this year. Fmoc-Tyr[$(PO(OMe)_2$]-OH, which is commercially available has been converted[153] into its stable fluoride (Fmoc-Tyr[$PO(OMe)_2$]-F), and used for an efficient coupling of phosphotyrosine to adjacent sterically-hindered amino acids, Aib and Ac_6c, as well as for difficult sequences in the phosphotyrosine peptide Stat 91.[695–708] The phosphate Me groups could be cleaved on solid phase using trimethylsilyl iodide, a protocol which has been proven[154] in the presence of Arg, His, Met and Trp. Bis-(2,2,2-trichloro)ethyl(Tc) groups as phosphate protectors have been assessed[155] for both Boc-protocols (using Boc-Tyr(PO_3Tc_2)OH) and Fmoc-protocols (using Fmoc-Tyr(PO_3Tc_2)-OH). The phosphate protection was shown to be incompatible with the piperidine used for Fmoc removal. The synthesis[156] of phosphoserine peptides related to heat shock protein HSP27 was carried out using both Boc strategy (O-phosphonoserine protected by either cyclopentyl or cyclohexyl groups) or the Fmoc protocol [Fmoc-Ser[PO(OBzl)OH]OH]. Thiophosphotyrosine peptides can be useful[157] as potential inhibitors of PTK, PTPase and cytosolic protein binding blockers. The key intermediate for their synthesis, Boc-Tyr[$PO(SCH_2CH_2CN)_2$]-OH, was used.

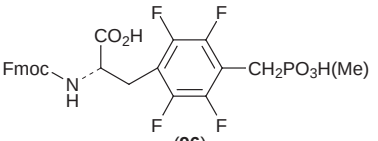
The alternative approach of 'global phosphorylation' has been used[158] for five phosphopeptides with the general structure Ac-Ala-Xaa(PO_3H_2)-Pro-Yaa-NH-p-nitroanilide (Xaa=Ser, Thr, Tyr; Yaa=Tyr-Lys). Global phosphorylation was on

resin-bound peptides using dibenzyl, N,N-diisopropyl-phosphoramidate/tetrazole followed by oxidation using t-butyl hydroperoxide. During post assembly global phophorylation formation of H-phosphonate peptides as by-products is a complication.[159] However, using dibenzyl N,N-diisopropylphosphoramidite for phosphitylation, followed by immediate oxidation with anhydrous t-butylhydroperoxide in dry THF under argon, minimises the problem.

More stable non-hydrolysable mimetics of phosphopeptides are in demand. H-Phosphono and methylphosphono tyrosyl peptides can be synthesised[160] on solid phase using the amidites $Et_2N\text{-}P(OBu^t)_2$ and $Et_2N\text{-}P(OBu^t)\text{-}Me$ respectively followed by oxidation with *m*-chloroperbenzoic acid and TFA cleavage. A facile synthesis[161] in 80-90% yield of F_2Pmp derivatives (95) involves CuCl mediated coupling of an iodophenylalanine with (diethylphosphoryl)difluoromethylcadmium bromide. This method could also be used for iodo-Phe containing peptides. A new non-hydrolysable phosphotyrosine analogue (96) (Fmoc-L-F_4Pmp) has been synthesised[162] with the pK_a of its side-chain determined at 6.9 (cf. phosphotyrosine pK_a 5.9). When incorporated into model peptides, the resulting peptides gave poorer affinities than the analogues Pmp containing peptides. A new analogue[163] of phosphoserine prepared via [4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino]butanoic acid has been reported.



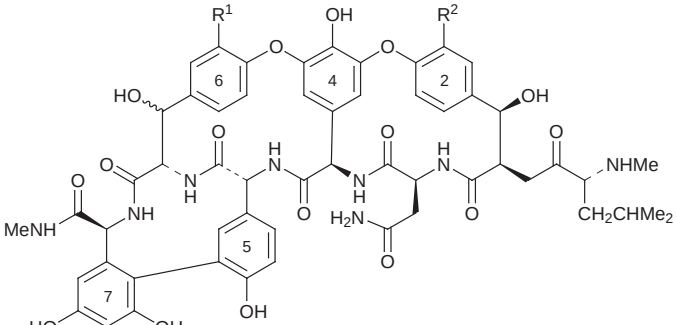
(**95**) X = Fmoc or Boc



(**96**)

Whereas phosphoesters of Ser, Thr and Tyr have been studied extensively, only limited information is available for other phosphate modified amino acids. Phosphohistidine derivatives[164] in proteins were originally discovered in prokaryotic organisms, but now have also been found in eukaryotic cells. Methods of synthesis and analysis have been discussed. However, so far, phosphorylated *cis* or *trans*-4-hydroxyprolines have not been found[165] *in vivo* but in preparation for that event model compounds bearing phosphorylated HOPro side chains have been made using global phosphorylation with O,O′-t-butyl-N,N-diethylphosphoramidite on solid phase. NMR showed that O-phosphorylation had a significant influence on the Gly-HOPro *cis-trans* isomerisation. The phosphotyrosine mimic, O-malonyltyrosine has been introduced[166] into cyclic peptides to improve the proteolytic stability and restrain conformation flexibility. Improved potency was recorded. Procedures known to harm phosphopeptide derivatives can be replaced[167] by milder conditions if enzymatic removal of heptyl esters at pH 7 and 37 °C is used.

3.2 Glycopeptide Antibiotics - A review has been published[168] on structural modification of glycopeptide antibiotics, and a more focussed account[169] of S_NAr macrocyclisation for biaryl ether formation covers synthetic aspects of relevance to this section. The need to develop efficient methods of biaryl coupling for

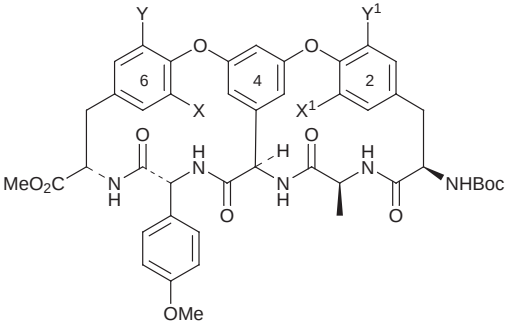


(**97**) $R^1 = R^2 = Cl$, $R^3 = H$
(**98**) $R^1 = R^3 = Cl$, $R^2 = H$

glycopeptide antibiotics has overlapped into other compounds, bearing similar structures, that do not necessarily lie comforably in this section. For convenience, however, we review these here as an extension of similar synthetic techniques.

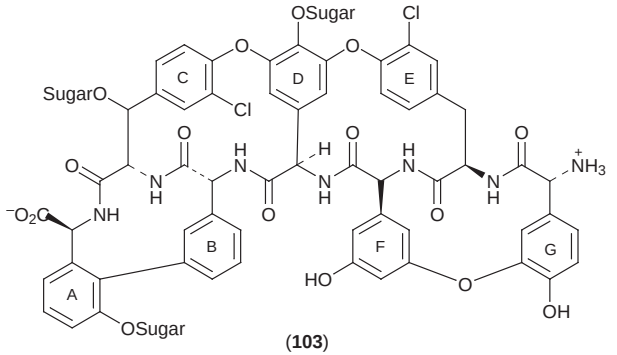
After years of synthesising large 'chunks' of the vancomycin aglycone molecule (97), the final linking of the jig-saw is almost complete with the synthesis[170] of bis-dechlorovancomycin, orienticin C (98). The final stages in the synthesis involved coupling of a pre-synthesised[171] bicyclic biaryl tetrapeptide core including rings 4, 6, 5 and 7, with a protected N-terminal tripeptide, which included ring 2 as an ortho-nitro fluoride derivative to enable intramolecular nucleophilic substitution to give the final biaryl ether link. The preparation[171] of the fully functionalised (4-6) (5-7) tetrapeptide core involved the key step of oxidative biaryl cyclisation between 5-7 with VOF_3, $BF_3{\cdot}OEt_2$, $AgBF_4$ and CF_3COOH to give the highly strained unnatural *R*-atropisomer, which had to be atropisomerised to the *S*-atropisomer. The macrocycle incorporating the 4-6 residues has a profound influence on the kinetic and thermodynamic stability of the atropisomers, stabilising the *S*-biaryl form (>98:2) and the bias for *cis* conformation of the 5-6 amide bond found in natural vancomycin. Thallium(III)-mediated intramolecular oxidative cyclisation[172] of phenolic residues to provide the 2-4 diaryl ether link has been shown to be remarkably sensitive to transannular effects across the 4-6 ring systems, especially the ring 6 chlorine substitution pattern. Chlorine atoms in ring 6 appear to be essential for a successful oxidative 2-4 cyclisation. The conformational bias imparted by a coupled 5-7 ring system is also important. Studies[173] on the fully functionalised 6-4-2 system have confirmed earlier studies on 6-4 and 4-2 ring systems separately, that it is only the 4-2 system that seems to undergo atropisomer isomerisation at 130 °C in *ortho*-dichlorobenzene. This allows control of the natural product atropisomer stereochemistry.

Synthesis[174] of all four atropdiastereoisomers (99)-(102) of a model 6-4-2 ring system has been carried out by sequential cycloetherification based on the S_NAr methodology. Full details[175] have also appeared on the fully substituted 6-4 and 4-2 ring systems in vancomycin, constructed via the S_NAr method. A similar approach[176] following assembly of the peptide backbone on solid phase has been used for the efficient synthesis of OF4949 derivatives. With the ultimate aim of



(**99**) X = Y^1 = NO_2, Y = X^1 = H
(**100**) X = X^1 = NO_2, Y = Y^1 = H
(**101**) X = X^1 = H, Y = Y^1 = NO_2
(**102**) X = Y^1 = H, Y = X^1 = NO_2

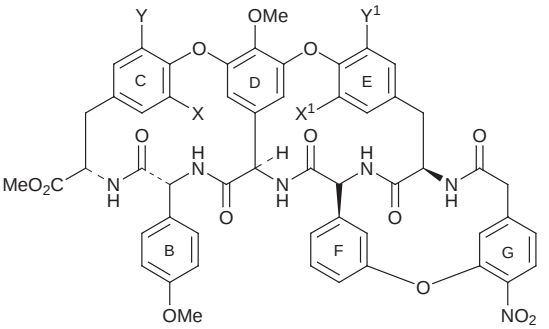
synthesising teicoplanin (103), studies[177] have demonstrated the remarkable efficiency of intramolecular S_NAr cycloetherification in making the precursors (104) and (105). In conjuction with Evans's oxidative ring coupling of rings A-B, the authors predict a total synthesis of teicoplanin soon. To add further clarification to the atropdiastereoselection issue during the coupling of ring 2 to ring 4 in vancomycin, *ortho*-nitrofluoro groups have been placed[178] in ring 4 precursors with ring 2 carrying *o*-chlorophenol substitution. However, reversing the substitution pattern in this way had insignificant effect on atropdiastereoselectivity. Sequential intramolecular S_NAr reactions have offered[179] a start to the total synthesis of the bicyclic left part of kistamycin A and B, as summarised in



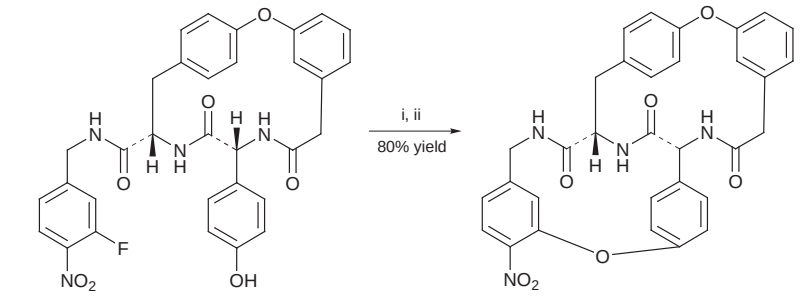
(**103**)

Scheme 2, and formation of ring I in kistamycin has been modelled[180] via the synthesis of (106) using a similar approach. Ring closure of a linear tetrapeptide bearing *o*-nitrofluorine ring neighbouring onto a phenolic ring has also been effective[181] in the making of (107) which constitutes the 'western' part of chloropeptins I and II.

Although the compounds themselves do not readily qualify for entry in this

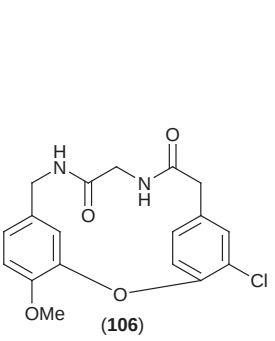


(**104**) X = Y^1 = H, Y = X^1 = NO_2
(**105**) X = X^1 = H, Y = Y^1 = NO_2

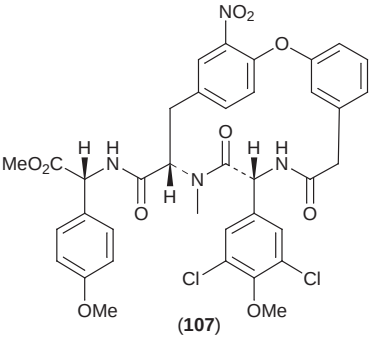


Reagents: i, $KHCO_3$/DMF; ii, 18-crown ether

Scheme 2



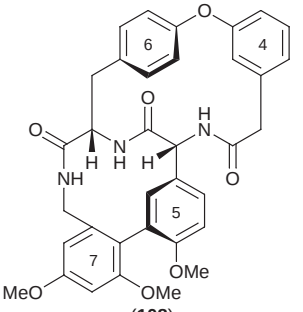
(**106**)



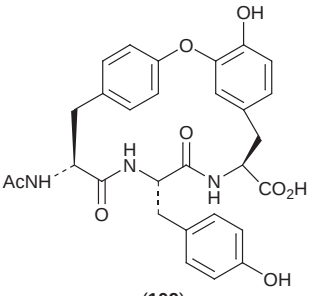
(**107**)

sub-section, it is convenient to report on the successful S_NAr macrocyclisation to aromatic diaryl ethers, using ruthenium π-arene complexes, in the synthesis[182] of two natural products K13 (109) and OF4949-I (110).

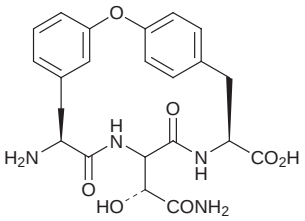
A new synthetic approach[183] to the synthesis of 6-4 and 4-2 ring systems in vancomycin utilises coupling between a phenolic tyrosyl ring and a dibromotriazinyl substituted aromatic ring, in the presence of CuBr-Me_2S/K_2CO_3 and



(**108**)



(**109**)



(**110**)

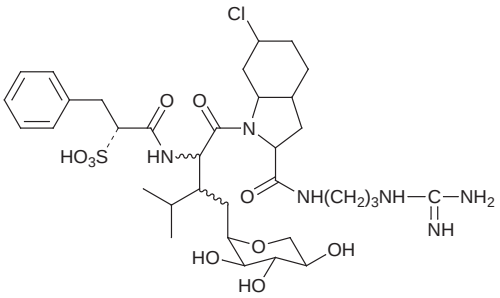
pyridine in MeCN at 75 °C. The critical biphenyl ring formation between the 5-7 rings in vancomycin has been achieved[184] via the Suzuki coupling reaction, but acknowledged to be greatly assisted by the presence also of a completed 6-4-diaryl ether bridge in the precursor leading to (108). A mild method[185] for construction of the 5-7 link in a model compound involves treating iodo-aromatic ring precursors with $(Ph_3P)_2NiCl_2/Zn/Ph_3P$. Synthesis[186] of an epimer of a previously synthesised model for teicoplanin, using arene ruthenium chemistry for cyclo-diaryl formation, confirmed the formation of the epimer at the Phe unit in early cycloamidation processes. Attempted cyclisations[187] of pre-formed biaryl linkages in the complestatin and chloropeptin series have both met with failure, but the S_NAr macrocyclisation of a model for OF4949 synthesis has been successfully carried out[188] on solid phase.

The first crystal structure of vancomycin has been determined[189] as its acetate complex. It confirms the asymmetric homodimer structure as seen in a recent determination of balhimycin, and predicted previously by NMR studies. In the longstanding debate about carboxylate recognition by vancomycin, a possible cooperative mechanism linking ligand binding and dimerisation is offered. In recent unpublished work, it has been shown that a semi-synthetic antibiotic, biphenylchloroeremomycin (BCE), or LY307599 owes its activity against vancomycin-resistant bacteria to its ability to dimerise and anchor to bacterial cell membranes. It can now be shown[190] that a second semi-synthetic glycopeptide MDL63246, produced from A40926 (related to teicoplanin), showed no evidence of dimerisation, but does contain a membrane-anchoring C_{11} chain attached via ring 4 sugar. This would make it similar to A40926. NMR studies[191] and molecular

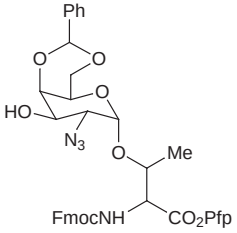
modelling in the presence of detergent micelles indicate that vancomycin interacts with the membrane, despite lacking a membrane anchor. The membrane appears to affect the conformation of vancomycin. Model cell membranes with cell wall analogues[192] anchored *via* a hydrophobic decanoyl chain bind selectively to vancomycin. Dipeptide, pentapeptide and hexapeptide analogues display enhanced binding at the model cell surface, while tetra- and tripeptides do not. Capillary electrophoresis[193] has been used to establish the dissociation constant of a synthetic dimeric vancomycin (V) (V-$NHCH_2$-*p*-$C_6H_4CH_2NH$-V) with a dimeric Ac-Lys-D-Ala-Ala-OH (dimerised through a succinic acid linker at Lys side-chain). An enhancement of ~10^3 in binding, relative to monomeric interaction, was established. The same techniques[194] also showed that acetylation of N-terminal vancomycin decreases its affinity for Di-Ac-Lys-D-Ala-D-Ala. The effect of dimethylation at the N-terminus has also been studied.[195]

3.3 Glycopeptides – Interest in this sector continues to flourish and a reflection of the importance of the subject are the number of reviews published during 1997. Synthesis[196] of selectively deprotected building blocks in O- and N-glycopeptides has been reviewed, and the synthesis of the peptide moieties[197] has been highlighted. O-Glycosyl amino acids as building blocks for glycopeptide synthesis has justified a 178 reference review.[198] The use of *O*-linked and *N*-linked solid supports and methods of dendrimeric synthesis have been explored[199] in a 169-reference compilation, while recent advances[200] in the general glycopeptide synthetic area runs to 212 references. Direct synthesis of glycosylated amino acids and Fmoc amino acids for biomedicinally interesting glycopeptides has been reviewed,[201] and publications on the chemical and chemoenzymatic assembly of glycopeptides have been compiled.[202] Large scale enzymic synythesis of glycopeptides,[203] and enzymic synthesis of peptide conjugates[204] (including phosphopeptides and lipopeptides) have been reviewed while shorter reviews on isolation and characterisation[205] and application of glycopeptides as antigens against cancer[206] have been published. Solid phase synthesis of glycopeptides and immunological studies involving T-cell stimulation have been reviewed[207] as novel applications in medicine.

3.3.1 O-Glycopeptides – The majority of papers cover the synthesis of O-glycopeptides, usually by the building block approach, but there is one report[208]



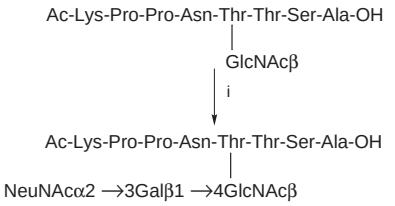
(**111**)



(**112**)

of the structure elucidation of aeroginosins 205A (111) and B, found as serine protease inhibitors in cyanobacterium *Oscillatoria agardhii* (NIES-205). Aeroginosin 205B differs from A in its stereochemistry at the 3-hydroxyleucine and phenyllactic acid-2-O-sulfate.

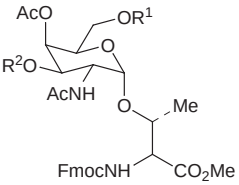
The building block approach, where the initial carbohydrate side chain is already attached to the protected amino acid derivative (usually Fmoc), remains the most popular method. Introduction[209] of the unit (112) into a solid phase protocol, gives glycopeptides with free hydroxyl groups which can be glycosylated further on-resin with perbenzoylated glycosyl trichloroacetimidates. A strategy has also been developed[210] for introducing unique functional groups (e.g. aldehydes or hydroxylamino groups) onto glycopeptides produced by the building block approach by using the commercially available enzyme galactose oxidase. A resin-bound O-glycoconjugate, assembled on a HYCRON linker, can be removed[211] under Pd(0) catalysed cleavage off the resin to yield a glycopeptide, which can be extended at the glycan side-chain using glycosyltransferases as depicted in Scheme 3. Different series of glycopeptides can be prepared[212] in multiple-column solid phase synthesis, and allow the synthesis of the mucins MUC2 and MUC3 containing oligosaccharides with core 1 (113), core 2 (114), core 3 (115) and core 4 (116) structures, based on a Fmoc-threonine building block similar to (112). Glucosamine donors[213] having N-TCP, N,N-diacetyl and N-trichloroethoxycarbonyl have been shown to give exclusively β-glycosides when reacted with Boc-Ser(or Thr)-OBzl or Fmoc-Ser(or Thr)-OPfp.

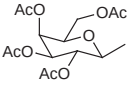


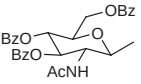
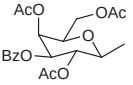
Reagent: i, glycosyltransferases

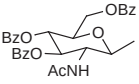
Scheme 3

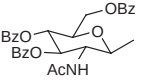
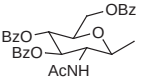
In order to examine and improve on the silver trifluoromethane sulfonate based glycosylation of Ser, Thr and HOPro, a detailed study[214] has revealed that using 2,3,4,6-tetracetyl-α-D-glycopyranosyl bromide produced significant quantities of disaccharide by-products. With the sugars L-rhamnose and D-mannose there were less by-products. Incorporation of the glycoamino acid derivatives into linear and cyclic Arg-Gly-Asp sequences gave glycopeptides which were equal or slightly less potent as platelet aggregation inhibitors, than the non-glycosylated parent peptides. The Fmoc derivative (117), as its Pfp ester, together with its Ser analogue have been specifically incorporated[215] into the model peptides (118) - (120) in order to study conformational effects on the peptide backbone. A note of warning about racemisation of the first assembled glycosylated residue on to a Wang resin has been expressed.[216] Linking of glycosylated Ser and Cys derivatives through activation by symmetrical anhydride, TBTU/DMAP, with or without HOBt, or pentafluorophenyl (Pfp) esters gave high levels



(**113**) R^1 = Ac, R^2 = 

(**114**) R^1 =  , R^2 = 

(**115**) R^1 = Ac, R^2 = 

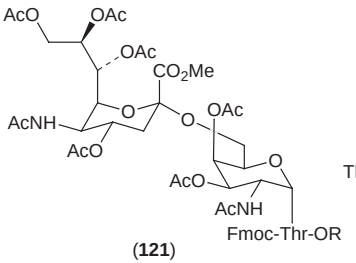
(**116**) R^1 =  , R^2 = 

(**117**) $R^1 = R^2$ = Ac

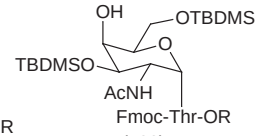
(**118**) R^1 = GlcNAcβ, R^2 = H
(**119**) R^1 = H, GlcNAcβ
(**120**) $R^1 = R^2$ = GlcNAcβ

of racemisation. The lowest % racemisation was achieved by 2,4,6-mesitylene sulfonyl-3-nitro-1,2,4-triazolide (MSNT), with glycosylated Cys lower than its Ser analogue. The synthesis[217] of the threonyl derivative (121), from Fmoc-Thr-OBu^t,3,4,6-tri-OAc-2-azido-2-deoxy-α-D-galactopyranosyl bromide and sialyl-xanthogenate in 5 steps, has given entry into a sialyl-T_N-glycoundecapeptide, which is part of the repeating unit of MUC1. The same approach[218] using solid phase and a HYCRAM allylic linker has given a 42% overall yield of sialyl-T_N-antigen "tandem repeat" of MUC1 mucin. Building blocks corresponding to T_N[α-D-GalNAc(1 →O)-Thr] and sialyl-T_N[α-D-Neu5Ac(2 →6)α-D-GalNAc(1 →O)-Thr] epitopes have been prepared[219] from 4-methylphenyl 2-azido-2-deoxy 1-thio-β-D-galactopyranoside using t-butylmethylsilyl groups (as in 122), rather than acetyl groups to circumvent β-elimination problems. A multiple antigen glycopeptide (MAG) carrying the T_N antigen has also been synthesised[220] using the Fmoc protocols as a possible approach to a synthetic carbohydrate vaccine. A study[221] of glycan T-cell specificity has been made using O-glycosylated and photoaffinity labelled glycopeptides synthesised by multiple column peptide synthesis. The mouse haemoglobin decapeptide (67-76), Val-Ile-Thr-Ala-Phe-Asn-Glu-Gly-Leu-Lys, was used as model non-imunogenic in CBA/J mice. It was converted into an immunogen by introduction of different tumour associated moieties, [β-D-GlcNAc-O-Ser/Thr, α-D-Gal-NAc-O-Ser/Thr ($T_N$antigen) core 1 (T-antigen) core 2 core 3 and core 4].

Mannose and mannose disaccharides with bis-trichloroethyl phosphate on Fmoc-Thr-OPfp have been used[222] on solid phase using side chain anchoring of the peptide, with the C-terminal carboxyl group protected as an allyl ester. Selective removal of this ester protection and head-to-tail cyclisation gave a high yield of cyclic peptide templates. The restricted conformation of the cyclic

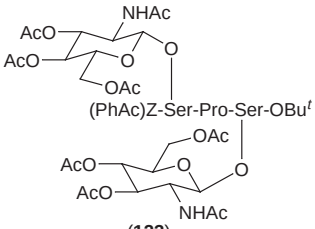


(**121**)

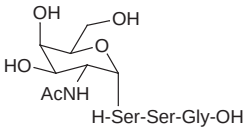


(**122**)

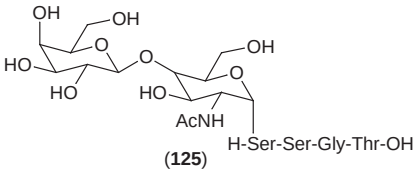
peptides decreased the binding at the mannose-6-phosphate receptor. Glycopeptide libraries can be built up[223] by stereoselective glycosylation of free hydroxy groups on resin (polyethyleneglycol based) using trichloroacetimidate glycosyl donors. A complex phosphoglycohexapeptide has been built up[224] from (123) by utilising the very mild conditions necessary for removal of (phenylacetyloxy)benzyloxycarbonyl with pencillin G acylase. Addition of Aloc-Ser[OPO(OAl)$_2$]-OH completed the sequence.



(**123**)



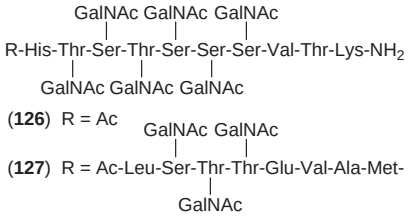
(**124**)

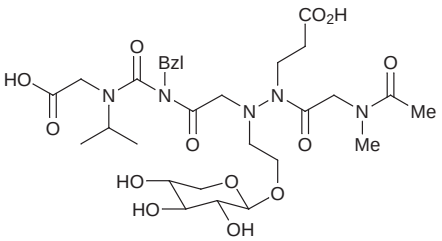


(**125**)

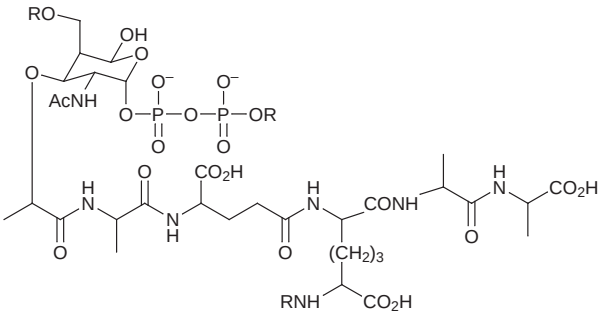
Synthesis[225] of suppressins A (124) and B (125), which suppress the production of the phytoalexin of pea, has involved, for A, condensation of 3,4,6-tri-OAc-2-azido-2-deoxy-α-D-galactopyranosyl bromide with Z-Ser-Ser(Bzl)-Gly-OMe, in the presence of trimethylsilyl trifluoromethansulfonate (TMSOTf), and, for B, treatment of Z-Ser-Ser(Bzl)-Gly-Asp(OBzl)-Thr(Bzl)OMe with 2,3,4,6-tetra-OAc-αD-galactopyranosyl-(1 →4)-3,6-di-OBz-2-azido-α-D-galactopyranosyl trichloroacetimidate in the presence of TMSOTf. Highly glycosylated peptides corresponding to human glycophorin A^N have been assembled[226] on solid phase *via* the building blocks Fmoc-Ser(Ac$_3$GalN$_3$)-OPfp and Fmoc-Thr(Ac$_3$GalN$_3$)-OPfp. NMR techniques were used to characterise the final products (126) and (127) which represent a fine achievement for the methodology.

A new prototype[227] glycopeptidomimetic (128) compound of *N*-substituted





(**128**)

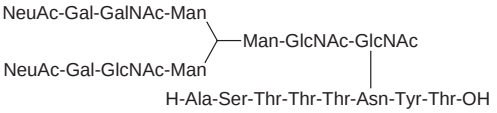


(**129**)

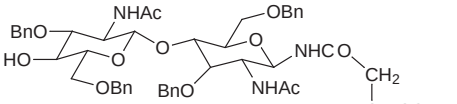
oligoglycine has been synthesised, and a substrate[228] (129) of Mur G transferase was synthesised from natural UDP-N-acetylmuramoyl pentapeptide.

3.3.2 N-Glycopeptides – The combination of enzymatic and chemical methodology has been successful in (a) glycosylated peptide T (130)[229] and (b) a glycosylated asparagine carrying a complex undecasaccharide N-glycan,[230] Neu-5-Acα(2→6)Galβ(1→4)GlcNAcβ(1→2)Manα(1→3)[Neu-5-Acα(2→6)Galβ-(1→4)GlcNAcβ(1→2)(Manα(1→6)]Manβ(1→4)GlcNAcβ(1→ 4)GlcNAc-Asn. In the former example, Fmoc-Asn(GlcNAc)-OH was incorporated using dimethylphosphinothioic mixed anhydride without protecting the hydroxy group, in order that the transglycosylation could be carried out using an endoglycosidase. A vicinally glycosylated fragment of the interleukin 8 receptor has been assembled on solid phase *via* the double insertion[231] of derivative (131). A benzylated heptasaccharide conjugate[232] assembled with a terminal azide, which can be

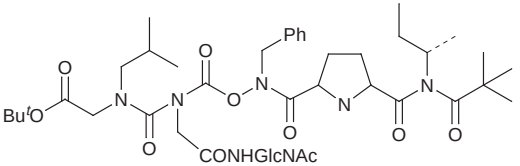
reduced to an amino group, has been acylated with Fmoc-Asp-OBu^t using DCCI/HOBt to give a useful building block for the synthesis of complex N-linked glycopeptides. A similar synthetic strategy[233] has been used with a benzylated pentasaccharide moiety for the solid phase synthesis of the glycopeptide portion of the CD52 antigen. The last stage removal of benzyl groups was carried out by 20% $Pd(OH)_2/C/H_2$ in aq. ethanol. Glycosylated peptoids[234] incorporating N-acetylgluosamine in different positions, as e.g. in (132), have been synthesised to improve the bioavailability of mimetics of the HIV-1 PR substrate sequence Leu^{165}-Ile^{169}.



(**130**)



(**131**)



(**132**)

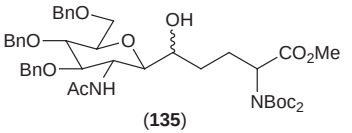
NMR and annealing calculation studies[235] on the peptidoglycan monomer from *Brevibacterium divaricatum*, GlcNAc-MurNAc-Ala-D-iGln-mDap-D-Ala-D-Ala, provide evidence for the conformational stabilising effects of the carbohydrate moieties. Peptide (133), a linear sequence based on A282-288 of hemaglutinin,[236] adopted an open and extended Asx-turn prior to glycosylation to (134), when it underwent a conformational change to a type I β-turn as analysed by 2D ^{1}H NMR. Ten new ureido sugar derivatives based on dipeptide combinations of (135) have been synthesised[237] and analysed by NMR methods.

Ac-Orn-Ile-Thr-Pro-X-Gly-Thr-Trp-Ala-NH_2

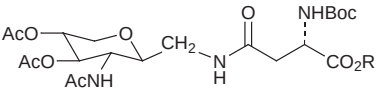
(**133**) X = Asn
(**134**) X = Asn(GlcNAc)$_2$

3.3.3 Other Linked Sugars – C-Glycosidic links have been explored via the stereoselective synthesis[238] of (135) which is a mimetic of Asn(βGlcNAc). The key link is made via a glycosyl dianion reacting with a side-chain aldehyde. The building block (136) has been incorporated[239] into (137), by initial chemical insertion followed by treatment with an *endo*-β-N-Ac-glucosoaminidase.

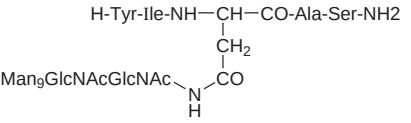
Analogue (137) shows inhibitory activity towards glycoamidases and has good enzymic resistance. Novel difluoromethylene-linked Ser-O-glycopeptide analogues have been prepared[240] stereoselectively using two complementary free radical reactions, and catalytic asymmetric hydrogenation[241] of a C-glysosyl enamide has yielded a series of carbon-linked glycopeptides. Neo-C-Glycopeptide building blocks (138) and (139) have been developed[242] to study carbohydrate-protein interactions. While *S*-β-D-glucosylated cysteine has been readily available, it is only now that the first synthesis[243] of *S*-α-D-glucosyl cysteine has been recorded. Condensation of N-phthaloyl-L-Cys-OH with 2,3,4,6-tetra-OBzl-α-glucopyranosyl trichloroimidate in the presence of BF_3 brought about a successful synthesis. Selective dimerisation[244] of cysteine residue with 2,2′-dithiopyridine does not affect sugar side-chains elsewhere on the peptide.



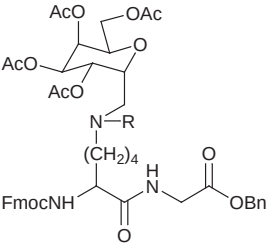
(**135**)



(**136**)



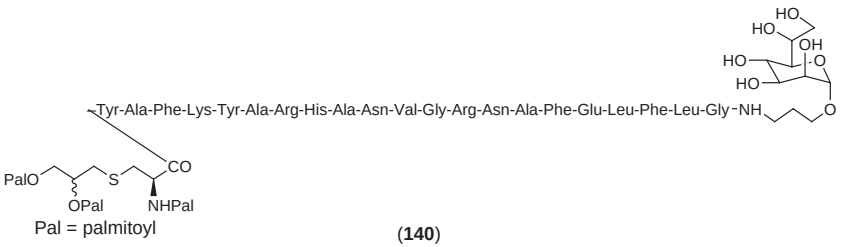
(**137**)



(**138**) R = H

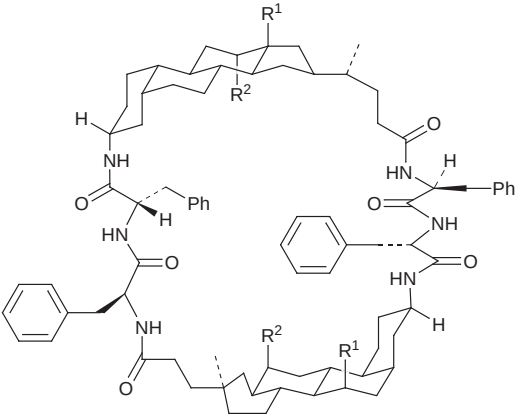
(**139**) R = AcO

3.4 Lipopeptides – The increased lipophilicity accompanying the post-translational event of incorporating farnesyl and geranylgeranyl moieties on to the side chain of cysteine often causes localisation of the resulting protein to the membrane, and may be essential for biological activity. A review[245] of methods of chemical and biochemical synthesis of prenylated peptides is therefore timely. Reaction of serine β-lactone with prenyl thiolate in the presence of sodium hydride is one recent method[246] for chemical synthesis of prenylated cysteines. Tandem mass spectrometry[247] of geranylgeranylcysteine will aid future analysis of samples. A combination molecule (140), designed for the development of a



(**140**)

synthetic vaccine against *Neisseria meningitidis* contains a carbohydrate B-epitope, a peptide T epitope and a lipopeptide adjuvant[248] and has been assembled via Fmoc-protocols on a HMPB-MBHA, including the addition of $(Pam)_3$ Cys at a late stage. This conjugate was released from the support, and then linked to an amino-sugar using EDC/HOBt coupling. N-Acylated serine and threonine containing D-glucosamine derivatives have been made[249] as mimics of lipid A disaccharide, and a series[250] of novel lipopeptides and lipomimetics have shown inhibitory power in monolayer assays. β-Loop, γ-loop and helical conformations have been found[251] in a series of cyclic peptides containing steroidal nuclei as in (141), which act as rigid spacers.

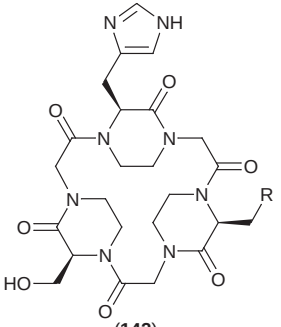


(**141**) a: $R^1 = R^2 = H$; b: $R^1 = H$, $R^2 = MeCO_2$; c: $R^1 = R^2 = MeCO_2$

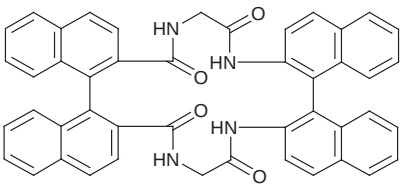
4 Miscellaneous Structures

As discussed in the introduction to the Chapter, most of the work reviewed conforms every year to a series of sub-sections. Yet annually a few ideas and structures do not conform to the routine and feel more comfortable in this miscellaneous section. Thus the 2-chlorotrityl resin has been highlighted[252] as a better medium for solid phase synthesis incorporating Fmoc-Tyr($SO_3^-Na^+$)-OH

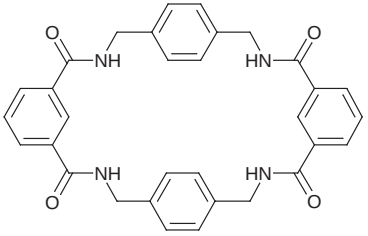
blocks, for synthesising sulfonated gastrin/cholecystokinin peptides. Cyclisations[253] using TBTU/HOBt/DIPEA or DPPA have been effective in making head to tail links when making cyclo-Gly-Ile-Ile-Gly-bradykinin, cyclo-Lys-Lys-bradykinin and cyclo des-Arg-bradykinin. Tris-bridged cyclic peptides containing Ser and His to mimic the catalytic site of lipase[254] have proved that carboxylic acid groups in position R of (142) aid catalysis. X-Ray studies[255] have revealed that the glycine residues in (143) are twisted when they are flanked between two *R*-1,1′-binaphthyls, but are planar when they are between *R*-1,1′-binaphthyl and *S*-1,1′-binaphthyl. Macrocycle (144) threaded on glycylglycyl chains [Ph_2CHCO-



(142)

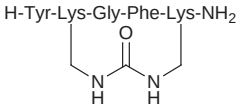


(143)



(144)

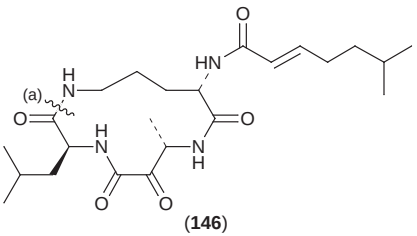
Gly-Gly-$O(CH_2)_3]_2X$, X=CH_2CH_2, $(CH_2)_{10}$ or S have been prepared[256] and shuttling rates calculated from NMR studies. The redox potential of selenocystine in unconstrained cyclic peptides such as glutaredoxin reveals[257] a preference for Se-Se-bridges rather than mixed Se-S bridges. Solution synthesis,[258] using a pentafluorophenyl ester to cyclise side chains at the tripeptide stage, has yielded a potent cyclic amide linked [Sar^1,Lys^3,Glu^5] angiotensin II (cyclised between Lys^3 and Glu^5). Cyclic constraints have been inserted[259] into the 'address' sequence of dynorphin A. Cyclo[D-Asp^6,Dap^9]Dyn A(1-13)NH_2, showed both high κ receptor affinity and potent agonist activity in the GPI assay, while cyclo[D-Asp3,Dap6]Dyn A(1-13)NH_2 showed very weak binding at all receptors. Generally cyclised versions showed decreased μ receptor affinity, while κ receptor affinity was retained or improved. Cyclic analogues of enkephalin cyclised via the side chains of Pen have produced[260] analogue H-Tyr-[D-Pen-Gly-Phe(*p*-F)-Pen]-Phe-OH with a δ versus μ receptor selectivity ratio of 45,000. A cyclo[N^{ε},$N^{\varepsilon 1}$-



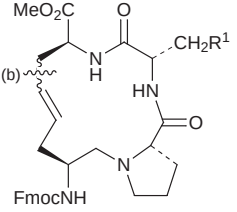
(**145**)

carbonyl-D-Lys^2,Lys^5]enkephalinamide, (145) showed a preference for μ over δ opioid receptors.[261]

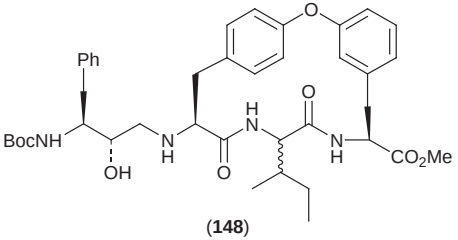
Acyl cyano-phosphorane has been used[262] in the synthesis of the α-ketoamide unit in the protease inhibitor eurystatin A (146). Macrocyclisation was carried out at point (a) in (146) using DPPA. Polymer bound substrates such as (147) cyclise rapidly[263], e.g. at point (b) using Grubbs' ruthenium metathesis catalyst $Cl_2(Pcyclohexyl_3)_2$-Ru:CHPh, when exhibiting favourable conformations. The methodology for constructing cyclic biphenyl ethers has been discussed extensively under the glycopeptide antibiotics section. It has also been shown[264] that these biaryl ethers can function as β-strand mimetics and HIV protease inhibitors. Thus inhibitory values of K_i=15 nM and 900 nM were achieved for the ether (148) and its isomer (149).



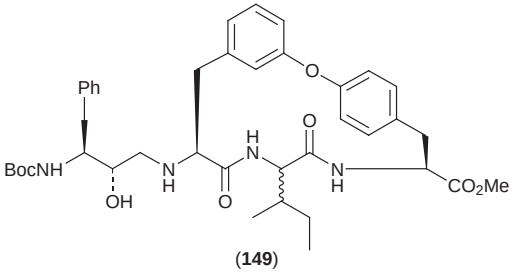
(**146**)



(**147**) R^1 = polymer (tritylpolystyrene)



(**148**)



(**149**)

References

1. J.S. Davies in "Amino-acids, Peptides and Proteins", Specialist Periodical Reports, Sr. Rep. G.T. Young, The Chemical Society, 1969 Chapter 4, p. 211.
2. C.A. Selects on Amino Acids, Peptides and Proteins, published by the American Chemical Society and Chemical Abstracts Service, Columbus, Ohio.
3. "Methods in Enzymology", Volume 289: Solid Phase Synthesis, ed. G.B. Fields, Academic Press Inc., 1997, 780 pp.
4. "Peptides 1996", Proc. of the 24th EPS, eds. R. Ramage and R. Epton, Mayflower Scientific UK, 1998, 971 pp.
5. R. Hirschmann in "Peptides 1996", Proc. of the 24th EPS, eds. R. Ramage and R. Epton, Mayflower Scientific, UK, 1998, p. 3.
6. S. Arttamangkul, B. Arbogast, D. Barofsky and J.V. Aldrich, *Lett. Pept.Sci.*, 1997, **3**, 357.
7. C. Blackburn and S.A. Kates, *Methods Enzymol.*, 1997, **289**, 175.
8. A.F. Spatola and P. Romanovskis, *Comb.Pept.Nonpept.Libr.*, 1996, 327.
9. A.G. Beck-Sickinger, *Methods.Mol.Biol.*, 1997, **73**, 61.
10. J. Eichler and R.A. Houghten, *Protein Pept.Lett.*, 1997, **4**, 157.
11. B. Hargittai and G. Barany, *Methods Enzymol.*, 1997, **289**, 198.
12. J.A. Camarero and T.W. Muir, *J.Chem.Soc.Chem.Commun.*, 1997, 1369.
13. J.P. Tam and Y-A. Lu, *Tetrahedron Lett.,* 1997, **38**, 5599.
14. L. Zhang and J.P. Tam, *Tetrahedron Lett.,* 1997, **38**, 4375.
15. L. Zhang and J.P. Tam, *J.Am.Chem.Soc.,* 1997, **119**, 2363.
16. R. Liu, P.Zhang, T. Gan and J.M. Cook, *J.Org.Chem.*, 1997, **62**, 7447.
17. M. Varoglu, T.H. Corbett, F.A. Valeriote and P. Crews, *J.Org.Chem.*, 1997, **62**, 7078.
18. T. Gan and J.M. Cook, *Tetrahedron Lett.,* 1997, **38**, 1301.
19. Y. Yonezawa, K. Shimizu, M. Uchiyama, N. Kagawa and C.G. Shin, *Heterocycles*, 1997, **45**, 1151.
20. H. Wang and H. Ganesan, *Tetrahedron Lett.,* 1997, **38**, 4327.
21. X. Fu, L-M. Zeng, J-Y. Su and M. Pais, *J.Nat.Prod.*, 1997, **60**, 695.
22. R. Hulst, Q.B. Broxterman, J. Kamphuis, F. Formaggio, M. Crisma, C. Toniolo and R.M. Kellogg, *Tetrahedron: Asymmetry*, 1997, **8**, 1987.
23. J. Thoen and M.A. Lipton, *Tetrahedron: Asymmetry*, 1997, **8**, 3947.
24. C.R. Noe, A. Weigand, S. Pirker and P. Liepert, *Monatsh.Chem.*, 1997, **128**, 301.
25. F. Guenoun, T. Zair, F. Lamaty, M. Pierrot, R. Lazaro and P. Viallefont, *Tetrahedron Lett.,* 1997, **38**, 1563
26. T.X.H. Le, J.C. Bussolari and W.V. Murray, *Tetrahedron Lett.,* 1997, **38**, 3849.
27. M. Falorni, G. Giacomelli, F. Nieddu and M. Taddei, *Tetrahedron Lett.,* 1997, **38**, 4663.
28. P.W. Baures, W.H. Ojala, W.J. Costain, M.C. Ott, A. Pradhan, W.B. Gleason, R.K. Mishra and R.L. Johnson, *J.Med.Chem.*, 1997, **40**, 3594.
29. M.E. Pfeifer, A. Linden and J.A. Robinson, *Helv.Chim.Acta*, 1997, **80**, 1513.
30. A.K. Szardenings, T.S. Burkoth, H.H. Lu, D.W. Tien and D.A. Campbell, *Tetrahedron*, 1997, **53**, 6573.
31. S.N. Danthi and R.A. Hill, *J.Heterocycl.Chem.*, 1997, **34**, 835.
32. A.R. Tapia-Benavides, H. Tlahuext and R. Contreras, *Heterocycles*, 1997, **45**, 1679.
33. E. Kasafirek, J. Moural, J. Vinsova, A. Sturc and J. Taimr, *Coll.Czech.Chem.-Commun.*, 1997, **62**, 941.
34. N. Walchshofer, M.E. Sarciron, F. Garnier, P. Delatour, A.F. Petavy and J. Paris, *Amino Acids*, 1997, **12**, 41.

35. M. Botta, A. Crescenza, W. Magara and F. Covelli, *Tetrahedron Lett.,* 1997, **38**, 2775.
36. P. Speers, C. Chan, R. Wilcock and K.T. Douglas, *Amino Acids*, 1997, **12**, 49.
37. P.V. Kostetsky, S.F. Arkhipova, I.V. Artem'ev, I.L. Rodinov, L.N. Rodinova and V.T. Ivanov, *Bioorg.Khim.*, 1997, **23**, 531.
38. C. Flouzat, F. Marguerite, F. Croizet, M. Percebois, A. Monteil and M. Combourieu, *Tetrahedron Lett.,* 1997, **38**, 1191.
39. J.M. Travins and F.A. Etzkorn, *J.Org.Chem.*, 1997, **62**, 8387.
40. S. Carret, M. Baudy-Floc'h, A. Robert and P. Le Griel, *Chem.Commun.*, 1997, 1441.
41. W.D. Clark, T. Corbett, F. Valeriote and P. Crews, *J.Am.Chem.Soc.,* 1997, **119**, 9285.
42. M.A. Sow, A. Molla, F. Lamaty and R. Lazaro, *Lett. Pept.Sci.*, 1997, **4**, 455.
43. C.N. Eid, T.I. Nicas, D.L. Mullen, R.J. Loneharich and J.W. Paschal, *Bioorg.Med.-Chem.Lett.*, 1997, **7**, 2087.
44. U. Schmidt and J. Langner, *J.Pept.Res.*, 1997, **49**, 67.
45. K. Akaji and Y. Kiso, *Tetrahedron Lett.,* 1997, **38**, 5185.
46. M. Tamaki, S. Komiya, M. Yabu, E. Watanabe, S. Akabori and I. Muramatsu, *J.Chem.Soc.Perkin Trans. 1*, 1997, 3497.
47. R. Haubner, D. Finsinger and H. Kessler, *Angew.Chem.Int.Ed.Engl.*, 1997, **36**, 1374.
48. J. Wermuth, S.L. Goodman, A. Jonczyk and H. Kessler, *J.Am.Chem.Soc.,* 1997, **119**, 1328.
49. M. Koppitz, M. Huenges, R. Gratias, H. Kessler, S.L. Goodman and A. Jonczyk, *Helv.Chim.Acta*, 1997, **80**, 1280.
50. J.S. Davies, J. Howe, J. Jayatilake and T. Riley, *Lett. Pept.Sci.*, 1997, **4**, 441.
51. A. Muller, F. Schumann, M. Koksch and N. Sewald, *Lett. Pept.Sci.*, 1997, **4,** 275.
52. J.P. Dirlam, A.M. Belton, N.C. Birsner, R.R. Brooks, S.-P. Chang, R.Y. Chandrasekaran, J. Clancy, B.J. Cronin, B.P. Dirlam, S.M. Finegan, S.A. Froshauer, A.E. Girard, S.F. Hayashi, R.J. Howe, J.C. Kane, B.J. Kamicker, S.A. Kaufman, N.L. Kolosko, M.A. LeMay, R.G. Linde II, J.P. Lyssikatos, C.P. MacLelland, T.V. Magee, M.A. Massa, S.A. Miller, M.L. Minich, D.A. Perry, J.S. Petitpas, C.P. Reese, S.B. Seibel, W.-G. Su, K.T. Sweeney, D.A. Whipple, and B.V. Yang, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 1139.
53. J.P. Lyssikatos, S.-P. Chang, J.P. Dirlam, S.M. Finegan, A.E. Girard, S.F. Hayashi, D.P. Larson, A.S. Lee, R.G. Ginde, C.P. MacLelland, J.W. Petitpas, S.B. Siebel and C.B. Vu, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 1145.
54. R.C. Linde II, N.C. Birsner, R.Y. Chandrasekaran, J. Clancy, R.J. Howe, J.P. Lyssikatos, C.P. MacLelland, T.V. Magee, J.W. Peptitpas, J.P. Rainville, W.-G. Su, C.B. Su and D.A. Whipple, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 1149.
55. H.J. Shin, H. Matsuda, M. Murakami and K. Yamaguchi, *J.Nat.Prod.*, 1997, **60**, 139.
56. I.R. Marsh, M. Bradley and S.J. Teague, *J.Org.Chem.*, 1997, **62**, 6199.
57. K. Fujii, K. Sivonen, K. Adachi, K. Noguchi, H. Sane, K. Hinayama, M. Suzuki and K-I Harada, *Tetrahedron Lett.,* 1997, **38**, 5525.
58. E.W. Schmidt, M.K. Harper and J.D. Faulkner, *J.Nat.Prod.*, 1997, **60**, 779.
59. Y.S. Yun, H. Morita, K. Takeya and H. Itokawa, *J.Nat.Prod.*, 1997, **60**, 216.
60. H. Morita, Y.S. Yun, K. Takeya and H. Itokawa, *Bioorg.Med.Chem.*, 1997, **5**, 2063.
61. H. Morita, Y.S. Yun, K. Takeya, H. Itokawa and O. Shirota, *Bioorg.Med.Chem. Lett.*, 1997, **5**, 631.
62. P.A. McDonnell, G.W. Caldwell, G.C. Leo, B.L. Podlogar and B.E. Maryanoff, *Biopolymers*, 1997, **41**, 349.

63. H.M.M. Bastianns, J.L. van der Baan and H.C.J. Ottenheijm, *J.Org.Chem.*, 1997, **62**, 3880.
64. C. Paulitz and W. Steglich, *J.Org.Chem.*, 1997, **62**, 8474.
65. K.L. Webster, T.J. Rutherford and D. Gani, *Tetrahedron Lett.,* 1997, **38**, 5713.
66. I. Vlattas, J. Dellureficio, R. Dunn, I.I. Sytwu and J. Stanton, *Tetrahedron Lett.,* 1997, **38**, 7321.
67. W.D.F. Meutermans, P.F. Alewood, G.T. Bourne, B. Hawkins and M.L. Smythe, *Lett. Pept.Sci.*, 1997, **4**, 79.
68. J.J. Wen and A.F. Spatola, *J.Pept.Res.*, 1997, **49**, 3.
69. H. Morita, T. Kayashita, A. Uchida, K. Takeya and H. Itokawa, *J.Nat.Prod.*, 1997, **60**, 212.
70. A. Bigot, R. Beugelmans and J. Zhu, *Tetrahedron*, 1997, **53**, 10753.
71. D.L. Boger, J. Zhou, R.M. Borzilleri, S. Nukii and S.L. Castle, *J.Org.Chem.*, 1997, **62**, 2054.
72. Y. Hitotsuyanagi, Y. Anazawa, T. Yamagishi, K. Samata, T. Ichihara, K. Nanaumi, N. Okado, S. Nakaike, M. Mizumura, K. Takeya and H. Itokawa, *Bioorg.Med.-Chem. Lett.*, 1886, **7**, 3125,
73. D. Lim and K. Burgess, *J.Am.Chem.Soc.,* 1997, **119**, 9632.
74. P. Cuniasse, I. Raynal, A. Yiothakis and V. Dive, *J.Am.Chem.Soc.,* 1997, **119**, 5239.
75. O. Mechnich, G. Hessler, H. Kessler, M. Bernd and B. Kutscher, *Helv.Chim.Acta*, 1997, **80**, 1338.
76. L.S. Bonnington, J. Tanaka, T. Higa, J. Kimura, Y. Yoshimura, Y. Nakao, W.Y. Yoshida and P.J. Scheuer, *J.Org.Chem.*, 1997, **62**, 7765.
77. H. Morita, T. Kayashita, K. Takeya, H. Itokawa and M. Shiro, *Tetrahedron*, 1997, **53**, 1607.
78. H. Morita, Y.S. Yun, K. Takeya and H. Itokawa, *Chem.Pharm.Bull.*, 1997, **45**, 883.
79. Y.-R. Zhao, J. Zhou, X.-K. Wang, H.-M. Wu, X.-L. Huang and C. Zou, *Phytochemistry,* 1997, **46**, 709.
80. L. Chao-Ming, T. Ning-Hua, M. Qing, Z. Hiu-Lan and H. Xiao-Jiang, *Phytochemistry,* 1997, **45**, 521.
81. H. Morita, K. Takeya and H. Itokawa, *Phytochemistry,* 1997, **45**, 841.
82. C. Auvin-Guette, C. Baraguey, A. Blond, J.-L. Pousset and B. Bodo, *J.Nat.Prod.*, 1997, **60**, 1155.
83. G. Oesapay, L. Prokai, H.-S. Kim, K.F. Medzihradszky, D.H. Coy, G. Liapakis, T. Reisine, G. Melacini, Q. Zhu, S.H.-H. Wang, R.H. Mattern and M. Goodman, *J.Med.Chem.*, 1997, **40**, 2241.
84. J.P. Lewis, N.H. Pawley and O.F. Sankey, *J.Phys.Chem.B.*, 1997, **101**, 10576.
85. A. Karlstrom and A. Unden, *Biopolymers*, 1997, **41**, 1.
86. T. Ishizu and S. Noguchi, *Chem.Pharm.Bull.*, 1997, **45**, 1202.
87. M. Cebrat, M. Lisowski, I.Z. Siemion, M. Zimecki and Z. Wieczorek, *J.Pept.Res.*, 1997, **49**, 415.
88. M. Cebrat, M. Lisowski, I.Z. Siemion and Z. Wieczorek, *Pol.J.Chem.*, 1997, **71**, 1401
89. H. Morita, A. Shishido, T. Matsumoto, K. Takeya, H. Itokawa, T. Hirano and K. Oka, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 1269.
90. C. Auvin, C. Baraguey, A. Blond, F. Lezenven, J.-L. Pousset and B. Bodo, *Tetrahedron Lett.,* 1997, **38**, 2845.
91. H. Morita, A. Shishido, T. Kayashita, K. Takeya and H. Itokawa, *J.Nat.Prod.*, 1997, **60**, 404.
92. H. Morita, A. Gonda, K. Takeya, H. Itokawa and O. Shirota, *Chem.Pharm.Bull.*, 1997, **45**, 161.

93. T. Suzawa, K. Shibata, T. Tanaka, Y. Matsuda and M. Yamasaki, *Bioorg.Med.-Chem.Lett.*, 1997, **7**, 1715.
94. H. Morita, A. Gonda, K. Takeya, H. Itokawa and Y. Iitaka, *Tetrahedron*, 1997, **53**, 1617.
95. D.L. Herald, G.L. Cascarano, G.R. Pettit and J.K. Srirangam, *J.Am.Chem.Soc.,* 1997, **119**, 6962.
96. F. Rossi, M. Saviano, P. Di Talia, B. Di Blasio, C. Pedone, G. Zanotti, M. Mosca, G. Saviano, T. Tancredi, K. Ziegler and E. Benedetti, *Biopolymers*, 1997, **40**, 465.
97. H. Mihara, J. Hayashida, H. Hasegawa, H.I. Ogawa, T. Fujimoto and N. Nishino, *J.Chem.Soc.Perkin Trans. 2*, 1997, 517.
98. M. Tamaki, S. Komiya, S. Akabori and I. Muramatsu, *Bull.Chem.Soc., Japan*, 1997, **70**, 899.
99. D. Andreu, S. Ruiz, C. Carreno, J. Alsina, F. Albericio, M.A. Jimenéz, N. de La Figuera, R. Herranz, M.T. Garcia-Lopez and R. Gonzalez-Muniz, *J.Am.Chem.Soc.,* 1997, **119**, 10579.
100. I. Bonnard, M. Rolland, C. Francisco and B. Banaigs, *Lett. Pept.Sci.*, 1997, **4**, 289.
101. K. Ishida, H. Matsuda, M. Murakami and K. Yamaguchi, *J.Nat.Prod.*, 1997, **60**, 724.
102. S.Y. Ko and R.M. Wenger, *Helv.Chim.Acta*, 1997, **80**, 695.
103. V. Havlicek, A. Jegorov, P. Sedmera and M. Ryska, *Chem.Listy*, 1997, **91**, 2.
104. F. Magni, I. Fermo, C. Arcelloni, L. Arnoldi, M. Del Puppo and R. Paroni, *J.Pept.Res.*, 1997, **49**, 191.
105. C. Papageorgiou, J. Kallen, J. France and R. French, *Bioorg.Med.Chem.Lett.*, 1997, **5**, 187.
106. T.-A. Tran, R.-H. Mattern, Q. Zhu and M. Goodman, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 997.
107. H. Morita, A. Gonda, K. Takeya, H. Itokawa, T. Hirano, K. Oka and O. Shirota, *Tetrahedron*, 1997, **53**, 7469.
108. M. Tamaki, S. Komiya, S. Akabori and I. Muramatsu, *J.Chem.Soc.Perkin Trans. 1*, 1997, 2045.
109. A.A. Van Dijk, L.L. Van Wijk, A. Van Vliet, P. Haris, E. Van Swieten, G.I. Tesser and G.T. Robillard, *Protein Sci.*, 1997, **6**, 637.
110. H. Sone, H. Kigoshi and K. Yamada, *Tetrahedron*, 1997, **53**, 8149.
111. G.R. Pettit, J.P. Xu, M.S. Williams, F. Hogan, J.M. Schmidt and R.L. Cerny, *Bioorg.Med.Chem.Lett.*, 1997, **7**, 955.
112. A.D. Patel, A.J. Freyer, L. Killmer, C. Chambers-Myers and R.K. Johnson, *Nat.Prod.Lett.*, 1997, **9**, 181.
113. L. Chill, Y. Kashman and M. Schleyer, *Tetrahedron*, 1997, **53**, 16147.
114. M.A. Ciufolini and Y.C. Shen, *J.Org.Chem.*, 1997, **62**, 3804.
115. S. Lociuro, P. Tavecchia, E. Marzorati, P. Landini, B.P. Goldstein, M. Denaro and R. Ciabatti, *J.Antibiotics*, 1997, **50**, 344.
116. G.R. Pettit, *Prog.Chem.Org.Nat.Prod.*, 1997, **70**, 1.
117. K. Yamada and H. Kigoshi, *Bull.Chem.Soc., Jpn.*, 1997, **70**, 1479.
118. G.R. Pettit, J-p. Xu, F. Hogan, M.D. Williams, D.L. Doubek, J.M. Schmidt, R.L. Cerny and M.R. Boyd, *J.Nat.Prod.*, 1997, **60**, 752.
119. J. Gerard, R. Lloyd, T. Barsby, P. Haden, M.T. Kelly and R.J. Andersen, *J.Nat.Prod.*, 1997, **60**, 223.
120. T. Okino, S. Qi, H. Matsuda, M. Murakami and K. Yamaguchi, *J.Nat.Prod.*, 1997, **60**, 158.
121. K. Ishida, H. Matsuda, M. Murakami and K. Yamaguchi, *J.Nat.Prod.*, 1997, **60**, 184.

122. M. Murakami, S. Kodani, K. Ishida, H. Matsuda and K. Yamaguchi, *Tetrahedron Lett.,* 1997, **38**, 3035.
123. F. Romero, F. Espliego, J.P. Baz, T.G. De Quesada, D. Gravalos, F. De la Calle and J.L. Fernandez Puertes, *J.Antibiotics*, 1997, **50**, 734; J.P. Baz, L.M. Canedo, J.L.C. Puentes and M.V.S. Elipe, *ibid*., 1997, **50**, 738.
124. Y. Kajimura and M. Kaneda, *J.Antibiotics*, 1997, **50**, 220.
125. C. Kempter, D. Kaiser, S. Haag, G. Nicholson, V. Gnau, T. Walk, K.H. Gierling, H. Decker, H. Zähner, G. Jung and J.W. Metzger, *Angew.Chem.Int.Ed.Engl.*, 1997, **36**, 498.
126. G.V. Subbaraju, T. Golakoti, G.M.L. Petterson and R.E. Moore, *J.Nat.Prod.*, 1997, **60**, 302.
127. K.M. Gardinier and J.W. Leahy, *J.Org.Chem.*, 1997, **62**, 7098.
128. D.E. Ward, R. Lazny and M.S.C. Pedras, *Tetrahedron Lett.,* 1997, **38**, 339.
129. S. Gangwar, G.M. Pauletti, T.J. Siahaan, V.J. Stella and R.T. Borchardt, *J.Am.-Chem.Soc.,* 1997, **62**, 1356.
130. B. Wang, S. Gangwar, G.M. Pauletti, T.J. Siahaan and R.T. Borchardt, *J.Org.Chem.*, 1997, **62**, 1363.
131. G. Jou, I. Gonzalez, F. Albericio, P. Lloyd-Williams and E. Giralt, *J.Org.Chem.*, 1997, **62**, 354.
132. J.M. Ramanjulu, X. Ding, M.M. Joullie and W-R. Li, *J.Org.Chem.*, 1997, **62**, 4961.
133. J.M. Ramanjulu, M.M. Joulli and W-R. Li, *Synth.Commun.*, 1997, **27**, 3259.
134. R.B. Bates, K.G. Brusoe, J.J. Burns, S. Caldera, C. Sriyani, W. Cui, S. Gangwar, M.R. Gramme, K.J. McClure, G.P. Rouen, H. Schadow, C.C. Stessman, S.T. Taylor, V.H. Vu, G.V. Yarick, J. Zhang, G.R. Pettit and R. Bontems, *J.Am. Chem.Soc.,* 1997, **119**, 2111.
135. T. Shioiri, T. Imaeda and Y. Hamada, *Heterocycles*, 1997, **46**, 421.
136. S.L. Belagali and P.J. Kocienski, *Indian J.Heterocyclic Chem.*, 1997, **6**, 211.
137. K.J. Hale and J. Cai, *Chem.Commun.*, 1997, 2319.
138. B.H. Lee, *Tetrahedron Lett.,* 1997, **38**, 757.
139. U. Schmidt, K. Neumann, A. Schumacher and S. Weinbrenner, *Angew.Chem.Int. Ed.Engl.*, 1997, **36**, 1110.
140. T. Mutou, K. Suenaga, T. Fujita, T. Itoh, N. Takada, K. Hayamizu, H. Kigoshi and K. Yamada, *Synlett*, 1997, 199.
141. T.Q. Dinh, C.D. Smith and R.W. Armstrong, *J.Org.Chem.*, 1997, **62**, 790.
142. T.Q. Dinh, X. Du, C.D. Smith and R.W. Armstrong, *J.Org.Chem.*, 1997, **62**, 6773.
143. F. Cavelier, R. Jacquier, J.L. Mercadier, J. Verducci, M. Traris and A. Vey, *J.Pept.Res.*, 1997, **50**, 94.
144. A.M. Sefler, M. Kozlowski, T. Guo and P.A. Bartlett, *J.Org.Chem.*, 1997, **62**, 93.
145. M.A. Ciufolini and N. Xi, *J.Org.Chem.*, 1997, **62**, 2320.
146. J.M. Villalgordo and H. Heimgartner, *Helv.Chim.Acta*, 1997, **80**, 748.
147. M. Nagarajan, V. Satish Kumar and B.V. Rao, *Tetrahedron Lett.,* 1997, **38**, 5835.
148. V.Z. Pletnev, V.T. Ivanov, D.A. Langs, B.M. Burkhart and W.L. Duax, *Biopolymers*, 1997, **42**, 645.
149. V.Z. Pletnev, S.N. Ruzeinikov, I.N. Tsigannik, V.T. Ivanov, S.V. Pletnev, D.A. Langs and W.L. Duax, *Biopolymers*, 1997, **42**, 651.
150. H. Rapaport, I. Kuzmenko, K. Kjaer, P. Howes, W. Bowman, J. Als-Nielsen, L. Leiserowitz and M. Lahav, *J.Am.Chem.Soc.,* 1997, **119**, 11211.
151. D. Ranganathan, V. Haridas, K.P. Madhusudanan, R. Roy, R. Nagaraj and G.B. John, *J.Am.Chem.Soc.,* 1997, **119**, 11578.
152. J.W. Perich, *Methods Enzymol.*, 1997, **289**, 245.

153. H. Fretz, *Lett. Pept.Sci.*, 1997, **4**, 171.
154. H. Fretz, *Lett. Pept.Sci.*, 1997, **3**, 343.
155. A. Paquet, B. Blackwell, M. Johns and J. Nikiforuk, *J.Pept.Res.*, 1997, **50**, 262.
156. T. Wakamiya, R. Togashi, T. Nishida, K. Saruta, J.I. Yasuoka, S. S. Kusumoto, S. Aimoto, Y.K. Kumagaye, K. Nakajima and K. Nagata, *Bioorg.Med.Chem.*, 1997, **5**, 135.
157. E-K. Kim, H. Choi and E-S. Lee, *Yakhak Hoechi*, 1997, **41**, 588.
158. A. Bernhardt, M. Drewello and M. Schutkowski, *J.Pept.Res.*, 1997, **50**, 143.
159. Q. Xu, E.A. Ottinger, N.A. Sole and G. Barany, *Lett. Pept.Sci.*, 1997, **3**, 333.
160. A. Tholey and R. Hoffman, *J.Pept.Sci.*, 1997, **3**, 186.
161. M.N. Qabar, J. Urban and M. Kahn, *Tetrahedron*, 1997, **53**, 11171.
162. W-Q. Liu, B.P. Roques and C. Garbay, *Tetrahedron Lett.,* 1997, **35**, 1389.
163. A.M. Kawamoto and M.M. Campbell, *J.Fluorine Chem.*, 1997, **81**, 181.
164. K.F. Medzihradszky, N.J. Phillipps, L. Senderowicz, P. Wang and C.W. Turck, *Protein Sci.*, 1997, **6**, 1405.
165. R. Hofmann, T. Hofmann, A. Tholey, A.C. Schultze and H.R. Kalbitzer, *J.Pept.Res.*, 1997, **49**, 163.
166. M.A. Akamatsu, P.P. Roller, L. Chen, Z-Y. Zhang, B. Ye and T.R. Burke, *Bioorg.Med.Chem.*, 1997, **5**, 157
167. D. Sebastian and H. Waldmann, *Tetrahedron Lett.,* 1997, **38**, 2927.
168. A. Malabarba, T.I. Nicas and R.C. Thompson, *Med.Res.Rev.*, 1997, **17**, 69.
169. J. Zhu, *Synlett*, 1997, 133.
170. D.A. Evans, J.C. Barrow, P.S. Watson, A.M. Ratz, C.J. Dinsmore, D.A. Evrard, K.M. DeVries, J.A. Ellman, S.D. Rychnovsky and J. Lacour, *J.Am.Chem.Soc.,* 1997, **119**, 3419.
171. D.A. Evans, C.J. Dinsmore, A.M. Ratz, D.A. Evrard and J.C. Barrow, *J.Am. Chem.Soc.,* 1997, **119**, 3417.
172. D.A. Evans, C.J. Dinsmore and A.M. Ratz, *Tetrahedron Lett.,* 1997, **38**, 3189.
173. D.L. Boger, O. Loiseleur, S.L. Castle, R.T. Beresis and J.H. Wu, *Bioorg.Med.Chem. Lett.*, 1997, **7**, 3199.
174. C. Vergne, M. Bois-Choussy, R. Beugelmans and J. Zhu, *Tetrahedron Lett.,* 1997, **38**, 1403.
175. D.L. Boger, R.M. Borzilleri, S. Nukui and R.T. Beresis, *J.Org.Chem.*, 1997, **62**, 4721.
176. K. Burgess, D. Lim, M. Bois-Choussy and J. Zhu, *Tetrahedron Lett.,* 1997, **38**, 3345.
177. M. Bois-Choussy, C. Vergne, L. Neuville, R. Beugelmans and J. Zhu, *Tetrahedron Lett.,* 1997, **38**, 5795.
178. A.V.R. Rao, M.K. Gurjar, P. Lakshmipathi, M.M. Reddy, M. Nagarajan, S. Pal, B.V.N.B.S. Sarma and N.K. Tripathy, *Tetrahedron Lett.,* 1997, **38**, 7433.
179. R. Beugelmans, E.G. Zamora and G. Roussi, *Tetrahedron Lett.,* 1997, **38**, 8189.
180. G. Roussi, E.G. Zamora, A-C. Carbonnelle and R. Beugelmans, *Tetrahedron Lett.,* 1997, **38**, 4405.
181. G. Roussi, E.G. Zamora, A-C. Carbonnelle and R. Beugelmans, *Tetrahedron Lett.,* 1997, **38**, 4401.
182. J.W. Janetka and D.H. Rich, *J.Am.Chem.Soc.,* 1997, **119**, 6488.
183. K.C. Nicolaou, C.N.C. Boddy, S. Natarajan, T-Y. Yue, H. Li, S. Braese and J.M. Ramanjulu, *J.Am.Chem.Soc.,* 1997, **119**, 3421.
184. K.C. Nicolaou, J.M. Ramanjulu, S. Natarajan, S. Brase, H. Li, C.N.C. Boddy and F. Rubsam, *Chem.Commun.*, 1997, 1899.
185. K.C. Nicolaou, X-J. Chu, J.M. Ramanjulu, S. Natarajan, S. Bräse, F. Rübsam and C.N.C. Boddy, *Angew.Chem.Int.Ed.Engl.*, 1997, **36**, 1539.

186. A.J. Pearson, P. Zhang and G. Bignan, *J. Org. Chem.*, 1997, **62**, 4536.
187. M.K. Gurjar and N.K. Tripathy, *Tetrahedron Lett.,* 1997, **38**, 2163.
188. K. Burgess, D. Lim, M. Bois-Choussy and J. Zhu, *Tetrahedron Lett.,* 1997, **38**, 3345.
189. P.J. Loll, A.E. Bevivino, B.D. Korty and P.H. Axelsen, *J. Am. Chem. Soc.,* 1997, **119**, 1516.
190. G.J. Sharman and D.H. Williams, *Chem. Commun.*, 1997, 723.
191. S.G. Grdadolnik and D.F. Mierke, *Biopolymers*, 1997, **42**, 627.
192. A.C. Try, G.J. Sharman, R.J. Dancer, B. Bardsley, R.M.H. Entress and D.H. Williams, *J. Chem. Soc. Perkin Trans. 1*, 1997, 2911.
193. J. Rao and G.M. Whitesides, *J. Am. Chem. Soc.,* 1997, **119**, 10286.
194. J. Rao, I.J. Colton and G.M. Whitesides, *J. Am. Chem. Soc.,* 1997, **119**, 9336.
195. S.G. Ang and Y.K. Peh, *Aus. J. Chem.*, 1997, **50**, 31.
196. H. Kunz in "*Prep. Carbohydr. Chem.*", ed. S. Hanessian, Dekker, 1997, p. 265.
197. D.G. Large and I.J. Bradshaw in "*Glycopept. Relat. Compd.*", eds. D.G. Large and C.D. Warren, Dekker, 1997, p.295.
198. G. Arsequell and G. Valencia, *Tetrahedron: Asymmetry*, 1997., 8, 2839.
199. S. Danishefsky and J.Y. Roberge in "*Glycopept. Relat. Compd.*", eds. D.G. Large and C.D. Warren, Dekker, 1997, p.245.
200. H. Kunz and M. Schultz in "*Glycopept. Relat. Compd.*", eds. D.G. Large and C.D. Warren, Dekker, 1997, p. 23.
201. J.W. Perich, *Methods Enzymol.*, (*Solid Phase Synthesis*), 1997, **289**, 221.
202. M. Meldal, *Curr. Opin. Chem. Biol.*, 1997, **1**, 552.
203. Y. Ichikawa, in "*Glycopept. Relat. Compd.*", eds. D.G. Large and C.D. Warren, Dekker, 1997, p. 79.
204. T. Kappes and H. Waldmann, *Liebigs Ann./Recueil*, 1997, 803.
205. C.W. Sutton and J.A. O'Neill, *Methods. Mol. Biol.* (*Totowa, N.J.*), 1997, 64.
206. R.R. Koganty, M.A. Reddish and B.M. Longenecker, in "*Glycopept. Relat. Compd.*", eds. D.G. Large and C.D. Warren, Dekker, 1997, p. 707.
207. J. Kihlberg and M. Elofsson, *Curr. Med. Chem.*, 1997, **4**, 85.
208. H.J. Shin, H. Matsuda, M. Murakami and K. Yamaguchi, *J. Org. Chem.*, 1997, **62**, 1810.
209. H. Paulsen, A. Schleyer, N. Mathieux, M. Meldal and K. Boch, *J. Chem. Soc. Perkin Trans. 1*, 1997, 281.
210. E.C. Rodriguez, K.A. Winans, D.S. King and C.R. Bertozzi, *J. Am. Chem. Soc*., 1997, **119**, 9905.
211. O. Seitz and C-H. Wong, *J. Am. Chem. Soc., 1997,* **119**, 8766.
212. N. Mathieux, H. Paulsen, M. Meldal and K. Bock, *J. Chem. Soc. Perkin Trans. 1*, 1997, 2359.
213. U.K. Sahha and R.R. Schmidt, *J. Chem. Soc. Perkin Trans. 1*, 1997, 1855.
214. H. Vegad, C.J. Gray, P.J. Somers and A.S. Dutta, *J. Chem. Soc. Perkin Trans. 1*, 1997, 1429.
215. U.K. Saha, L.S. Griffith, J. Rademann, A. Geyer and R.R. Schmidt, *Carbohydr. Res.*, 1997, **304**, 21.
216. E. Harth-Fritschy and D. Cantacuzene, *J. Pept. Res.*, 1997, **50**, 415.
217. B. Liebe and H. Kunz, *Helv. Chim. Acta,* 1997, **80**, 1473.
218. B. Liebe and H. Kunz, *Angew. Chem. Int. Ed. Engl.*, 1997, **36**, 618.
219. M. Elofsson, L.A. Salvador and J. Kihlberg, *Tetrahedron*, 1997, **3**, 369.
220. S. Bay, R. LoMan, E. Osinaga, H. Nakada, C. Leclerc and D. Cantacuzene, *J. Pept. Res.*, 1997, **49**, 620.

221. E. Meinjohanns, M. Meldal, T. Jensen, O. Werdelin, L. Galli-Stampino, S. Mouritsen and K. Bock, *J. Chem. Soc. Perkin Trans. 1*, 1997, 871.
222. H. Franzyk, M.K. Christensen, B.M. Joergensen, M. Meldal, H. Cordes, S. Mouritsen and K. Bock, *Bioorg. Med. Chem*, 1997, **5**, 21.
223. A. Schleyer, M. Meldal, M. Renil, H. Paulsen and K. Bock, *Angew. Chem. Int. Ed. Engl.*, 1997, **36**, 1976.
224. T. Pohl and H. Waldmann, *J. Am. Chem. Soc.*, 1997, **119**, 6702.
225. T. Kanemitsu, Y. Ogihara and T. Takeda, *Chem. Pharm. Bull.*, 1997, **45**, 643.
226. G. Klich, H. Paulsen, B. Meyer, M. Meldal and K. Bock, *Carbohydr. Res.*, 1997, **299**, 33.
227. J.M. Kim and R. Roy, *Carbohydr. Res.*, 1997, **298**, 173.
228. G. Auger, M. Crouvoisier, M. Caroff, J. van Heijenoort and D. Blanot, *Lett. Pept. Sci.*, 1997, **4**, 371.
229. K. Yamamoto, K. Fujimori, K. Haneda, M. Mizuno, T. Inazu and H. Kumagai, *Carbohydr. Res*., 1997, **305**, 415
230. C. Unverzagt, *Carbohydr. Res.*, 1997, **305**, 423.
231. Z.W. Guo, Y. Nakahara, Y. Nakahara and T. Ogawa, *Carbohydr. Res.*, 1997, **303**, 373.
232. Z.W. Guo, Y. Nakahara and T. Ogawa, *Tetrahedron Lett.*, 1997, **38**, 4799.
233. Z.W. Guo, Y. Nakahara, Y. Nakahara and T. Ogawa, *Angew. Chem. Int. Ed. Engl.*, 1997, **36**, 1464.
234. U.K. Saha and R. Roy, *Tetrahedron Lett.*, 1997, **38**, 7697.
235. H. Matter, L. Szilágyi, P. Forgó, Z. Marinic and K. Klaic, *J. Am. Chem. Soc.*, 1997, **119**, 2212.
236. S.E. O'Connor and B. Imperiali, *J. Am. Chem. Soc.*, 1997, **119**, 2295.
237. A. Temeriusz, B. Piekarska-Bartoszewicz and I. Wawer, *Carbohydr. Res.*, 1997, **304**, 335.
238. F. Burkhart, M. Hoffmann and H. Kessler, *Angew. Chem. Int. Ed. Engl.*, 1997, **36**, 1191.
239. L-X. Wang, M. Tang, T. Suzuki, K. Kitajima, Y. Inoue, S. Inoue, J-Q. Fan and Y.C. Lee, *J. Am. Chem. Soc.*, 1997, **119**, 11137.
240. T.F. Herpin, W.B. Motherwell and J.M. Weibel, *Chem. Commun.*, 1997, 923.
241. S.D. Debenham, J.S. Debenham, M.J. Burk and E.J. Toone, *J. Am. Chem. Soc.*, 1997, **119**, 9897.
242. P. Arya, S. Dion and G.K.H. Shimizu, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1537.
243. L. Kasbeck and H. Kessler, *Liebigs Ann./Recueil*, 1997, 165.
244. R. Hoffmann and L. Otvos, *Lett. Pept. Sci.*, 1997, **3**, 371.
245. F.R. Naider and J.M. Becker, *Biopolymers*, 1997, **43**, 3.
246. W.W. Epstein and Z. Wang, *Chem. Commun.*, 1997, 863.
247. S.M. Pyle and A.B. Marcus, *J. Mass Spectrom.*, 1997, **32**, 899
248. F. Reichel and P.R. Ashton, *Chem. Commun.*, 1997, 2087.
249. K. Miyajima, N. Gomi, K. Ikeda and K. Achiwa, *Chem. Pharm. Bull.*, 1997, **45**, 1089.
250. A. Nicolaou, G. Kokotos, V. Constantinou-Kokotou, C. Charitos, C. Noula, R. Verger and W.A. Gibbons, *J. Pept. Sci.*, 1997, **3**, 291.
251. D. Albert and M. Feigel, *Helv. Chim. Acta*, 1997, **80**, 2168.
252. K. Kitagawa, C. Aida, H. Fujiwara, T. Yagami and S. Futaki, *Tetrahedron Lett.*, 1998, **38**, 599.
253. M. Gobbo, L. Biondi, F. Cavaggion, F. Filira, T. Piek, P. Mantel and R. Rocchi, *J. Pept. Res.*, 1997, **50**, 336.
254. H. Uchida, T. Kato and K. Achiwa, *Chem. Pharm. Bull.*, 1997, **45**, 1228.

255. K. Nozaki, T. Terakawa, H. Takaya and T. Hiyama, *Tetrahedron: Asymmetry*, 1997, **8**, 3431.
256. A.S. Lane, D.A. Leigh and A. Murphy, *J.Am.Chem.Soc*, 1997, **119**, 11092.
257. D. Besse, F. Siedler, T. Diercks, H. Kessler and L. Moroder, *Angew.Chem.Int.Ed.Engl.*, 1997, **36**, 883.
258. J. Ancans, G.J. Moore, P. Roumelioti and J.M. Matsoukas, *Epitheor.Klin.Farmakol-Farmakokinet.Int.Ed.*, 1997, **11**, 108.
259. S. Arttamangkul, J.E. Ishmael, T.F. Murray, D.K. Grandy, G.E. DeLander, B.L. Kieffer and J.V. Aldrich, *J.Med.Chem.*, 1997, **40**, 1211.
260. V.J. Hruby, H. Bartosz-Bechowski, P. Davis, J. Slaninova, T. Zalewska, D. Stropova, F. Porreca and H.I. Yamamura, *J.Med.Chem.*, 1997, **40**, 3957.
261. D. Pawlak, N.N. Chung, P.W. Schiller and J. Izdebski, *J.Pept.Sci.*, 1997, **3**, 277.
262. H.H. Wasserman and A.K. Petersen, *J.Org.Chem.*, 1997, **62**, 8972.
263. J. Pernerstorfer, M. Schuster and S. Blechert, *Chem.Commun.*, 1997, 1949.
264. J.W. Jonetka, P. Raman, K. Satyshur, G.R. Flentke and D.H. Rich, *J.Am.Chem.Soc.,* 1997, **119**, 441.

5
β-Lactam Chemistry

BY CHRISTOPHER J. SCHOFIELD AND MAGNUS W. WALTER

1 Introduction

Even outside specialist circles the increase in bacterial resistance against antibiotics, a term very often used in conjunction with penicillins, is now widely perceived as a major threat to public health. Attempts to combat resistance by the development of new and more active β-lactam compounds were at the centre of academic and industrial research in this field during the period reviewed (1996-1997). However, major new structural variants are rare and the lengthy syntheses associated with their production (*e.g.* trinems) may be limiting their availability for commercialisation. Indeed, it is noteworthy that the more recently introduced group of carbacephems antibiotics appears not to have precipitated as much research interest as previously introduced groups, such as carbapenems. The discovery and development of commercially viable orally active β-lactam antibiotics and inhibitors of Class A and C β-lactamases remain significant challenges for medicinal chemists. Inevitably, major new trends in organic synthesis will be reflected in an important research area like β-lactam chemistry. Thus, solid phase chemistry and combinatorial approaches to compound optimization are increasingly being used in this area. Significant progress has been made in the elucidation of penicillin biosynthesis. The structure of IPNS was solved and further crystallographic studies have provided important insights into the molecular mechanisms of β-lactam biosynthesis. Whether better understanding of fundamental aspects of β-lactam chemistry and biology will lead to the development of new and clinically more potent antibiotics remains to be seen.

Review articles in β-lactam chemistry are listed in a separate section in the Appendix.

2 Biosynthesis

The techniques of modern molecular biology have already had a major influence on biosynthetic studies on β-lactams and on the development of new bioprocesses. They have also facilitated the production of large amounts of protein for biophysical analyses, which have provided insights into isopenicillin N synthase, the only β-lactam forming enzyme to be so characterized. In the near future it seems that all the individual steps in the biosynthesis of the β-lactams

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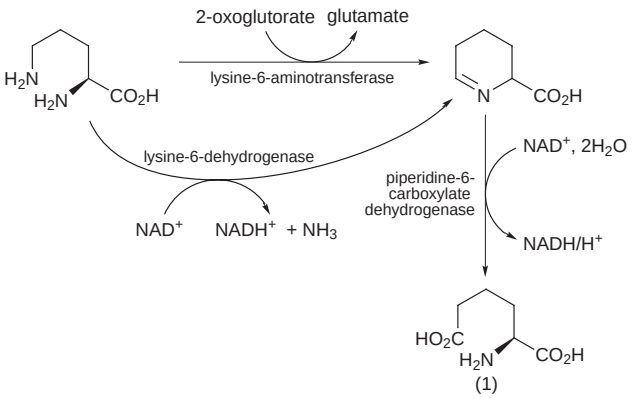
other than penicillins or cephalosporins, *e.g.* the clavams and carbapenems, will also be determined. Genome sequencing studies (see *e.g.* Kunst *et al.*[1a]) may also provide clues to the extent and origins of β-lactam biosynthesis and resistance. Elucidation of biosynthetic pathways and the structures/mechanisms of individual enzyme should set the stage for their rational manipulation. Objectives will not only be to produce existing β-lactams more efficiently, but also to produce new types of β-lactam, perhaps by hybridizing elements from the different biosynthetic pathways, *e.g.* to ferment clavulanic acid with a hydroxyethyl or 6-acetamido side-chain, thus far elusive goals for synthetic chemists.

An historical interesting analysis on the commercial improvement of *Penicillium chrysogenum* uses modern techniques to map penicillin titres with amplifications of the penicillin biosynthesis gene cluster.[1b] Evidence was presented for a tandem array of copies, arranged in a head-to-tail manner and that amplification is generated by unequal sister chromatid exchange. β-Lactam biosynthesis by *S. clavuligerus* has been studied in microgravity with apparently deleterious effects on antibiotic levels.[2]

2.1 Early Stages of Penicillin and Cephalosporin Biosynthesis and ACV Synthetase – (*L*)-α-Aminoadipic acid (1) biosynthesis in *Streptomyces clavuligerus* has been the subject of study, with both the lysine ε-aminotransferase[3] and piperidine-6-carboxylate dehydrogenase enzymes having been purified.[4] (Scheme 1).

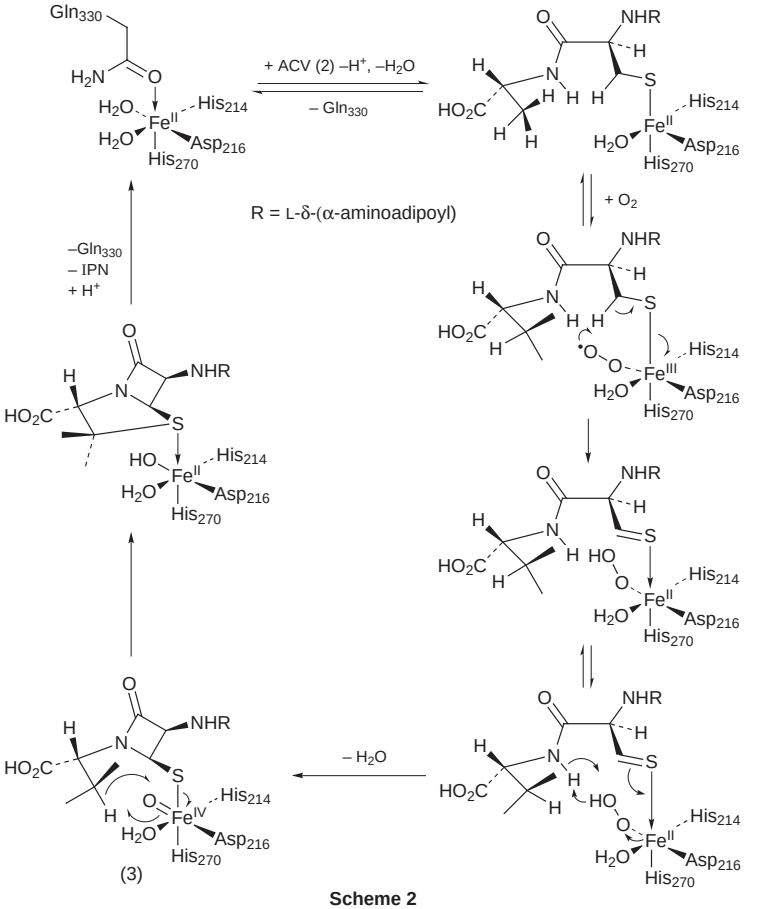


Scheme 1

Evidence has been provided that *(L)*-δ-(α-aminoadipoyl)-(*L*)-cysteinyl-(*D*)-valine synthetase (ACVS) is a rate-limiting enzyme for penicillin production in *Aspergillus nidulans*.[5] ACVS from *Penicillium chrysogenum* has been purified to homogeneity and characterized.[6] The native enzyme was apparently monomeric with an estimated mass of 470 kDa. Kinetic parameters were reported for the reaction and the dimer of ACV was found to give feedback inhibition. Experiments using di[^{18}O]valine as a substrate for ACVS from *Cephalosporium acre-*

monium demonstrated that dipeptides can be formed without loss of label, implying that thioester formation is not obligatory during the peptide synthetase catalysed formation of amide bonds. The observation suggests that there may be more than one possible mechanism for the peptide synthetases.[7]

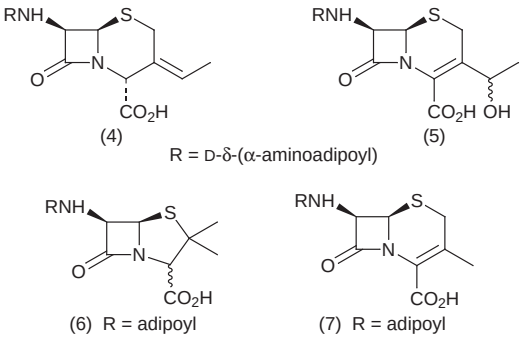
2.2 Isopenicillin N Synthase (IPNS) – IPNS has been crystallised in the presence of iron and its tripeptide substrate under anaerobic conditions.[8] A comparison of EXAFS studies in solution and the crystalline phase was carried out. Most significantly, the high-resolution structure of the IPNS-Fe-ACV complex was solved in the presence and absence of NO (acting as a dioxygen analogue[9]). Based on the structure a mechanism was proposed in which both rings of the penicillin nucleus were formed without the assistance of acid/base catalysis from amino acid residues (Scheme 2). Instead reactive species derived



Scheme 2

from the dioxygen molecule were responsible for the cleavage of cysteinyl and valine β-(C-H) bonds and the N-H bond of the cysteinyl-valine amide link. One interesting feature of the structure IPNS-Fe-ACV was the observation that the valine β-(C-H) bond which must be cleaved was directed away from the iron centre. It was proposed that rotation about the valine Cα-Cβ bond occurs after formation of the monocyclic ferryl intermediate (3). Mutagenesis studies on two conserved histidine and one aspartate residues are consistent with crystallographic and sequence analyses indicating these residues complex the active site iron.[10,11] In the Mn-IPNS (*A. nidulans*) crystal structure a fourth ligand, Gln-330, was observed to ligate the active site iron. Deletion and substitution mutagenesis studies have demonstrated this residue is not required for *in vitro* catalysis by both the *A. nidulans* and *Streptomyces jumonjinensis* IPNS enzymes.[12,13] The synthesis[14a,b] and incubation of analogues of ACV in which the valine residue was replaced by a labelled 2-methylcylopropylglycine residue has been described. Interpretation of the labelling patterns in the bicyclic products led to support for an insertion-homolysis mechanism, in which 'free radical character is manifested only after cleavage of the cyclopropane ring'. Analogues of ACV in which the NH of the aminoadipoyl-cysteinyl link was 1,3-transposed into the aminoadipoyl side chain were not substrates for IPNS.[15]

2.3 Cephalosporin Biosynthesis – Deacetoxycephalosprin C synthase (DAOCS) has been shown to convert 3-ethylidene cephalosporin C (4) into two diastereomeric alcohols (5). In contrast to previous studies with DAOCS, but consistent with those with DAOC/DAOCS, DAOC was shown to ring expand adipoyl-penicillanic acid (6) to the adipoyl cephalosporin (7).[16] This observation is supportive of the conversion of (6) into (7) occurring during a commercial process for the preparation of 7-aminodeacetoxycephalosporin C, involving the use of *P. chrysogenum* transformed with DAOCS. See previous report.



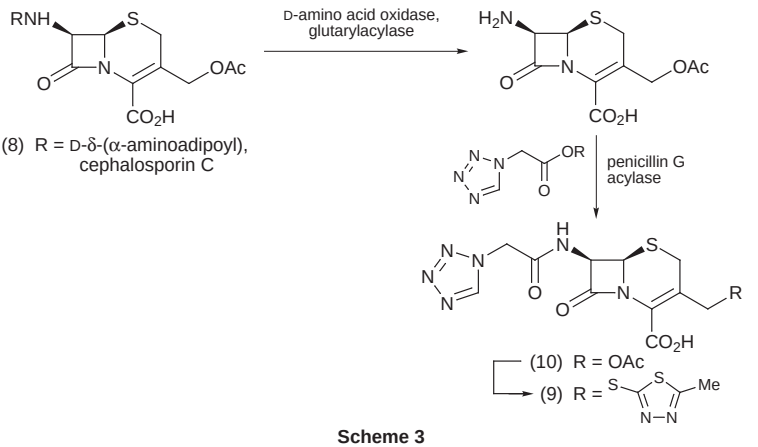
2.4 Penicillin Acylases – A penicillin V amidohydrolase has been isolated from two species of *P. chrysogenum*.[17] The activity with penicillin G was only 1.5%

that of penicillin V and no acyl-penicillin formation from 6-aminopenicillanic acid (6-APA) and phenoxyacetyl or phenylacetyl coenzyme A was observed. Thus, the enzyme differs from the phenylacetyl CoA:6-APA acyltransferase involved in penicillin biosynthesis. The regulation of the phenylacetic acid uptake system of *A. nidulans* has been studied[18] and found to be strongly induced by phenylacetic acid and less so by phenoxyacetic acid.

A detailed kinetic study on penicillin G acylase gave results characteristic of a mechanism involving an acyl-enzyme intermediate.[19] Pencillin G acylase continues to find use for the removal of phenylacetyl protecting groups,[20,21] sometimes coupled with resolution.[22,23] Nice examples of its use can be found in phosphopeptide and nucleopeptide synthesis.[24,25] The synthesis of acylated penicillins using penicillin G acylase and methyl esters has been investigated in various solvents.[26,27] Immobilized penicillin G acylase has also been used for the *N*-phenylacetylation of amino acids in biphasic systems.[28]

Interest in the optimization of penicillin acylases for the cleavage of penicillin side chains remains high, with many studies being reported.[26,27,29–31] A penicillin acylase has been purified *from Bacillus magaterium*.[32]

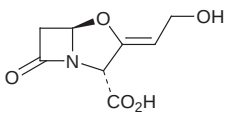
2.5 Cephalosporin Acylases/Acyltransferases and Acetylases – The gene coding for glutaryl-7-aminocephalosporanic acid (GL-7-ACA) acylase from *Pseudomonas sp* has been cloned.[33] Kinetic analysis of mutants, including those involving all the cysteines of the GL-7-ACA enzyme, have been carried out.[34] A one-pot chemo-enzymatic procedure for the conversion of cephalosporin C (8) into Cefazolin (9) has been described.[35,36] The procedure employs D-amino acid oxidase to deaminate the side chain and hydrolysis of the resultant glutaryl side chain by glutaryl amidase. Finally penicillin G acylase catalyzed introduction of the side chain to give (10) and displacement of the acetoxy group with 5-mercapto-5-methylthiadiazole to give Cefazolin (9) (Scheme 3).



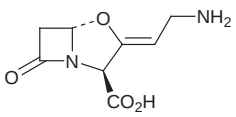
Scheme 3

The cephalosporin C-3 deacetylesterase gene from *Bacillus subtilis* was cloned into *E. coli*.[37] A lipase from *Aspergillus niger* was purified and shown to hydrolyse the 3-O-acetate of cephalosporin C and other cephalosporins, Cephalotin and Cefotaxime.[38] A recombinant acetylase from *Thermoanaerobacterium* has sequence similarity with the *B. subtilus* deacetylesterase and was shown to have thermostable activity.[39]

2.6 Clavam Biosynthesis – Disruption of the caR regulatory gene for clavulanic acid (11) and cephalosporin biosynthesis in *S. clavuligerus* resulted in the loss of the ability to synthesize antibiotics. Amplification of it resulted in an increase in antibiotic synthesis.[40] Labelling studies designed to identify the primary metabolic precursors of the three carbons of the β-lactam ring of the clavams have been carried out by two groups. They revealed that the hydrogen at C-2 of glycerate is lost in the conversion into clavulanic acid[41] and that pyruvate is more likely than glycerate to be the direct primary metabolic precursor of the β-lactam carbons.[42] The 2-oxoglutarate dependent oxygenase clavaminate (12) synthase (CAS), which catalyzes three steps in clavam biosynthesis, remains the subject of interest. Spectroscopic studies indicate that its active site iron is ligated by two histidine residues and a carboxylate ligand, similar to IPNS and DAOC.[43] However, primary sequence analyses indicate little sequence similarity between CAS and the oxygenases of penicillin and cephalosporin biosynthesis, so its three-dimensional structure will be of interest. Full details of the use of CAS for the preparation of 5,5 γ-lactams (13) and (14) have been described. Whilst these compounds are unlikely to display antibacterial or β-lactamase inhibitory activity due to their stereochemistry, one of them (13) has proved difficult to prepare *via* chemical synthesis. In the future CAS, possibly in combination with other enzymes, may prove useful for the preparation of new γ-lactam enzyme inhibitors.[44] Labelled clavaminic acid (12) has been synthesized in a chemo-enzymatic manner by incubation of labelled proclavaminic acid (15) with CAS. The resultant (12) was used to provide evidence that the branch-point between clavulanic acid (11) biosynthesis and that of other clavams occurs at a late stage in the biosynthetic pathways, possibly at clavaminic acid (12) itself.[45] A new synthesis of proclavaminic acid (15) has been reported.[46]



(11)



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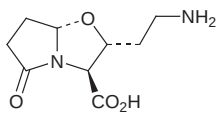
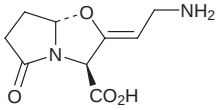


(13)



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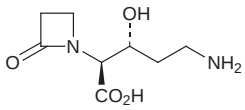
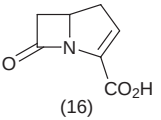


(15)

2.7 Carbapenem Biosynthesis – Analysis of the biosynthetic genes for carbapenem biosynthesis (16) has revealed surprisingly that two of them are related to genes encoding for enzymes involved in clavulanic acid (11) biosynthesis.[47] One of them shows sequence similarity to clavaminate synthase. The other is also related to asparagine synthetase. The position of the asparagine synthetase related genes in both clavulanic acid and carbapenem operon suggest these enzymes catalyse early steps in their respective biosynthetic pathways. Together with previous work from the same group this study paves the way for the identification and characterization of the individual enzymes catalyzing steps in carbapenem biosynthesis.

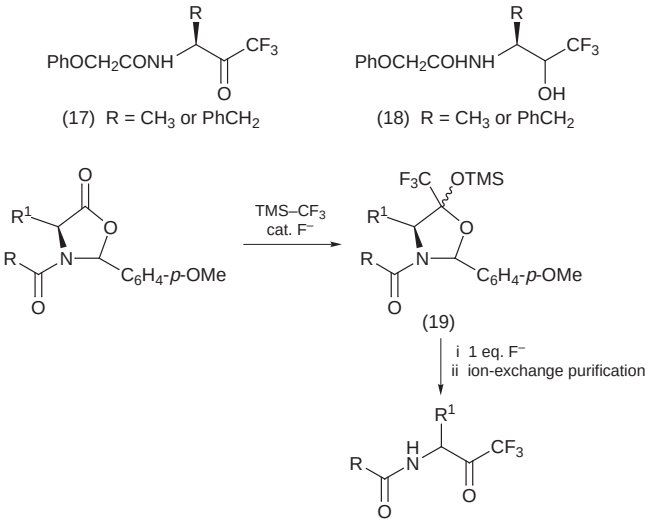


(16)

3 β-Lactamases and Related Enzymes

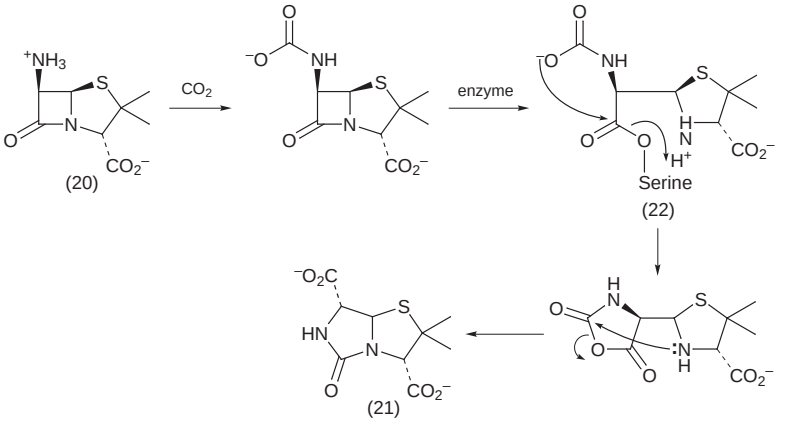
The period of review has seen major advances in the study of metallo (Class B) β-lactamases, which are of increasing clinical importance. Structures were reported for the enzymes from *Bacteroides fragilis*[48,49a] and *B. cereus*.[49b] Two zinc binding sites were observed in both enzymes, but whether the binding of zinc at both sites is required for catalysis is uncertain. In the *B. fragilis* enzyme, one Zn was tetrahedrally coordinated and the other had trigonal bipyramidal coordination. However, only a single Zn was observed in the *B. cereus* enzyme. It was proposed that one or both zinc ions polarise a water molecule for attack onto the β-lactam carbonyl. Spectroscopic studies demonstrated that the *B. fragilis* enzyme binds two Zn ions and, together with mutagenesis studies, implies a role for both zinc ions in catalysis.[50] However, studies on the metallo β-lactamase from *Aeromonas hydrophilia* indicate only one tightly bound zinc is required for activity with zinc binding at the second site actually inhibiting the enzyme.[51] Both thiols[52] and thiol esters[53,54] have been shown to be inhibitors of the Class B enzymes. In the latter case analysis of inhibited protein was consistent with a mechanism involving initial cleavage of the thiol ester releasing a thiol which formed a disulfide with the active site cysteine under the aerobic assay conditions. Trifluoromethyl ketones (17) and alcohols (18) have also been shown to be inhibitors with competitive inhibition being observed for three of the enzymes, but irreversible inhibition being observed (surprisingly) for the *Aeromonas hydrophilia* enzyme.[55,56] A new route for the synthesis of trifluoromethyl derivatives of amino acids was developed in which the key step involved reaction of Ruppert's reagent ($TMS\text{-}CF_3$) with oxazolid-5-ones (19) (Scheme 4).

The precise details of the mechanisms of the Class A and C (serine) β-lactamases remain of interest and despite crystal structures remain the subject of some controversy. The details are beyond the scope of this review but



Scheme 4

careful mutagenesis and kinetic studies have been carried out.[57–59] The most controversial aspect is the nature of the general base in the acylation step of the Class A enzyme mechanism. pK_a calculations indicate that Glu-166 is a much more likely candidate than Lys-73.[59] In the presence of bicarbonate the *E. cloacae* P99 enzyme catalyzed hydrolysis of 6-APA (20) unexpectedly led to 8-hydroxypenicillic acid (21).[60] A mechanism involving carbamate formation followed by acylation of the enzyme and rate-limiting intramolecular cyclisation of the carbamate onto the acyl-enzyme (22) was proposed (Scheme 5).



Scheme 5

The structure of the *E. coli* TEM-1 β-lactamase complexed with the β-lactamase inhibitory protein (BLIP) reveals that the β-hairpin loop of BLIP inserts into the active site mimicking the binding of a penicillin nucleus.[61] The results may lead to the design of non β-lactam β-lactamase inhibitors. A β-lactamase encoded for in the cephamycin/clavam gene cluster of *S. clavuligerus* was purified and found to bind benzylpenicillin, a result reflecting a rate-limiting deacylation step. The *in vivo* role of this enzyme in a clavulanic acid (11) producing strain is uncertain and of interest.[62]

Details of the β-lactamase induction pathways are being elucidated. β-Lactamase induction in *E. cloacae* is linked intimately to peptidoglycan recycling, and has been investigated by careful analysis of cell wall fragments of *E. coli*.[63] It was concluded that the signal molecule for β-lactamase production in *E. cloacae* is an anhydromuramyl-pentapeptide.

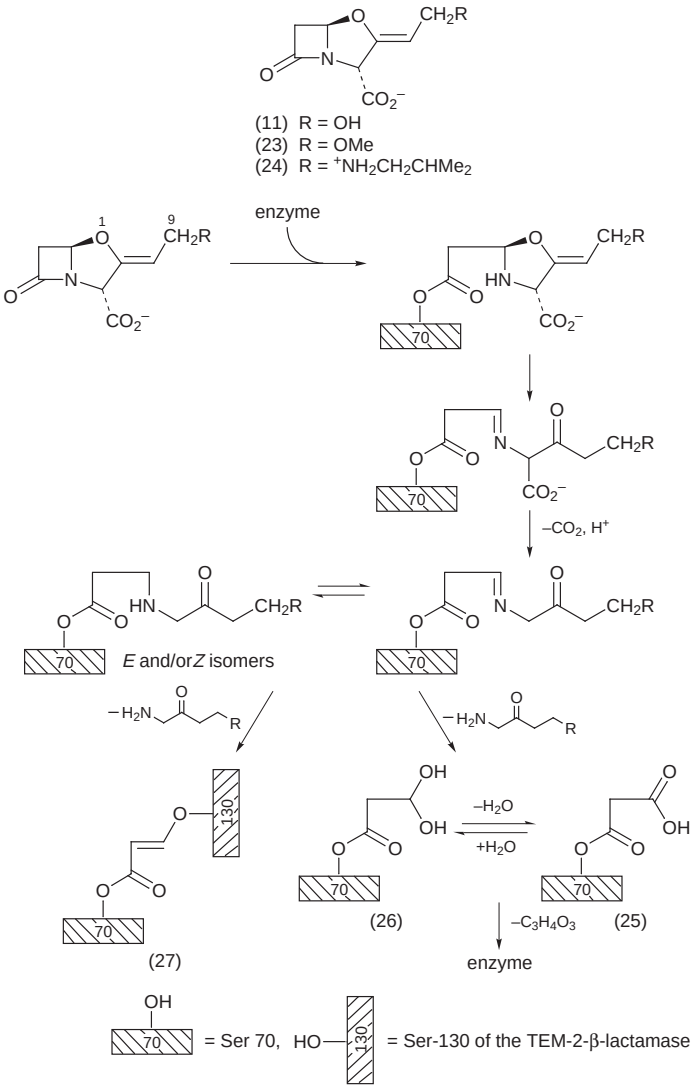
Studies by electrospray ionisation MS (ESMS) and HPLC on the inhibition of the TEM β-lactamase by clavulanic acid (11) and analogues (23) and (24)[64,65] have led to a revised mechanism in which initial acylation of Ser-70 and ring opening of the 4-membered ring is followed by that of the 5-membered ring. Decarboxylation and subsequent hydrolysis lead to aldehyde (25). Both aldehyde (25) and hydrated aldehyde (26) were observed, suggesting the aldehyde protects the ester link with Ser-70 from hydrolysis. Alternatively cross-linking with Ser-130 can occur to give a vinyl-ether (27) (Scheme 6).

A series of tripeptides including (28) were reported to be potentiators of methicillin against MRSA. The mode of action is unknown, but may be related to the ability of the peptides to form β-turns.[66]

4 Penicillins and Cephalosporins

The need for a broad spectrum β-lactamase inhibitor, active against Class A and C enzymes, continues to grow. The success of Sulbactam and Tazobactam has rendered the penam sulfones of particular interest. 2β-Acyl derivatives (29) were prepared *via* alcohol (30) and acid (31). One of these compounds (32) showed activity (in combination with Ceftazidine) comparable to that of Tazobactam.[67] 2-Acyl-derivative (33) also showed promising activity.[68] 2β-Oximinomethyl (34) and 2β-hydrazinomethyl penam sulfones are reported to show improved synergy with Ceftazidine versus Class C β-lactamase producing organisms.[68] Addition of a (6*R*)-6α-hydroxybenzyl side chain as in (35) did not give the hoped for improvement in synergy versus *P. aeruginosa* strains.

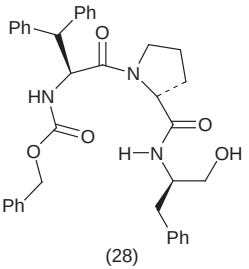
α-Aminosulfonopeptide (36) was prepared as an analogue of penicillin/acyl-D-Ala-D-Ala peptides, but proved to be very unstable. Methyl ester (37) had a half-life of < 10 min. in MeOH/water at pD 5.[69] Penicillin functionalised norbornene polymers were synthesized *via* metathesis polymerization using a Ru-catalyst.[70] Penicillin analogues with a styryl group at the 6-position (38) were prepared from 6α-bromopenicillanic acid *via* a cobaloxime cross-coupling procedure.[71] Norpenicillin sulfoxides (34), synthesized *via* hydrogenation of exomethylene penam (40), have been shown to undergo the Morin ring expansion to give cephems (41), with



Scheme 6

a requirement for the β-orientation of both the methyl and sulfoxide groups in the penicillin.[72] As with other sub-families of β-lactams, penicillins are being used in the construction of libraries, *e.g.* in a search for new inhibitors of elastase[73] and HIV protease.[74] Penicillins can be released efficiently from Merrifield/Wang resins using $AlCl_3$.[75]

Penicillins continue to attract the attention of spectroscopists and theoreticians



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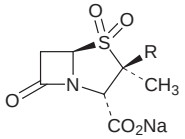
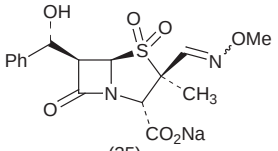


(29) R = COR
(30) R = CH_2OH
(31) R = CO_2H
(32) R = COEt
(33) R = COMe
(34) R = CH=N(OMe)



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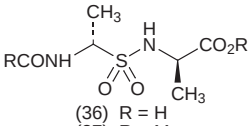
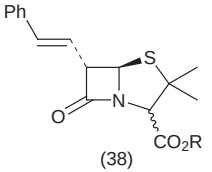
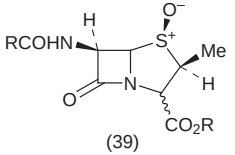


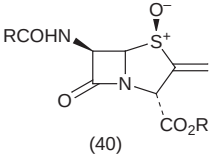
(36) R = H
(37) R = Me



(38)



(39)



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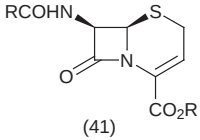
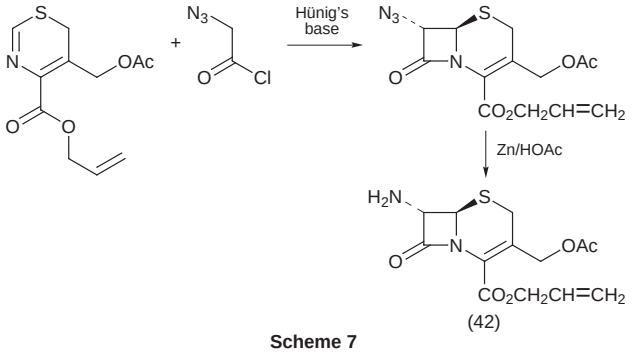


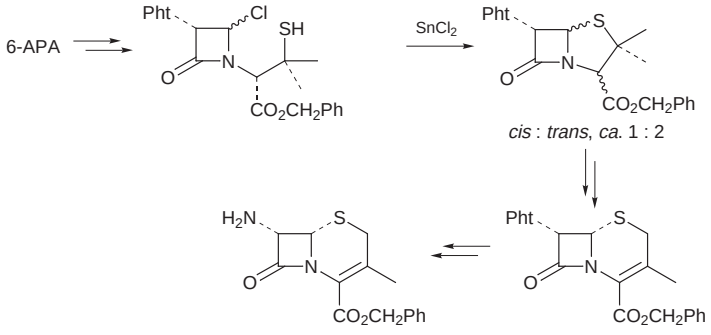
(41)

with reports on calculations on their hydrolysis,[76] transition metal complexes[77–78] and an interesting combined NMR/X-ray study on the dynamics of phenyl ring motion in pencillin V crystals.[79]

Cephalosporins: 7-Aminocephalosporanic acid (7-ACA) was prepared by total synthesis *via* a 7-*epi*-ACA derivative.[80] The route employed a ketene-imine cycloaddition (Scheme 7) to make the β-lactam ring in 38% yield. Epimerisation of (42) was achieved *via* reported methods. An HPLC separation system for all four stereoisomers of 7-ACA was described and led to the conclusion that the natural material had an e.e. of >99.95%. Practical (i.e applicable to multi-gram



Scheme 7

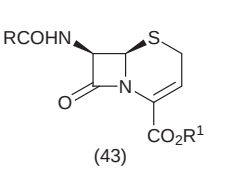


Scheme 8

scale) synthetic routes to *ent*-cephalsoporins and *6S*-cephalosporins starting from 6-APA have been described.[81] In one approach inversion of the stereochemistry at the penicillin C-5 and C-6 positions was achieved before ring expansion (Scheme 8), whilst in the other stereochemistry at the C-5 position was inverted before ring expansion and the C-7 cephem stereochemistry was inverted after rearrangement.[81]

The synthetic work of Torii *et al*. has been a highlight of the review period. A new route to 3-nor-cephems (43) by reaction of the dichloride (44), available in several steps from 6-APA, with tributyltin hydride/CuCl in *N*-methylpyrrolidone has been described. The reaction is proposed to proceed *via* enolate (45).[82] The same group also describe full details of how treatment of allene carboxylate (46) with nucleophiles, *e.g.* morpholine, azide, chloride in the presence of $CaCl_2$, leads to 3-subsbtiuted Δ-3 cephems.[83] In the case of chloride reaction, the formation of the unwanted 3-phenylsulfonyl byproduct (47), formed by reaction of allene (46) with benzenesulfinate, was eliminated by *in situ* oxidation of the latter to the less nucleophilic sulfonate.[84] Reaction of allenecarboxylate (46) with allylic and benzylic halides R-X to give cephems (48) was achieved in a reductive addition/cyclisation process employing a 'three metal redox system' consisting of {Al-metal, cat. $PbBr_2$, cat. $[NiCl_2(bipyr)]$}.[85]

Ring expansion of a penicillin sulfoxide (49) into an exomethylene cepham (50)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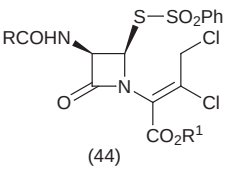
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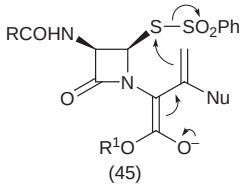
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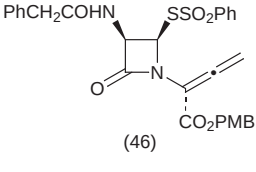
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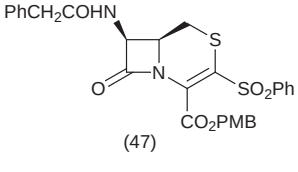
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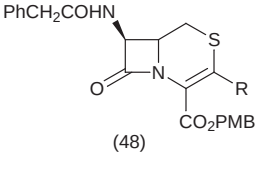
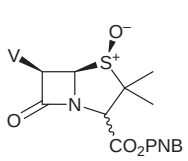

(48)

is a key step in the industrial preparation of Cefaclor. The process proceeds *via* conversion of sulfoxide (49) to sulfenyl chloride (51) which is treated with $SnCl_4$/MeOH to give exomethylene cepham (50). The production of a chlorinated byproduct (52) is minimized by the use of alkene scavengers.[86]

Ozonolysis of Δ-2 cephems (53) leads to enols (54), together with some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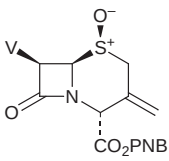
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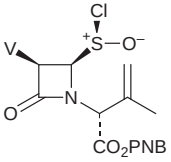
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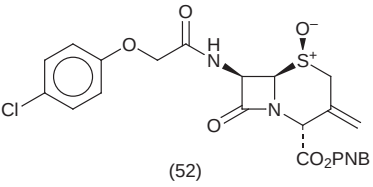
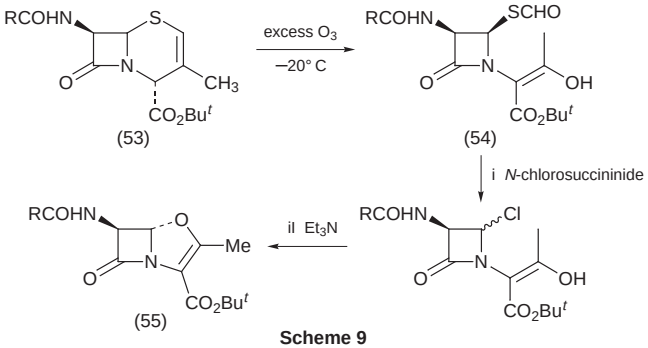

(52)

sulfoxide (not shown).[87] Treatment of one of the enols (54) with *N*-chlorosuccinimide followed by base gave oxapenem (55) and constitutes a new entry to these compounds (Scheme 9). t-Butylhydroperoxide in the presence of catalytic camphorsulfonic acid oxidises cephalosporins to their sulfoxides. In contrast to the use of m-CPBA, a mixture of α- and β-sulfoxides is obtained.[88] A facile route to the t-butyl esters of cephalosporins employs t-butylacetate in the presence of $BF_3.Et_2O$ rather than sulfuric acid as previously used.[89]

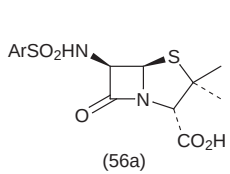
Extensive SAR studies continue on the cephalosporins in efforts to obtain broad spectrum, preferably orally active antibiotics. Most modifications are made at the C-7 and or C-3 positions. Description of the biological activities of these compounds is beyond the scope of a review concentrating on chemistry.



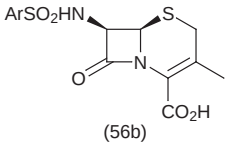
Scheme 9

Only modifications involving new directions/chemistry are discussed here. Others are listed in the Appendix.

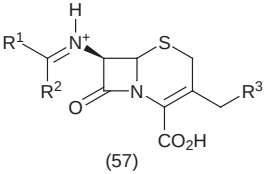
C-7 modified compounds: Replacement of the C-6, penicillin or C-7, cephem acetamido side-chain with a sulfonamido one to give (56a) or (56b) results in the retention of significant activity against a range of bacteria.[90] The susceptibility of these compounds to β-lactamase mediated hydrolysis will be of interest. C-6 Penams and C-7 cephams (including those with leaving groups at the C-3'-position) react with (cyclo)ketones under acidic conditions to form surprisingly stable alkylideneammonio salts, *e.g.* (57) and (58), which are useful as intermediates.[91] Phloroglucide analogues of polyketide antibiotics have been linked to both the C-3′ and C-7 positions of cephalosporins.[92] Some of the compounds, *e.g.* (59), showed resistance to β-lactamases whilst still displaying interesting levels of antibacterial activity. Given their potential as metal-chelators, these compounds should be tested as metallo β-lactamase inhibitors.



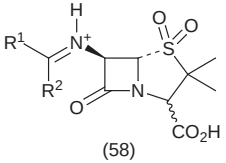
(56a)



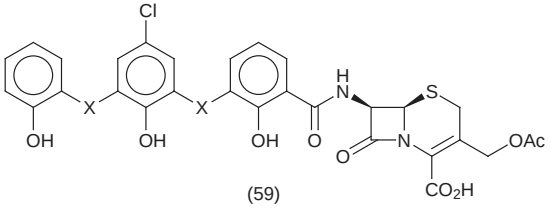
(56b)



(57)



(58)

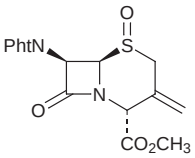


(59)

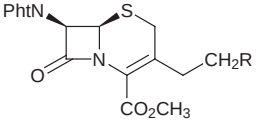
C-3 modified compounds: An alternative to the Stille protocol, which uses Bu_3SnH, for the reduction of 3-chloro or 3-triflate cephems to give 3-H cephems, is to use Et_3SiH in the palladium catalyzed reaction. 1.1′-bis-Diphenylphosphino-

ferrocene or PPh_3 were used as palladium ligands and high yields were obtained under optimised conditions.[93] A one-pot procedure for the preparation of 3-(alkenyl) cephems from 3-hydroxymethylcephems *via* Wittig chemistry has been described.[94] Pummerer reaction of exomethylene sulfoxide (60) allows trapping with aromatic or olefinic nucleophiles giving Δ^2-C-3′ substituted cephems (61).[95] Cephem 3-triflates underwent Stille coupling to give 3-vinyl sulfoxide (62), potential β-lactamase inhibitors. None of the compounds synthesized displayed any β-lactamase inhibitory activity, but two compounds displayed activity versus MRSA.[96]

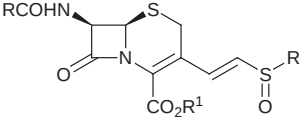
In an investigation into the reaction of 3-chloromethylcephem (63) with



(60)



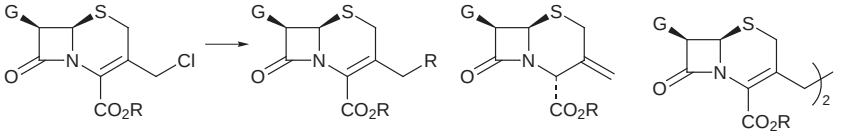
(61)



(62)

organotin reagents in the presence of CuCl in the presence of various additives, Torii *et al.* optimised yields for the desired products (64). Exomethylene cephem (65) and dimer (66) were isolated as byproducts under some conditions (Scheme 10).[97]

Several studies on cycloaddition reactions with cephalosporins have been

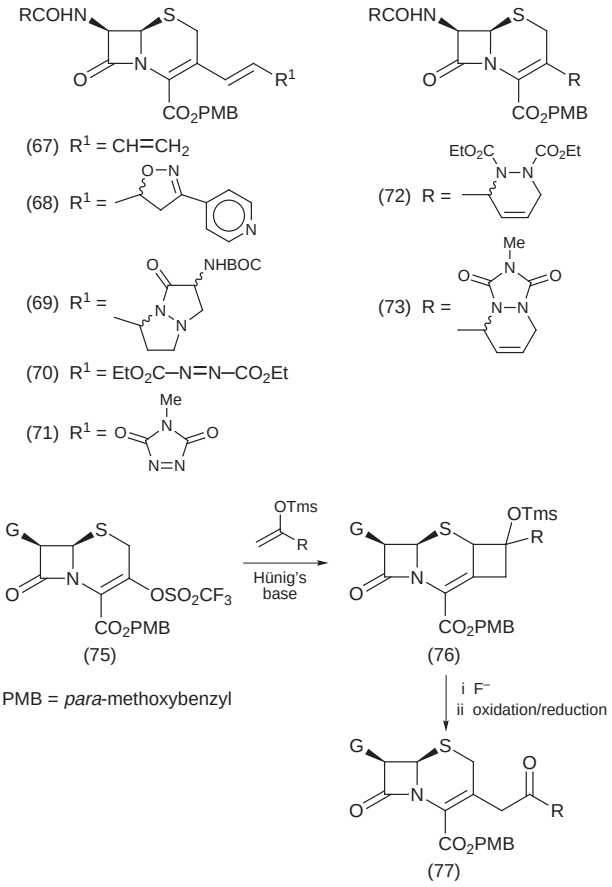


(63) (64) (65) (66)

Scheme 10

reported.[98–101] 3-(1,3-Butadienyl) cephem (67) was reacted in both [3+2] and [4+2] cycloadditions. The [3+2] cycloadditions occurred only at the terminal alkene and were successful with nitrile oxide and azomethine imine to give adducts, including (68) and (69). Diels-Alder reaction with two dienophiles, (70) and (71), gave a mixture of diene diastereomers (72) or (73).[100] Cycloaddition reaction of 3-cephem triflate (75) with silylenol ethers or silylketene acetals leads to cyclobutanes (76) (Scheme 11), which can be fragmented by treatment with fluoride giving a mixture of Δ^2/Δ^3 cephem isomers, which was converted into the Δ^3-isomers (77) solely by the known oxidation (MCPBA)/reduction (PBr_3) procedure.[101]

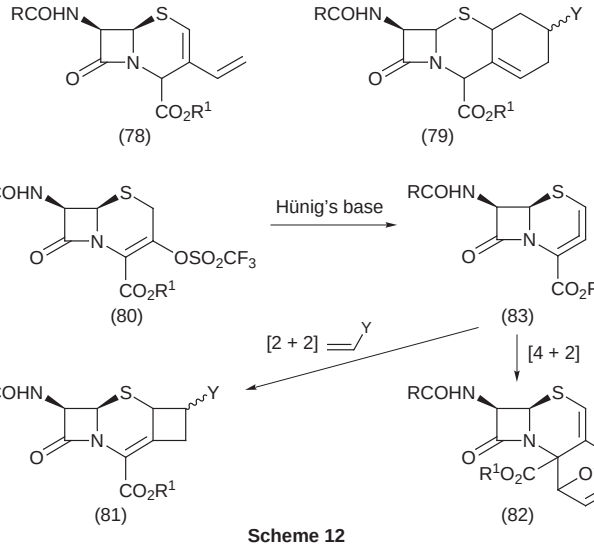
3-Vinyl-Δ^2-cephem (78), synthesized *via* CuCl promoted Stille coupling, underwent reaction with dienophiles to give tricyclic cephams (79). Dimerization of (78) was also observed on heating. The most detailed study on cycloaddition reactions on cephems comes from Elliott *et al.*[99] Cephem 3-triflates (80) were



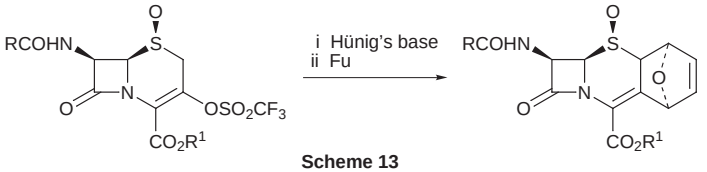
Scheme 11

treated with Hünig's base and reacted with a wide variety of alkenes and alkynes in [2+2] cycloadditions to give (81) and with dienes, including furan, to give [4+2] cycloadducts, such as (82) (Scheme 12). In the latter case the regiochemistry of the products is determined by the oxidation state of the cephem sulfur (cf. Schemes 12 and 13). Detailed mechanistic studies were carried out and the reaction products rationalized by invoking a strained 6-cyclic allene (83) intermediate.

Elastase inhibition is a known application for cephalosporins. Several groups are exploring the effects of substitution of the cephem ring on elastase inhibitory activity. Buynak has extended his work with 7-alkylidenecephalosporins as β-lactamase inhibitors to show that 7-vinylidene (84) and 7-alkylidene (85) benzhydryl esters are elastase inhibitors.[102] Note, optimum activity was obtained as the sulfide rather than the sulfones. Deprotonation of cephem sulfone (86) with NaH, followed by reaction with carbon disulfide and an alkyl halide gives 2-(1,3-

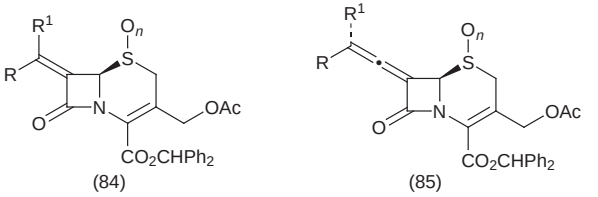


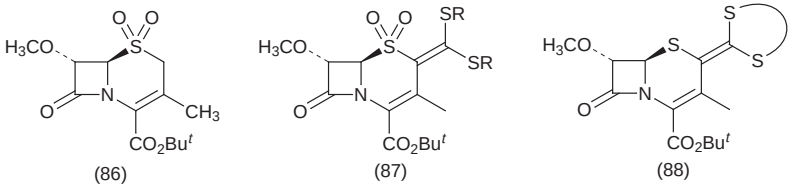
Scheme 12



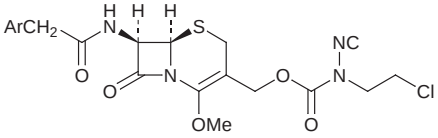
Scheme 13

diothiolan-2-ylidene)cephems (87) or (88), which were reported to be potent elastase inhibitors.[103]





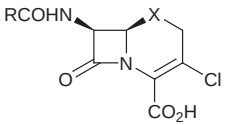
The potential use of β-lactams in antibody directed prodrug therapy continues to be investigated. *N*-Nitrosochloroethyl-cephem (89), prepared from cephalothin, was found to be a good substrate for a Class C β-lactamase and is designed to release a chloroethyl diazo species in the presence of an antibody β-lactamase conjugate.[104]



(89)

5 Carbacephems

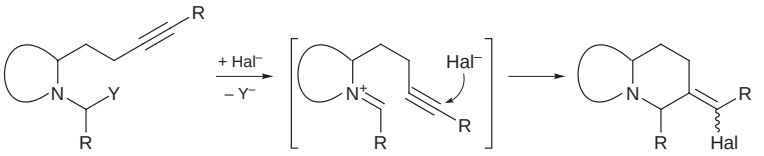
The first commercial carbacephem, Loracarbef (90), was introduced for the treatment of various infections in 1992. While being a potent, orally active antibiotic, the carbacephem motive ($X = CH_2$) increases the chemical and hydrolytic stability relative to the cephem analogue Cefaloclor (91, X = S). As carbacephems are to-date only available by total or semi-synthesis, new approaches to the parent ring system are of considerable interest.



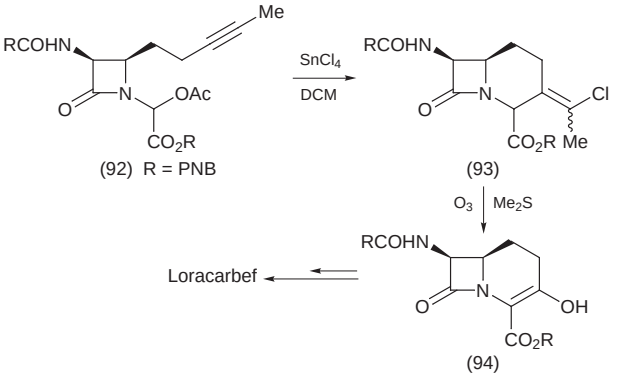
(90) Loracarbef X = CH_2
(91) Cefaloclor X = S

Overman's halide terminated *N*-acyliminium ion alkyne cyclisation is a powerful method for the construction of complex *N*-containing heterocycles (Scheme 14). Its successful application to the carbacephem nucleus, in a formal total synthesis of Loracarbef (90), has been reported[105]The crucial cyclisation step of (92), obtained in several steps from a commercially available precursor, was initiated by $SnCl_4$ and proceeded in 60% yield to afford vinyl chloride (93). Ozonolysis of (93) led to carbacephem enol (94), which was converted into the triflate and subjected to nucleophilic displacement affording loracarbef and various analogues (Scheme 15).

Another approach to the carbacephem nucleus utilises the related *N*-acyl iminium ion cyclisation (Scheme 16).[106] Lewis acid-mediated cyclisation of *N*-hydroxy-methylene precursor (95) proceeded in moderate to excellent yield, depending on the nature of the substituents. Attempts to use similar conditions for the cyclisation of *N*-α-hydroxy esters did – perhaps surprisingly – not afford the desired C-2 carboxylate-substituted product. However, it was found that electron richer, *i.e.* more nucleophilic, cyclisation precursor (96)

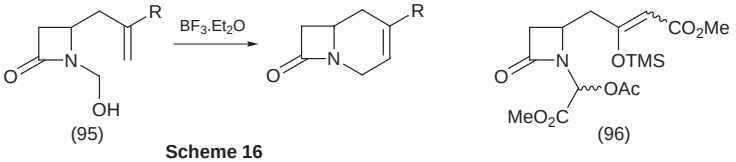


Scheme 14



Scheme 15

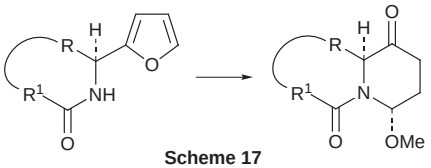
gave the ring-closed product in 50% yield. In a chiral modification of this approach, optically active *N*-hydroxy-methylene precursor (95) was obtained by enzymatic resolution involving lipase-mediated transesterification with vinylacetate.[107]



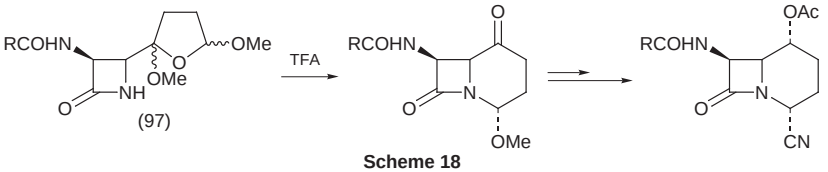
Scheme 16

The acid-mediated conversion of furylamides into heterocycles is known as the aza-Achmatowicz rearrangement (Scheme 17). Treatment of 3-furyl azetidinone derivative (97) under similar conditions (Scheme 18) figures as key step in a novel approach to the carbacephem skeleton.[108] Whereas the cyclisation proceeded in excellent yield, conversion of the C-4 methoxy group into a cyano group required a number of steps.

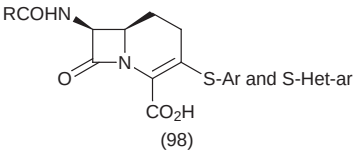
As in the case of other β-lactam antibiotics, the emergence of resistant bacterial strains has reduced the efficacy of carbacephems in clinical use. The SAR around C-3 has been further studied with the aim of improving in particular the activity against such resistant strains. Aromatic and heteroaromatic thiophenols (98) as C-2 substituents were shown to have the desired effect *in vitro*.[109]



Scheme 17



Scheme 18



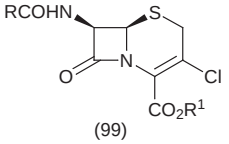
(98)

6 Penems

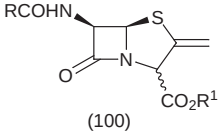
Interest in the 6-acetamido penems, first synthesized by Woodward, waned when it was discovered that they were insufficiently stable for clinical use. Subsequently, more stable penem derivatives with potent antibacterial (including MRSA) and β-lactamase inhibiting activities have been discovered. However, optimized or new synthetic procedures may be required in order to minimize production costs if these penems are to be used clinically.

Torii and co-workers have described an interesting and potentially useful method for the ring contraction of 3-substitured cephems into 2-exomethylene penams and/or 2-methylpenems.[110] Thus, treatment of cephems (99) with Al/$BiCl_3$/$AlCl_3$ led, in *N*-methyl-2-pyrrolidinone, to exomethylene penam (100) and/or penem (101), with the product ratios depending upon the temperature, reaction time and amount of $AlCl_3$ present. Switching the solvent to methanol led to the apparent exclusive production of (102). Although the precise mechanism of the conversion of (99) is unclear, it seems likely that reductive elimination to give allene (103) is followed by subsequent Michael addition to give exomethylene penam (100), which may undergo isomerization to (101). In MeOH, two-electron reduction of (99) followed by rapid protonation occurs and gives (102).

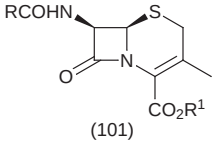
Pfaendler has reported that, as for the oxapenems, 2-tert-butyl substituents substantially increase the hydrolytic stability of the penems.[111,112] The intramolecular Wittig reaction proved useless for the synthesis of these compounds. However, Budt's modified 'oxalimide' route proved to be successful. The half-life of (104) was extended by about 10-fold relative to its 2-unsubstiuted penem analogue. 2-Hydroxyethyl derivative (105) was also significantly more stable than



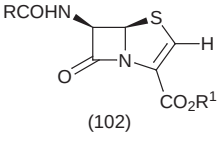
(99)



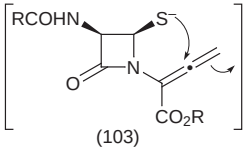
(100)



(101)

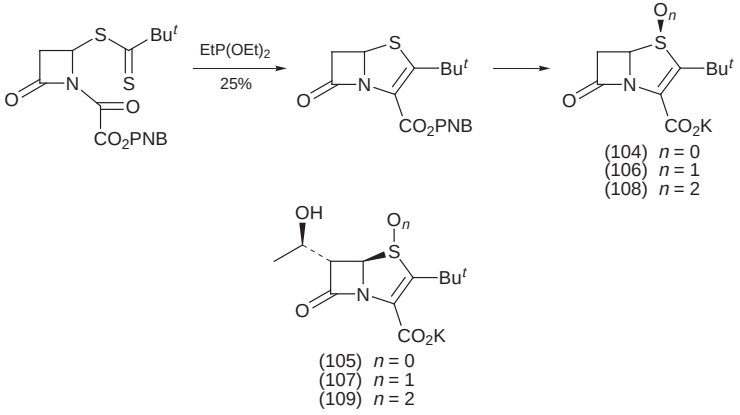


(102)



(103)

its oxapenem analogue. The antibacterial and β-lactamase inhibitory activity of (105) was compared with that of (104). The increased stability of the 2-susbtituted penems allowed the synthesis of the sulfoxides (106), (107) and the corresponding sulfones.[113] As anticipated sulfur oxidation resulted in a decrease in stability. The sulfoxides (106) and (107) also displayed reduced antibacterial activity compared to the parent sulfide. The sulfone (109), whilst inactive in antibacterial asssays (presumably due to its instability), was a potent inhibitor of both the *E. cloacae* and *E. coli* TEM β-lactamases.

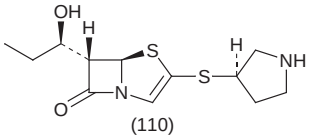


(104) $n = 0$
(106) $n = 1$
(108) $n = 2$

(105) $n = 0$
(107) $n = 1$
(109) $n = 2$

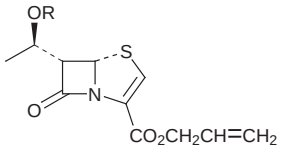
Crystal structures of a series of penems have been used in an attempt to rationalize their antibacterial activities.[114] The results were consistent with an experimental study which demonstrated that 5,6-*cis*-penems, including (110), possessed potent antibacterial activity versus a range of bacteria, including MRSA and β-lactamase producing strains.[115] The structure-activity results were discussed in terms of the formation of a hydrolytically stable acyl-enzyme complex with the target enzymes.

More details of the synthesis of penems of general formula (111), which are



(110)

inhibitors of bacterial signal peptidases[116] have been reported. The synthesis employed established methods for the preparation of 5*S*-penems followed by photoisomerisation to give the desired *5R*-penems.



(111)

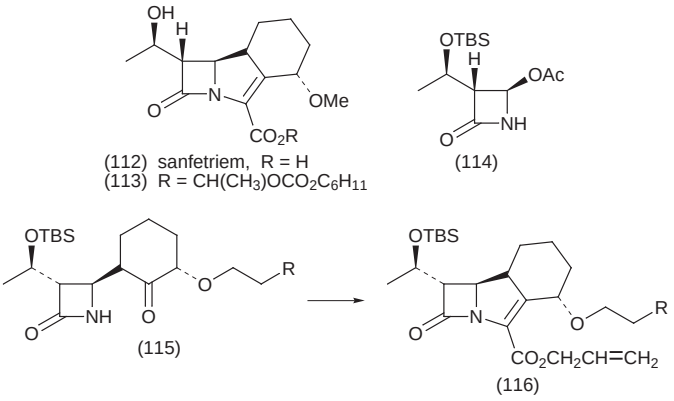
7 Clavams and Oxacephems

Chmielewski and co-workers continue to exploit their route to chiral clavams using [2+2]-cycloaddition of chlorosulfonyl isocyanate to sugar-vinyl ethers[117] and a stereochemical model rationalizing the observed stereoselectivity has been proposed.[118] The products of the cycloaddition have been subjected to intramolecular *N*-alkylation to give 1-oxabicyclic β-lactams fused to six- or seven-membered rings.[117] See section 4 for a new entry into the oxopenem ring system, viz. (54) to (55).[87] Clavam analogues have been used for the generation of azomethine ylids which were successfully employed in the synthesis of carbapenems *via* [1,3]-dipolar cycloaddition chemistry. This new approach by Gallagher *et al.* is described in section 9. The stereoselective cycloaddition of chlorosulfonyl isocyanate to 3-O-vinyl ethers derived from sugars has also led to the synthesis of series of oxacephems, one of which displayed weak antibacterial activity.[119]

8 Trinems and Related Compounds

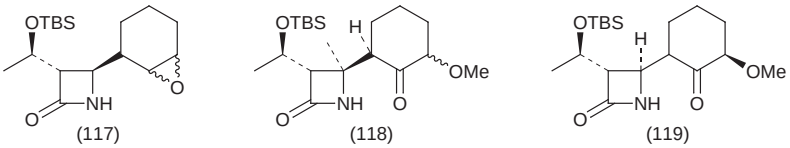
The trinems (a.k.a. the tribactams), discovered in the laboratories of Glaxo Wellcome (Verona), have been the subject of several detailed reports, with acid GV104326, Sanfetrinem, (112) and metabolically labile ester GV118819 (113) being presently in clinical trials. Structure-activity studies on 8-methoxy and 4-methoxy trinems, involving the synthesis of various isomers, indicated that (112) was the best compound in terms of antibacterial activity and β-lactamase stability.[120,121] These Glaxo Wellcome compounds were prepared by total synthesis from acetoxy azetidinone (114) and production costs may be a factor in compounds to be chosen for clinical use. Cyclisation of the bicyclic precursor

(115) was achieved by *N*-acylation with allyl oxalyl chloride in the presence of triethylamine followed by reflux of the resultant oxalimide in xylene with triethyl phosphite, to give (116) (Scheme 19).[120]



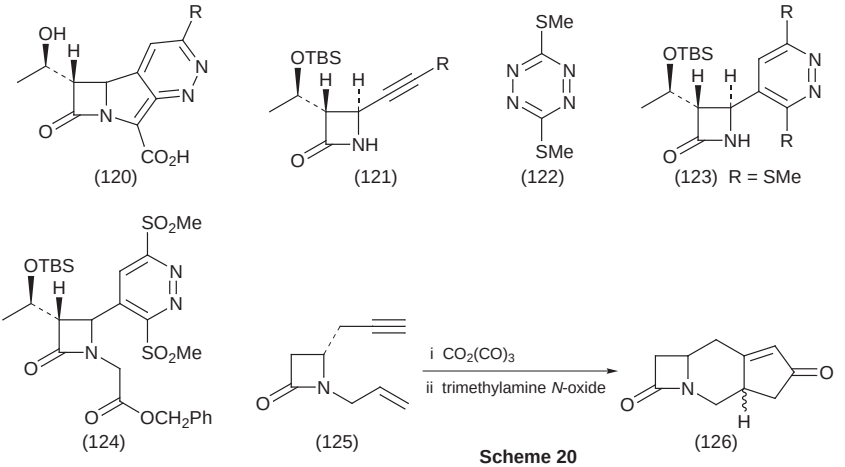
Scheme 19

Full details for preparation of the intermediate isomeric epoxides (117) have been reported.[122] In an alternative trinem synthesis, not requiring a chiral cyclohexanone, and also starting from (114), a key step was the deprotonation of epimeric (118), followed by formation of a zinc enolate complex which was quenched with diethylmalonate and then ammonium chloride to give (119) as the



major (>15:1) isomer.[123] Alternative trinem variants with different substituents at the 4-position have been of particular interest with 4-alkoxy,[121,124,125] 4-ureido,[126] and 4-amino[127] derivatives having been reported. Some of these show improved *in vitro* activity with respect to (112). Using modified versions of this scheme, 5- and 7-oxa-trinems with differing stereochemistries at C-8 have been prepared.[120] The results emphasize the importance of an allylic oxygen (at C-5 or C-7) in the 6-membered ring coupled with the *S*-stereochemistry at the 8-position. Since substitution with oxygen at C-5 or C-7 increases activity the results suggest the oxygen is not important for initial binding, but may activate the β-lactam or stabilize the acyl-enzyme complex. Since mechanistic studies have not yet been reported it cannot be ruled out that an elimination reaction occurs after acylation of the target enzymes.[128] However, the significant inhibitory activity observed for the 5α-hydroxyethyl compounds would argue against this.[128]

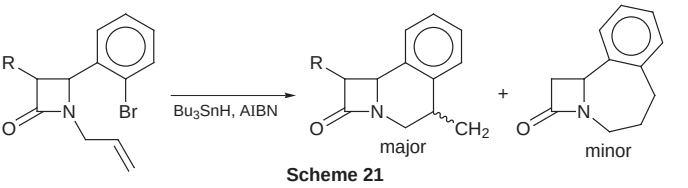
Stereo-controlled syntheses of Sanfenitrem (112) and its enantiomer have also been achieved using the ester enolate *N*-trimethylsilylimine approach.[129] Development of the Glaxo Wellcome compounds will probably kindle interest in other tricyclic β-lactams. Tricyclic diazo carbapenems (120), analogues of Sanfenitrem, have been synthesized.[130] The route involved inverse Diels-Alder reaction of (121) with (122) to give (123). *N*-Alkylation with benzylbromoacetate, followed by oxidation gave sulfone (124). Base (LHMDS) mediated cyclisation gave the desired tricyclic ring system. The Pauson-Khand reaction has been used for the synthesis of tricyclic β-lactams, treatment of enynes (125) with $Co_2(CO)_8$ followed by thermally or trimethylamine *N*-oxide mediated decomposition of the cobalt complexes gave tricyclic β-lactams (126) (Scheme 20).[131] An aryl radical cyclisation route has also been used to prepare tricyclic β-lactams. In the cyclisation step 6-exo-cyclisation predominated, but some 7-endo cyclisation was observed (Scheme 21).[132] Acylation of methylthioimidates (127) with substituted acetyl chlorides led to either β-lactam (128) or *N*-acyl products (120) depending on the structure of the bicyclic starting material and the substituent on the acetyl chloride.[133] Zwitterions (130) were proposed as intermediates (Scheme 22).



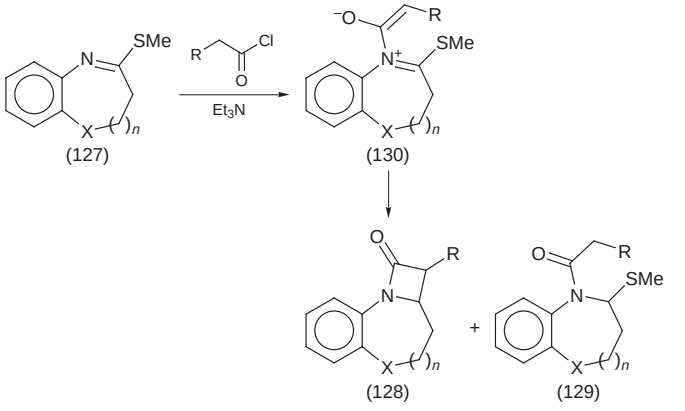
Scheme 20

9 Carbapenems

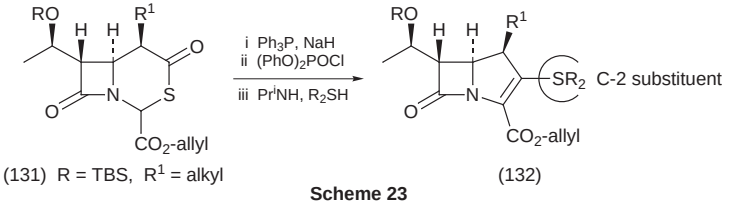
Whilst the development of new synthetic routes to the carbapenem nucleus remains an important area of research, exploration of the structure-activity relationship (SAR) around the C-2 substituent (see (132) in Scheme 23) is another focus of attention. In the light of resistance against β-lactam antibiotics in general an increase in potency and hydrolytic stability of carbapenems appears to be a viable strategy to combat bacterial infections. Hence another area of considerable interest concerns the study and improvement of the biological profile of carbapenems.



Scheme 21

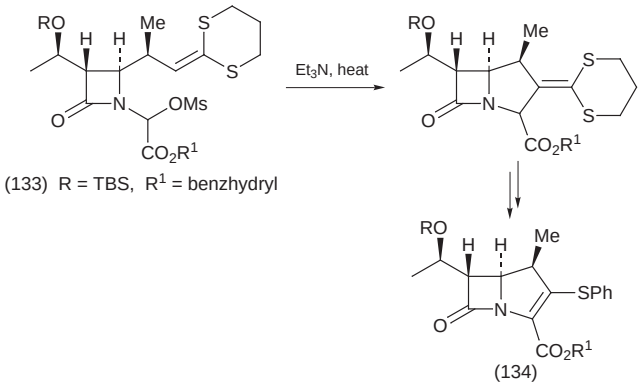


Scheme 22



Scheme 23

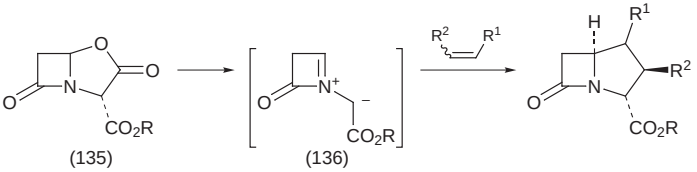
An Eschenmoser sulfide contraction reaction has been used as a key step for the construction of the carbapenem nucleus.[134] Thus, 1,3-thiazinone (131) was directly converted into (132) in a one-pot procedure in good yields (Scheme 23). The same authors have described a ketene dithioacetal terminated cyclisation approach to carbapenems.[135] The intermediate acyliminium ion was generated *in situ* by base-mediated elimination of mesylate (133) (Scheme 24). Transformation into 1β-methyl-carbapenem (134) was accomplished using standard procedures. Gallagher *et al.* have employed novel 1,3-dipolar cycloaddition chemistry to access the carbapenem nucleus.[136] Thermal decarboxylation of β-lactam based oxazolidinone (135) gave intermediate 1,3-dipole (136) which was trapped with a variety of dipolarophiles (Scheme 25) in moderate to good yields (see also the section on clavams).[137] Biologically more important Δ^2-carbapenems were only available *via* oxidation/elimination of C-2 selenides. Another approach to carba-



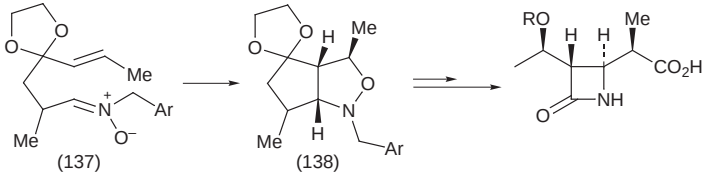
Scheme 24

penems employing 1,3-dipoles has been reported by Jung and Vu.[138] Intramolecular cycloaddition of (137) gave isoxazole (138) as a single diastereomer in 84% yield (Scheme 26). β-Amino acid carbapenem precursors were prepared using organo-cobalt chemistry (Scheme 27).[139] A modified Nicholas reaction gave acetylenic acid (139) which was converted by a Curtius rearrangement into *N*-BOC protected β-amino acid (140). An interesting feature of this chemistry is the control of relative and absolute chemistry at three contiguous carbon centres. In a formal synthesis of thienamycin a diastereoselective conjugate addition reaction of chiral allylamide (141) to (*E*)-t-butyl pentane-2,4-dienoate followed by a stereoselective aldol reaction figured as the key steps[140]. A Pd-mediated ring-closure with subsequent hydrogenation has been used to access 1β-methyl carbapenems (142) in good yields (Scheme 28). Pure 1β-isomer was isolated in 70% yield with the asymmetric induction being exerted by the stereogenic centre present in the rigid bicyclic ring system.[141]

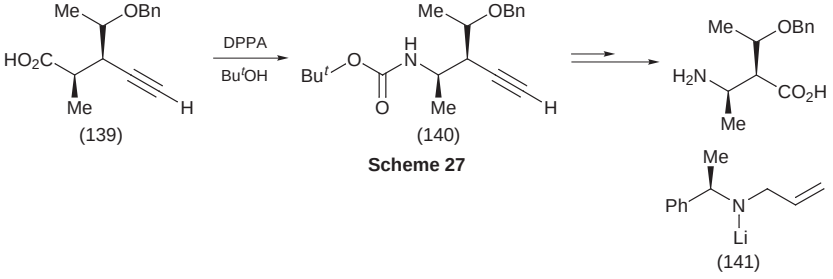
Improved syntheses of known carbapenem precursors have been reported (see also the section on azetidinone chemistry). Thus, bis(hydroxymethyl) acetaldehyde (143) was used in a protecting group controlled asymmetric induction approach to carbapenem precursor (144).[142] Two new approaches to (114) made use of (L)-threonine as a starting material.[143,144] Another synthesis of carbapenem precursors started from isoxazolidine derivatives (145).[145,146*a*] The latter were obtained *via* [1,3]-dipolar cycloaddition of appropriately functionalised nitrones and crotonates. *trans*-Ethyl crotonate has been used as inexpensive starting material in a synthesis of carbapenem precursor (146).[146*b*] Pyrrolidenes figured as starting materials in a conceptually novel approach to simple carbapenem analogues. The sequence involved a cyclisation of bis-acid (147).[147] Several approaches to crucial intermediate (149) for the synthesis of 1β-methyl carbapenems have been reported (Scheme 29). A number of steps were needed to obtain (149) from *gem*-dimethyl precursor (148) (route A in Scheme 29).[148] Azetidinone (149) was also obtained by $ZnCl_2$-mediated reaction of thioenolether (150) with (114) (route C in Scheme 29)[149]. The products can be transformed into



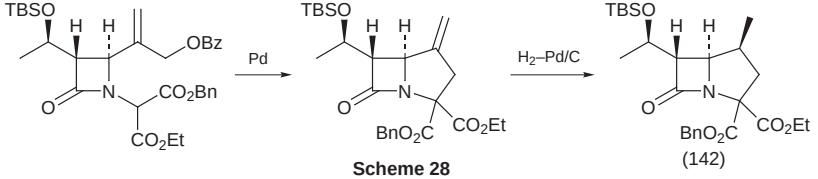
Scheme 25



Scheme 26



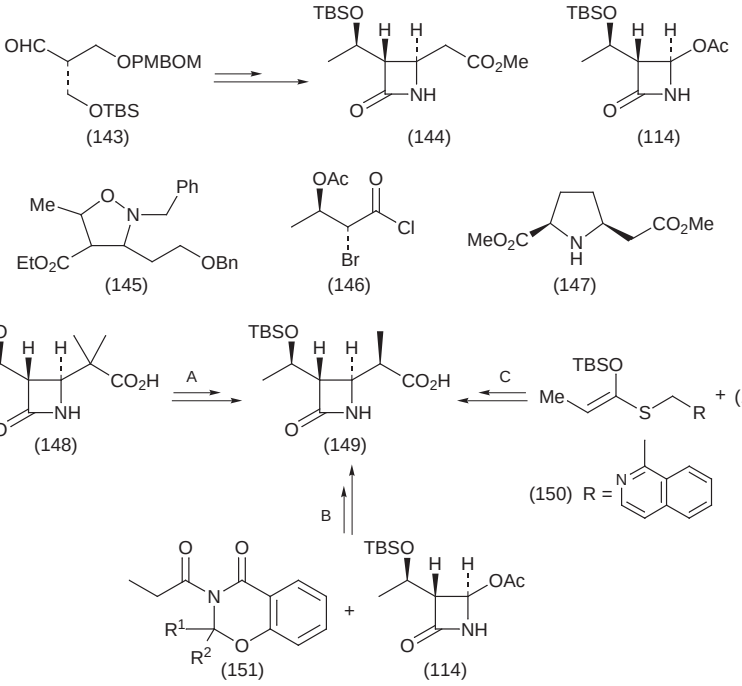
Scheme 27



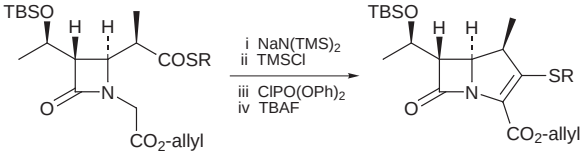
Scheme 28

common 1β-methyl carbapenem precursors. Another new route to (149) featues a reaction of 3-acetoxy-azetidinone (114) with propionyl lactam (151) (route B in Scheme 29).[150–152]

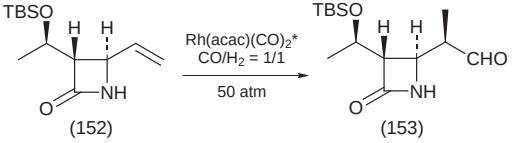
Dieckmann cyclisation followed by displacement of an intermediate vinylphospate by thiolates gave the bicyclic 1β-methyl carbapenem products in 52-62% overall yield (Scheme 30).[153,154] Asymmetric formylation of C-4 vinyl β-lactam (152) using a Rh(II)-catalyst with chiral phosphine ligands was shown to provide intermediate (153) (Scheme 31).[155] The reaction exhibited modest to good stereo- and regioselectivity. Interest in employing radical chemistry methodology for the synthesis of β-lactams continues. Ishibashi *et al.* reported a synthesis of the methyl ester of key intermediate (149) for carbapenem synthesis involving a moderately diastereoselective radical cyclisation (Scheme 32).[156,157] The com-



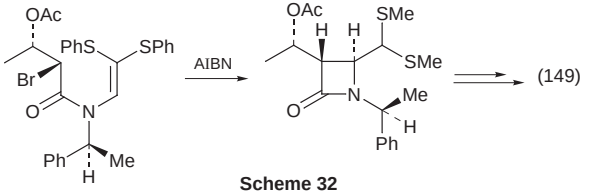
Scheme 29



Scheme 30



Scheme 31



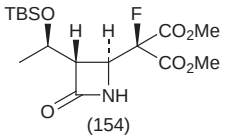
Scheme 32

bined effect of chiral centres in the C-3 side chain and as *N*-substituent led to a 3:1 ratio of diastereomers in the cyclisation step.[158]

Fluorinated azetidinone (154) was used as starting material in the synthesis of a 1β-fluoro-carbapenem.[159] Carbapenems with activity against methicillin-resistant *Staphylococcus aureus* (MRSA) are particularly interesting with regard to the current problems of bacterial resistance against β-lactam antibiotics. A series of lipophilic C-2 substituted carbapenems (*e.g.* 155) was evaluated for MRSA activity and found to have potent *in vitro* activity.[160] Dithiocarbamate carbapenems (156) were prepared *via* literature procedures and showed interesting potency against MRSA. The activity of some analogues was comparable to that of vancomycin.[161] The same authors report on the activity of pyrrolidine substituents at the C-2 position of 1β-methyl carbapenems. Several members of this new group of carbapenem derivatives were prepared.[162,163] On the basis of its good biological profile and physical properties Lenapenem (157) was chosen as a development candidate.[164] Armstrong *et al.* have described an efficient synthesis of the Lenapenem side chain starting from BOC-(L)-*trans*-4-hydroxyproline.[165] The parent compound of this series is Meropenem (158) which is one of three carbapenems approved for clinical use. More detailed SAR studies of Meropenem have been carried out.[166] Basicity of the side chain was found to influence antibiotic activity against some bacterial strains. The 1β-methyl group generally improved the stability against Gram-negative bacteria. The role of the 1β-methyl substituent was also studied using MM calculations and ^{1}H NMR spectroscopy.[167] The results suggest that this group interacts with the C-2 side chain and imparts a relative linear conformation on the molecule. The interest in SAR around the C-2 position has prompted the development of new chemistry. Nucleophilic displacement of C-2 sulfone groups by various thiols has been used in the synthesis of carbapenem analogues. The presence of $MgBr.Et_2O$ was found to be crucial for this reaction (Scheme 33).[168] The same authors accomplished a highly diastereoselective synthesis of (159) which can be converted into the corresponding carbapenem using reported literature procedures.[169]

Several examples for the use of organo-palladium chemistry for the introduction of modified C-2 side chains have been reported. Narukawa reported cross-coupling of enoltriflates (160)/(161) with organoboranes in good yields (64%-85%).[170] Direct alkylation of related enoltriflate (160) with allylacetate was achieved using a Heck reaction (Scheme 34).[171] The reaction was found to be restricted to the use of $Pd(dba)_2$ or $Pd(OAc)_2$ as catalysts. Evidence for differences in the hydrolysis mechanism of carbapenems and cephalosporin esters has been obtained.[172] The former appear to be more stable and hydrolyse through β-lactam ring opening whereas the latter decompose *via* initial Δ^3-Δ^2 double bond isomerisation. Variation of the C-6 substituent showed that the hydroxyethyl group is crucial for optimal activity.[173] A new route for the synthesis of the pyrrolidin-4-yl-thio side chain which is found in Meropenem has been reported in which thiolactone (162) figured as key intermediate.[174]

Lee *et al.* have investigated the influence of different pyrrolidine side-chains on antibiotic potency. Analogues (163) and (164) were found to exhibit the best combination of *in vivo* potency and stability.[175] Another group studied the



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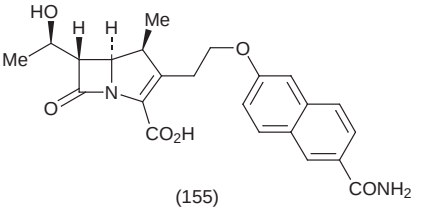

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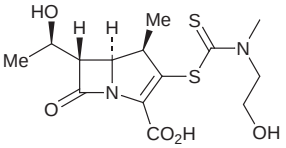
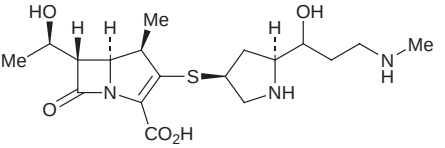
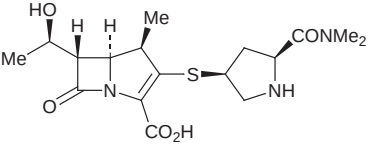


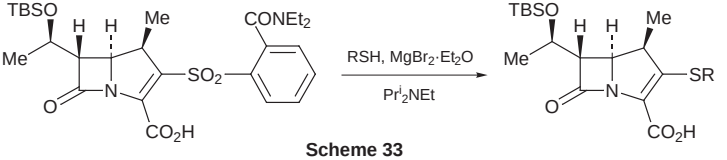
(156)



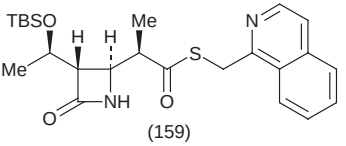
(157) Lenapenem



(158) Meropenem

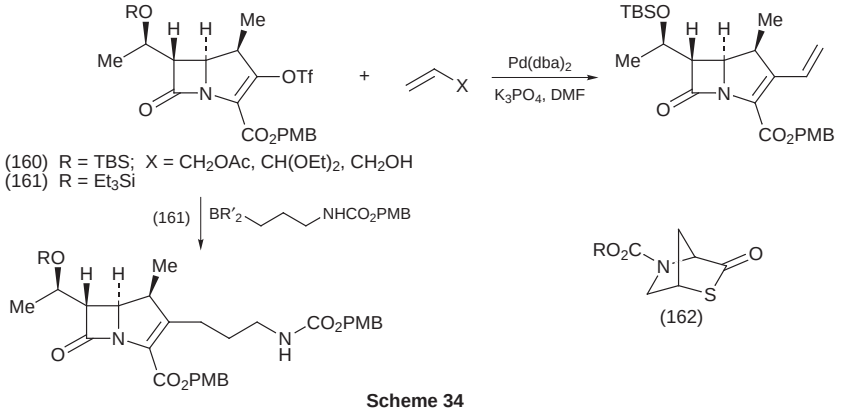


Scheme 33



(159)

influence of a sulfamoylamino group in the same position.[176] Compound S-4661 (165), prepared using Mitsunobu chemistry, was reported to have the best combination of properties.[177] The effect of heterocycles in the C-2 position on biological activity has been investigated. Saturated compounds such as (166) and semi-saturated ones such as (167) were prepared using standard chemistry. Despite some potent activity the overall activity profile and the stability against DHP-1 were found to be inferior to Meropenem.[178,179] C-2 α-(Hydroxy)benzyl-substituted carbapenems (168) were prepared in the same laboratories and showed a better activity/stability profile, similar to the standard Meropenem.[180] Tetrahydrofuranyl carbapenems (169) were prepared and shown to have mod-

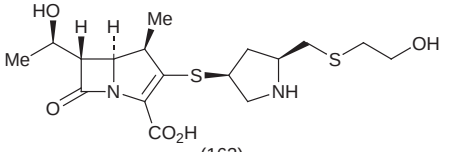


Scheme 34

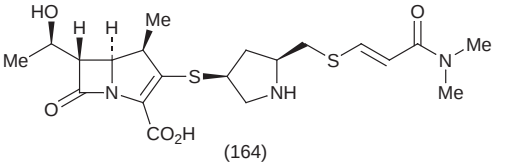
erate activity against some species, whilst being completely inactive towards MRSA.[181] The activity of diazabicyclo-substituted (169a) has been investigated.[182] Oxetane carbapenems as analogues of tetrahydrofuranyl substituted (169) were obtained by literature methods. Their significant biological activity was offset by their hydrolytic instability towards renal DHP.[183] The latter property could be improved by introduction of a vinyl substituent in the C-2 position as reported by Yamada *et al.*[184] Remarkably, (169b) lacking the 1β-methyl substituent was both active and stable towards hydrolysis. These findings might show the way for an alternative to the 1β-methyl strategy for imparting hydrolytic stability onto carbapenems.

10 Azetidinones

10.1 Synthesis of Azetidinones – Despite the wealth of previous work in this area, efforts are still being made to improve existing syntheses or even develop new routes to the azetidinone ring system. With respect to the latter, enantioselective approaches are particularly noteworthy. Several reports concern stereoselective versions of the Staudinger reaction. Double stereodifferentiating [2+2] cycloadditions of chiral aminoketenes (Evans-Sjögren ketene derived from (170)) with chiral imines (171) have been studied and matched and mismatched cases were characterized.[185] The same authors reported the synthesis of *N*-methylidene[bis(trimethylsilyl)methyl]amine (172), the first isolable and stable monomeric methanimine for use in the Staudinger reaction.[186] Cycloaddition of (172) to ketenes occurs in moderate to good yields. The bis(trimethylsilyl)-moiety can be removed under mildly oxidative conditions using CAN (cerium ammonium nitrate). Imine substituents of enolisable α-(trimethylsilyl)methyl imines and their effects on [2+2] cycloaddition were investigated in detail.[187,188] These compounds were then applied for the construction of the quaternary centre in 4,4-disubstituted azetidinones.[189] Several reports concern the use of new chiral auxiliaries in



(163)



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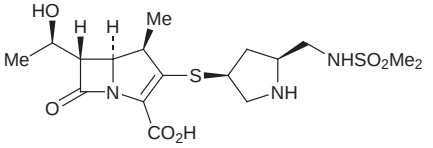
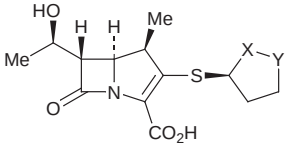
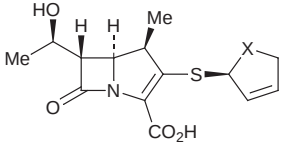


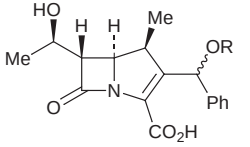
(165) S4661



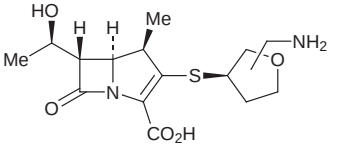
(166) X = N, O; Y = N



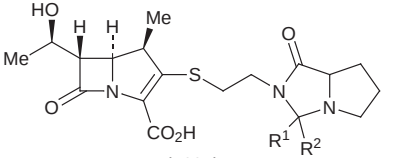
(167) X = O, S



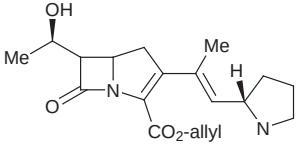
(168) R = H, Me



(169)



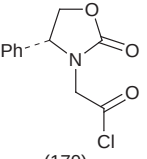
(169a)



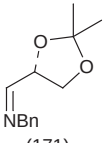
(169b)

the Staudinger reaction. Thus, imines (173) containing a bicyclic chiral auxiliary derived from (+)-3-carene were shown to exhibit high diastereofacial selectivity leading to β-lactams applicable to the synthesis of the anticancer drug taxol (see also section on further uses of azetidinones).[190,191] Use of chirally modified imine (174) led exclusively to 3,4-*cis* products in good to excellent yields. Further modification of the products gave access to pyrrolidinones and other heterocyclic

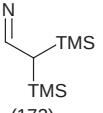
products.[192,193] The Oppolzer sultam auxiliary has been successfully used for the diastereoselective synthesis of azetidinones. Staudinger reaction of imines with sultam-derived ketenes proceeded in good chemical yield to give the *cis*-products as single diastereomers.[194] Chiral auxiliary (175) was used for chiral Staudinger reactions with phthalimidoketene. The diastereoselectivity was found to depend on the diol protecting groups in (175).[195]



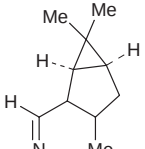
(170)



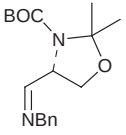
(171)



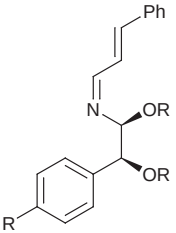
(172)



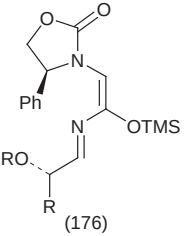
(173)



(174)



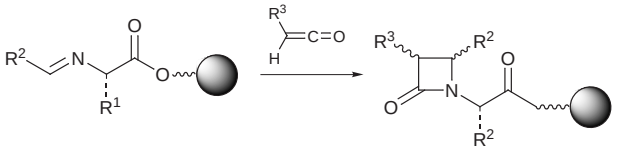
(175)



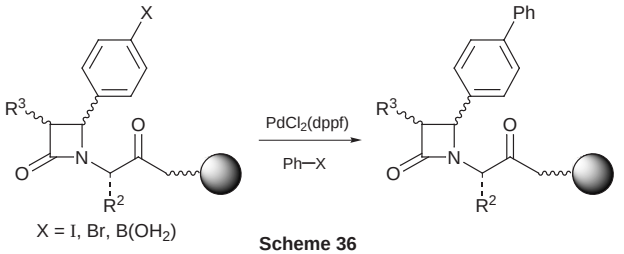
(176)

High *trans*-selectivity regardless of substituents and solvents was observed in a new two-step Staudinger reaction proceeding *via* chirally modified intermediate (176).[196] Overall this procedure constitutes an enantioselective approach to 3-amino-4-substituted azetidin-2-ones. The increasing importance of solid-phase chemistry is also reflected in the field of β-lactams. Staudinger reaction of ketenes with amino acid derived imines attached to solid phase *via* a photolabile linker has been reported to proceed in generally good yields with varying degrees of diastereoselectivity (Scheme 35).[197] Further modification of solid-phase supported 4-arylazetidinones is possible using Suzuki and Heck-type coupling reactions (Scheme 36).[198] Another approach to ketenes for use in the Staudinger rection involves the photochemical rearrangement of amino acid diazoketones. The latter are readily available by Wolff rearrangement of amino acids.[199,200] The reaction of azadienes (177) with ketenes has been studied and shown to afford azetidinones under kinetic reaction conditions.[201] Absolute asymmetric synthesis of azetidinones *via* irradiation of single crystals of substituted 2-pyridinones has been further investigated.[202] Photochemical conversion of optically active crystals of oxo-amides which are themselves optically inactive into chiral azetidinones has also been reported.[203]

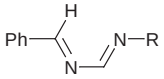
Enzyme-mediated methods for the synthesis of optically pure azetidinones have been further explored. Enantiopure 3-amino-azetidinone was prepared by kinetic enzymatic resolution with Penicillin G Acylase.[204] Chiral β-lactam precursor (178) was obtained by Baker's yeast mediated reduction of the



Scheme 35

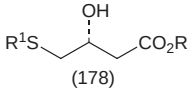


Scheme 36

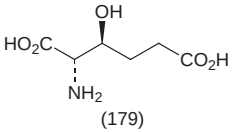


(177)

corresponding β-keto ester.[205] Serinehydroxymethyltransferase has been used for the synthesis of β-lactam precursor (179).[206] Lipase-mediated resolution of azetidinones has also been investigated.[207]

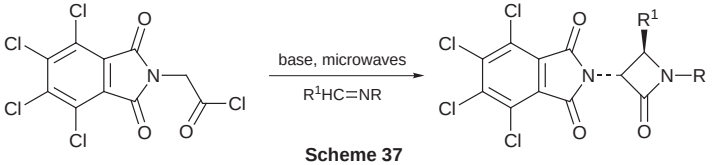


(178)



(179)

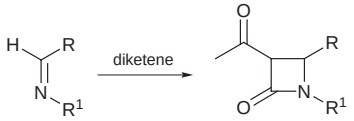
The emergence of microwaves from obscurity to becoming a standard tool in preparative organic synthesis is also reflected in the field of azetidinones. 3-Amino-substituted azetidinones were obtained from tetrachlorophthaloyl-protected acid chlorides and imines in a microwave-assisted Staudinger reaction (Scheme 37).[208] Short reaction times, high yields, and predominant formation of the *trans*-isomer illustrate the potential advantage of microwave irradiation over



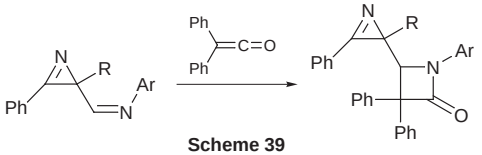
Scheme 37

conventional heating methods. Whilst these studies were carried out in a domestic oven, no technical details were disclosed in another report about microwave-assisted Staudinger-type synthesis of 3-chloro-azetidinones.[209]

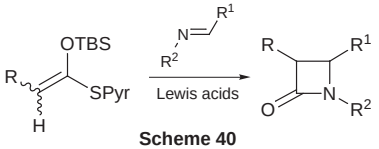
The use of diketene in the Staudinger reaction has been studied in more detail.[210] This approach provides direct access to 3-acetyl-substituted azetidinones (Scheme 38). Aziridine-containing imines react with diphenylketene leading exclusively to formation of azetidinones (Scheme 39).[211] Silylketene thioacetals derived from 2-pyridylthioesters have been shown to give azetidinones in a Lewis-acid promoted condensation reaction with imines (Scheme 40).[212] Apart from previously known Lewis acid promoters for this reaction, $AlBr_3$ and $EtAlCl_2$ were also shown to effect the condensation of 2-pyridylthioesters with imines.[213] The reaction proceeds in good yields for a variety of substrates and gives predominantly 3,4-*trans* products. $Yb(TfO)_3$ as Lewis acid can be used in catalytic amounts and permits the reaction to be carried out as a one-pot two- or three-component condensation reaction.[214]



Scheme 38



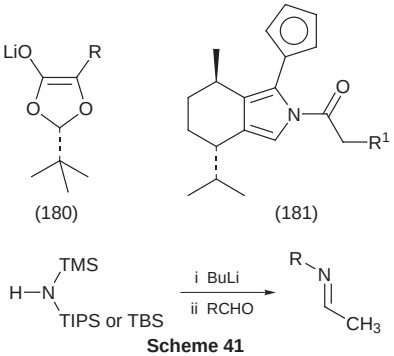
Scheme 39



Scheme 40

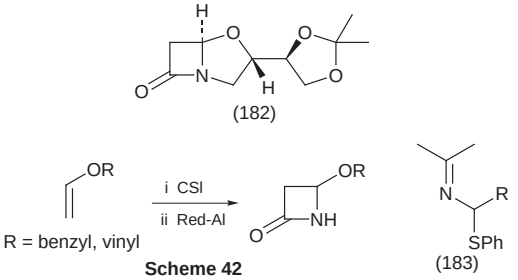
Reaction of optically active enolate (180) with imines gave chiral azetidinones with full stereocontrol at C-3.[215] Chiral pyrrazole (181) has been employed in a diastereoselective reaction with imines to give chiral azetidinones.[216] The same auxiliary has been used in a diastereoselective 1,3-dipolar cycloaddition to give isoxazoles which were further converted into azetidinones in moderate yield.[217] A new synthesis of *N*-silylated imines from unsymmetrical disilylamines by treatment with butyllithium has been disclosed (Scheme 41).[218]

The use of carbohydrates as chiral auxiliaries for the [2+2] cycloaddition of chlorosulfonylisocyanate (CSI) to double bonds continues to be investigated.[219] Using previously described chemistry a synthesis of dioxolanylclavam (182) from tartaric acid has been achieved by [2+2] cycloaddition of CSI and carbohydrate substituted vinylethers.[220] *N*-Unsubstituted 4-benzyloxy- and 4-vinyloxy-azetidi-



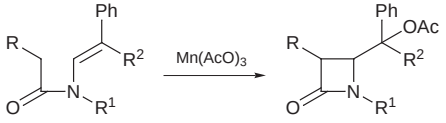
Scheme 41

nones were prepared for the first time directly by reaction of readily available vinyl or benzyl ethers and CSI (Scheme 42).[221,222] The reaction of isocyanates and 2,3-dihydrofuran has been shown to occur under pressure.[223] Another route to *N*-unsubstituted azetidinones involves reaction of imines (183) with acid chlorides in the presence of base followed by oxidative deprotection.[224]



Scheme 42

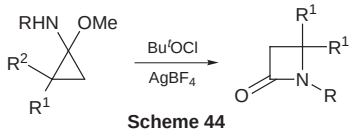
Several reports investigate new radical-based methodology for the synthesis of azetidinones. $Mn(OAc)_3$ has been used as promoter for radical cyclisations of substituted enamines to azetidinones in varying yields (23-73%) and with exclusive formation of the 3,4-*trans* isomer (Scheme 43).[225] A similar approach using CAN as radical initiator gave also predominantly 3,4-*trans* products, but proceeded in slightly lower yields.[226] The same group has disclosed a practical route to the enamide starting material for these reactions.[227]



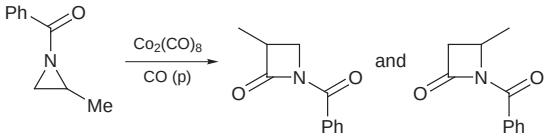
Scheme 43

Ring-expansion routes to azetidinones have been relatively little explored so far, presumably due to the difficulties associated with the preparation and

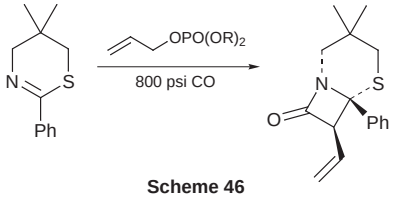
properties of the three-membered ring precursors of β-lactams. It has now been shown that silver-induced ring-expansion of 2,2-disubstituted 1-methoxycyclopropylamines affords azetidinones in 40-95% overall yields (Scheme 44).[228] The same authors report the preparation of 2-methylene-aziridines, which can serve as precursors to azetidinone derivatives.[229] Cobalt tetracarbonyl anion as catalyst has been shown to effect carbonylation of aziridines to azetidinones (Scheme 45).[230] The mechanism was investigated in detail and shown to involve nucleophilic ring-opening of the heterocycle leading to inversion. Carbonylative coupling and cyclisation of 1,3-thiazines and allylphosphates has been reported to give access to bicyclic β-lactams. The reaction proceeds stereoselectively to give *cis*-products (Scheme 46). Electrochemical generation of an azetidin-2-one derived *N*-acyl iminium ion followed by trapping with a known [3+2]-annulation reagent gave 4-substituted azetidinones in good yield (Scheme 47).[231]



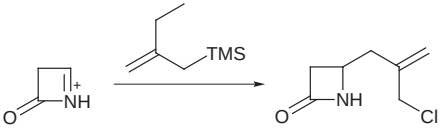
Scheme 44



Scheme 45



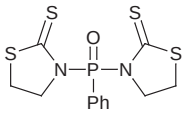
Scheme 46



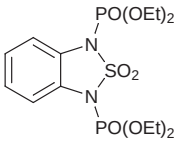
Scheme 47

Dehydration of β-amino acids provides a convenient entry to azetidinones. Phosphorus-containing derivatives (184)[232] and (185)[233] have been used as dehydrating reagents for β-amino acids. Reformatsky reaction of α-amino nitriles has been shown to produce several products including β-lactams.[234]

Theoretical investigations into the chemistry and properties of azetidinones continue.[235–238] Properties of carbocations derived from β-lactams and thio-β-



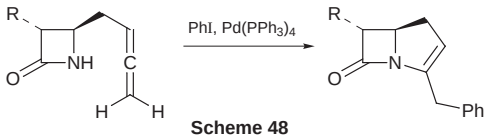
(184)



(185)

lactams have been investigated both experimentally and computationally.[239] A number of structures of azetidinones obtained by conventional methods have been studied by X-ray single crystal analysis.[240–244] Several reports of new azetidinones and their chemistry and properties have appeared.[245–253] A synthesis of a ^{3}H and ^{14}C labelled azetidinone has been reported.[254] 3-Methylazetidinone, the simplest optically active β-lactam has been prepared and its optical properties have been fully characterized for the first time.[255] Azetidinones have been shown to be occurring as fragmentation products of electron-mass impact fragmentation of 2-piperidones.[256]

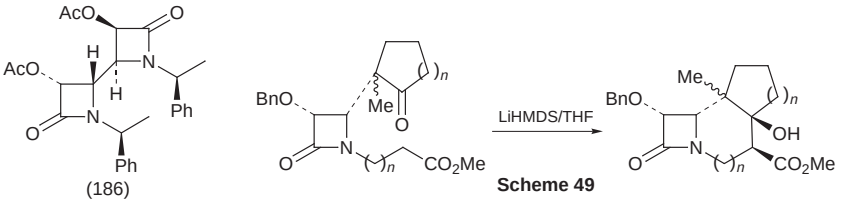
10.2 *N*-Chemistry – Pd-catalysed reaction of ω-2,3-butadienyl (allene) substituted azetidinones with aryliodides afforded bicyclic ring systems (Scheme 48).[257] *N*-Protection of β-lactams with 4,4-dimethoxybenzhydrol has been reported to occur in acetic acid under H_2SO_4 catalysis.[258] *N*-Arylsulfonation of azetidinones has been investigated. The yield of these reactions could be substantially improved by using excess of arylsulfonating reagents at low temperatures.[259]



Scheme 48

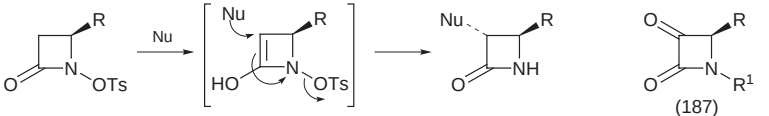
10.3 C-3, C-3′ and C-4, C-4′ Chemistry – Further details of the Baeyer-Villiger oxidation of β-lactams have been reported.[260] This stereoselective reaction constitutes one of the few examples for the preferred migration of a carbon atom over hydrogen in an aliphatic aldehyde under Baeyer-Villiger conditions. The same authors studied the synthesis of C-4, C-4′-bis-β-lactams (186) and their base induced rearrangement to fused bis-γ-lactams.[261] Several types of bi- and tricyclic β-lactams were obtained by Dieckmann cyclisation of azetidinone precursors (Scheme 49). Addition of nucleophiles to *N*-tosyloxyazetidinones in the 3-position has been further studied and details for the addition of amines have been reported. The reaction depends on the steric bulk and the basicity of the amine. Under carefully adjusted reaction conditions predominantly 3,4-*trans* products were obtained (Scheme 50).[262] Allylation of 3-oxo-azetidinone (187) was achieved in aqueous media *via* an indium-mediated Barbier reaction which resulted in the predominant formation of the 3,4-*trans* product.[263] An approach to Asperenomycin analogues from 3-vinyl and 3-isopropenyl azetidin-2-ones has been disclosed.[264] Electrophilic fluorination of 3-acetyl-azetidinones has been reported to

give 3-fluoro-azetidinones with reasonable diastereomeric excess at low temperature. The reaction proceeds best with fluorosulfinimides whilst fluoropyridinium triflates do not give any reaction products.[265] Fluorothioacetic acid was used as starting material in another synthesis of 3-fluoro-azetidinones *via* enolate condensation with imines.[266]



(186)

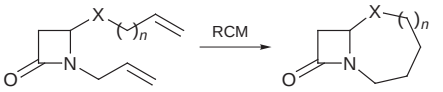
Scheme 49



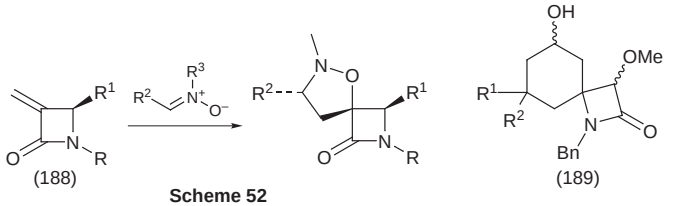
(187)

Scheme 50

Ring closing metathesis (RCM) has received much attention as a convenient method for the synthesis of medium- to large-sized rings. Its application to the synthesis of functionalised mono- and bicyclic β-lactams has been reported for the first time using the Schrock and the Grubbs catalysts (Scheme 51).[267,268] The related enyne metathesis of an appropriately functionalised precursor was achieved with the latter catalyst.[269] Ozonolysis of C-4 styrylazetidinones has been shown to proceed more smoothly in the presence of metal chlorides, such as $ZnCl_2$, $CaCl_2$, $AlCl_3$, and $TiCl_4$.[270] Resolution of 3-hydroxyazetidinones which are used in the semi-synthetic preparation of taxol has been achieved *via* a glycosylation reaction.[271,272] 1,3-Dipolar cycloaddition of nitrones to (188) has been studied (Scheme 52). The products were converted into 3-hydroxyazetidinones.[273] The intermediacy of spiro-β-lactams such as (189) in the photore-



Scheme 51

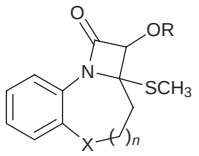


(188) (189)

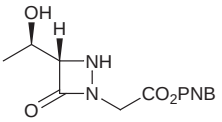
Scheme 52

arrangement of *N*-alkanoyl β-enaminones to α-amino-β,γ-unsaturated amides in a single step has been investigated.[274]

10.4 Other Chemistry – A previously reported unusual cyanide-mediated β-lactam ring cleavage affording acyclic N,O- and N,S-acetals has been studied in detail.[275] The trifluoroacetic acid mediated ring-opening of tricyclic azetidinones (190) has been studied in detail and a mechanism for the formation of products has been proposed.[133,276] 1,2-Diazetidinones (191) were prepared and used in the synthesis of azacarbapenems.[277]

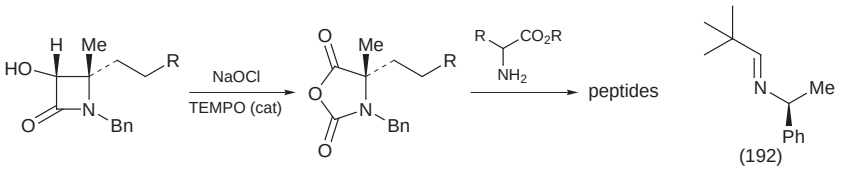


(190) X = O, S; n = 0, 1



(191)

10.5 Further Uses of Azetidinones and New Applications – TEMPO-oxidation of C-4 quaternary substituted azetidinones has been reported to give α-amino acid *N*-carboxy anhydrides ('UNCA's') in a one-pot reaction (Scheme 53).[278] The latter were incorporated into peptides and used for the first direct synthesis of peptidyl nucleoside antibiotics.[279] Use of imine (192) for this approach gave access to otherwise difficult to prepare (*S*)- and (*R*)-tert leucine.[280] A chiral dirhodium(II) azetidinone-carboxylate catalyst was shown to provide high levels of enantioselectivity in intermolecular cyclopropanation reactions of diazoacetates.[281] The use of norbornane-fused azetidinones as starting materials in the synthesis of quinazoline alkaloids has been reported.[282] 3-Acetoxy-azetidinone was used as starting point in a synthesis of 3-amino-3-vinyl-3-propionic acid which was converted further into aspartate semialdehyde hydrochloride salt.[283]

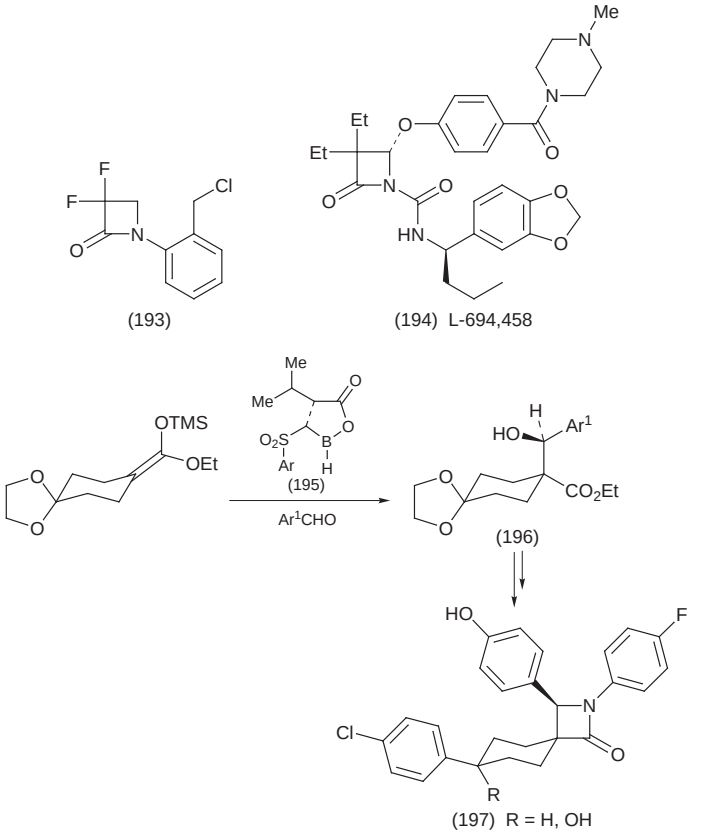


(192)

Scheme 53

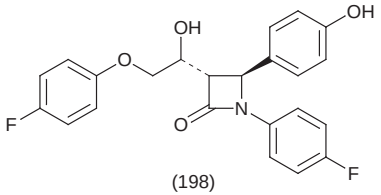
Several reports concern the use of azetidinones for non-classical applications, *i.e.* outside the antibiotic area. Inhibition of human leucocyte elastase (HLE) with *N*-(2-chloromethylphenyl)-3,3-difluoroazetidinone (193) possessing a latent methylene quinoniminium function has been further investigated.[284] Known potent HLE inhibitor L-694,458 (194) was prepared enantioselectively by lipase mediated resolution of an azetidinone intermediate.[285] Inhibition of cholesterol absorption appears to become an incresingly important application for azetidi-

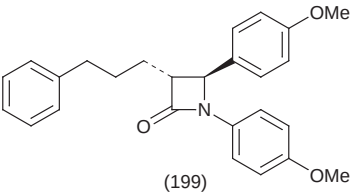
nones. Cholesterol absorption inhibitor (197) was prepared *via* β-hydroxy acid (196) obtained from a catalytic Mukayama-type aldol addition involving amino acid derived catalyst (195) (Scheme 54).[202]

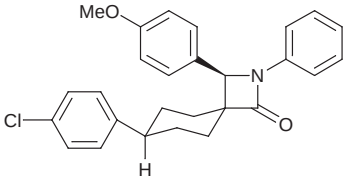


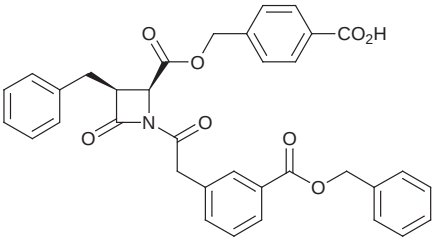
Scheme 54

More conventional methodology was used for the preparation of (198),[286] (199)[287] and (200)[288] as inhibitors of the same metabolic pathway. A Reformatsky reaction of chirally modified bromo-acetates with imines was used for the preparation of optically pure azetidinones. The latter were intermediates in another synthesis of cholesterol absorption inhibitors.[289] Design and synthesis of azetidinone inhibitor (201) of prostate specific antigen (PSA), an important serine protease, has been reported.[290] Azetidinone intermediates were used as intermediates for the synthesis of a thrombin inhibitor[291]. β-Lactams also figured as key-intermediate in the synthesis of β-turn mimetics.[292,293] Simple azetidinone (202) was prepared as a Phe-Gly methyl ester dipeptide analogue and shown to be a non-time-dependent inhibitor of α-chymotrypsin, carboxypeptidase A and other

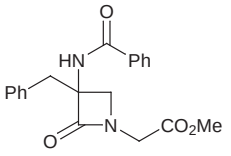

(198)

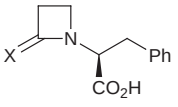

(199)

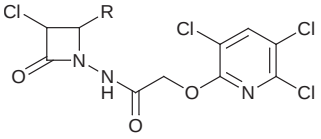

(200)

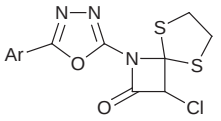

(201)

proteases.[294] A solution phase combinatorial approach to azetidinone libraries has been reported. A 126-member library was prepared *via* the Ugi multi-component-condensation reaction with the aim of discovering new protease inhibitors. The exploration of hybrid systems of known pharmocophores continues. Fusion of the enediyne motive with β-lactam rings has already been described and further details of this chemistry have been disclosed.[296] Carboxypeptidase A is competitively inhibited by compound (203).[297] Azetidine derivatives (204)[298] and (205)[299] were synthesized using standard methodology and shown to have herbicidal properties.

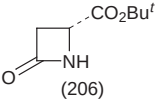

(202)


(203) X = O,S


(204)

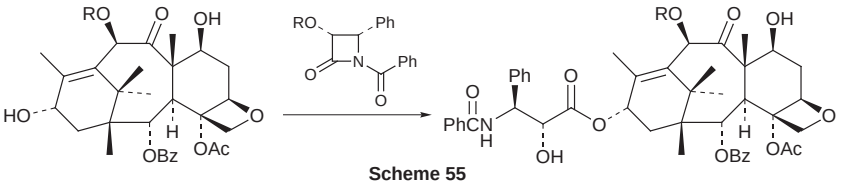

(205)

Interest in the use of azetidinones in polymer preparation continues.[300] Polymerization of (D)-aspartate-derived azetidinone (206) gave a chiral polymer whose physico-chemical properties have been characterized.[301, 302]



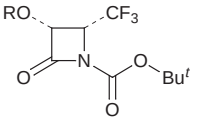
(206)

A very important application of azetidinones lies in the semi-synthesis of the potent anti-cancer drug taxol. The process involves β-lactam-mediated acylation of baccatin III to give the natural product taxol (Scheme 55). The former is relatively readily available from natural sources. This route is not only of commercial importance, but was also used as final step in another total synthesis of the actual anticancer compound.[303]

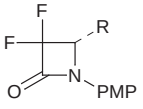


Scheme 55

Further progress in the treatment of ovarian and breast cancer with taxol will rely on the exploration of its SAR using new analogues of the parent compound. Structurally modified azetidinone precursors for the introduction of new side chains are a valuable tool in this respect. Fluorinated analogues appear to have received particular attention recently. Thus, second generation taxoids possessing a trifluoromethyl-group instead of the 3′-phenyl group were prepared from azetidinone (207) and shown to have good *in-vitro* activity against several different human cancer cell lines.[304, 305] Following a similar approach 2,2-difluoro-analogues of taxol were prepared from precursor (208). Their biological activity was comparable and in some cases superior to the natural product.[306] Other SAR-studies include change of the linker between the β-amino acid side chain and the polycyclic nucleus of taxol from an ester to an amide moiety. This was found to result in a complete loss of activity. Several other reports concern modification of the polycyclic nucleus in which introduction of the side-chain by azetidinone methodology figured as the final steps.



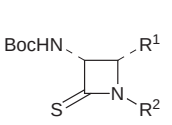
(207)



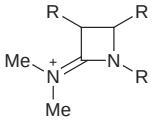
(208)

11 Related Systems

Thiono-monobactam derivatives (209)[307] and C-4 side chain thio and dithiocarbamate derivatives[308] were prepared and found to have weak antibiotic properties. Preparation and properties of 2-azetidin-iminium (210) salts have been systematically studied.[309]

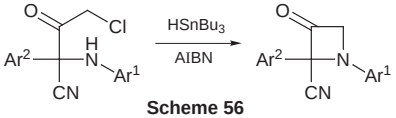


(209)

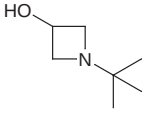


(210)

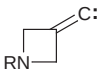
Azetidon-3-ones were prepared by radical-mediated cyclisation in good yields (Scheme 56). Formation of the four-membered ring was confirmed using extensive NMR-studies.[310] Acylative dealkylation of *N-tert*-butyl azetidines (211) followed by oxidation has been disclosed as a new entry to azetidin-3-ones.[311] The stability of azetidin-3-one derived carbene (212) has been investigated.[312] Properties and chemistry of 4,4-disubstitued 1,2-thiazetidin-3-one (213) have been studied.[313,314] Unusual quinazolines were obtained by [2+2]-cycloaddition of ketenimines with imines.[315]



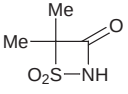
Scheme 56



(211)



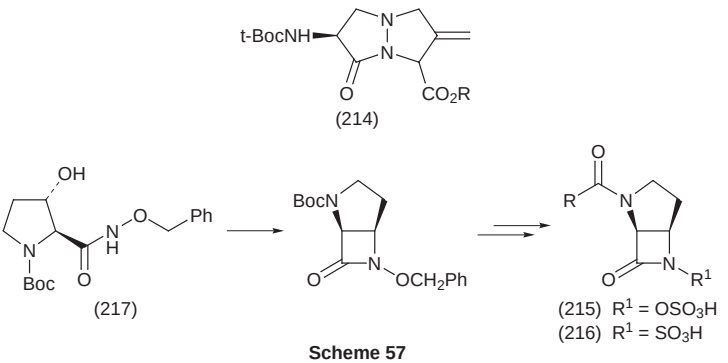
(212)



(213)

12 Major Structural Variants

A procedure for the preparation of 3-keto bicyclic pyrazolidinone (214), an intermediate for the synthesis of bioactive bicyclic pyrazolidines, has been reported[316]. A strategy for the incorporation of the construction of combinatorial libraries of β-sultams has been described:[317] polymer bound imines, derived by reaction of aldehydes with polymer bound amines were reacted with chlorosulfonyl acetates to give β-sultams bound to the solid phase. Bicyclic β-lactams (215) and (216), first synthesized by researchers at Hoffman La Roche, are potent inhibitors of Class C β-lactamases. A short synthesis, from *trans*-3-hydroxyproline derivative (217) of these new structural variants has been developed in which the key step is a Mitsunobu cyclisation (Scheme 57).[318]



Scheme 57

13 Appendix A: Review Articles

Biosynthesis

1. Molecular regulation of penicillin biosynthesis in *Aspergillus* (Emericella) *nidulans*.[319]
2. Engineering antibiotic producers to overcome the limitations of classical strain improvement programmes.[320]
3. Manufacture of β-lactam antibiotics by microorganisms. Application potentials of gene technology in biotechnological manufacturing of novel antibiotics derivates.[321]
4. Comparative genetics and molecular biology of β-lactam biosynthesis.[322]
5. Advances in the molecular genetics of β-lactam antibiotic biosynthesis.[323]

Resistance

1. Emerging antibiotic resistance.[324]
2. Mechanisms of methicillin resistance in staphylococci.[325]
3. Penicillin-binding proteins. Wall peptidoglycan assembly and resistance to penicillin: facts, doubts and hopes.[326]
4. Extended-spectrum β-lactamases: phenotype and detection.[327]
5. Evolution of penicillin-binding protein genes of sensitive streptococci into resistance determinants of *Streptococcus pneumoniae*.[328]
6. β-Lactam resistance determinants in *Streptococcus pneumoniae*: perspectives for new antibiotic targets.[329]
7. Penicillin binding proteins. Target of β-lactam antibiotics. Activity mechanism of β-lactamases and their inhibitors.[330]
8. β-Lactamases: quantity and resistance.[331]
9. Acquired carbapenemases.[332]
10. β-Lactamases: quality and resistance.[333]

11. Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics.[334]
12. The evolution of resistance to cephalosporins.[335]
13. Molecular bases for interactions between β-lactam antibiotics and β-lactamases.[336]
14. Broad-spectrum β-lactam antibiotics with β-lactamase inhibitors.[337]
15. Inhibitor-resistant β-lactamases.[338]
16. Structure and evolution of β-lactamase genes from *Streptomyces*.[339]
17. Complete mutagenesis of the gene encoding TEM-1 β-lactamase.[340]
18. New concepts of inhibition of penicillin sensitive enzymes.[341]
19. The *Aeromonas* metallo-β-lactamases: genetics, enzymology, and contribution to drug resistance.[342]
20. Atomic genetics and structure-function studies of PSE-4: a model enzyme for class A β-lactamases.[343]
21. Convergence of the β-lactamase induction and murein recycling pathways in enterobacteria.[344]
22. β-Lactamases and peptidoglycan recycling.[345]

Chemistry and Miscellaneous Topics

1. Rapid synthesis of β-lactams as intermediates for natural products *via* eco-friendly reactions.[346]
2. Preparations of two pivotal intermediates for the synthesis of 1-β-methyl carbapenem antibiotics.[347]
3. Trinems: synthesis and antibacterial activity of a new generation of antibacterial β-lactams.[348]
4. Stereocontrolled synthesis of 1-oxabicyclic β-lactam antibiotics *via* [2+2]-cycloaddition of isocyanates to sugar vinyl ethers.[349]
5. From penicillin to the proliferation of β-lactams. A fifty-year history.[350]
6. Switching of stereochemistry using different metal enolate species for construction of β-lactam skeletons.[351]
7. The chemistry of trinems.[352]
8. Developments in liquid membrane separation of β-lactam antibiotics.[353]
9. Penicillin and beyond: evolution, protein fold, multimodular polypeptides, and multiprotein complexes.[354]
10. Strategy and tactics in combinatorial organic synthesis. applications to drug discovery.[355]
11. Nucleophilic addition to the α-carbon of β-lactams.[356]
12. Drug chirality: a consideration of the significance of the stereochemistry of antimicrobial agents.[357]
13. Use of heteroatom-containing small cyclic compounds for enzyme inhibitor design.[358]
14. Asymmetric Pummerer-type reactions induced by O-silylated ketene acetals.[359]

15. Purification of microbially produced clavulanic acid by new sorption systems.[360]
16. Asymmetric synthesis of building-blocks for peptides and peptidomimetics by means of the β-lactam synthon method.[61a]
17. The synthesis of β-amino acids and their derivatives from β-lactams.[361b]
18. Syntheses of β-lactam antibiotics *via* ester enolate-imine condensation routes.[362]
19. Four-membered ring systems.[363]
20. β-Amino acids and β-lactams: synthesis and applications.[364]
21. β-Lactam chemistry.[365]
22. Cure for a crisis?[366]
23. A new strategic intermediate for β-lactam antibiotic synthesis. Allenecarboxylates.[367]

14 Appendix B: Additional articles

Penicillin Acylases

1. Enzymic conversion of cephalosporin C into glutaryl 7-aminocephalosporanic acid. A study in different reactor configurations.[368]
2. D-Amino acid oxidase from *Trigonopsis variabilis*: immobilization of whole cells in natural polymeric gels for glutaryl-7-aminocephalosporanic acid production.[369]
3. An engineered penicillin acylase with altered surface charge is more stable in alkaline pH.[370]
4. Synthesis of antibiotics (cephaloglycin) catalysed by penicillin G acylase: evaluation and optimization of different synthetic approaches.[371]

Cephalosporin Acylases/Acyltransferases and Acetylases

1. Synthesis of antibiotics (cephaloglycin) catalysed by penicillin G acylase: evaluation and optimization of different synthetic approaches.[372]
2. Cephalosporin modification: an extracellular glutaryl-7-ACA acylase from *Bacillus* sp.[373]

Clavam Biosynthesis

1. A straightforward synthesis of proclavaminic acid, a biosynthetic precursor of clavulanic acid.[46]

β-Lactamases and related enzymes

1. Synthesis of a new monobactamic acid as a potential β-lactamase inhibitor.[374]

Penicillins

1. Synthesis of new derivative of 6-aminopenicillin.[375]
2. Contributions to the development of new substitution patterns of penicillins: synthesis of new penicillin derivatives and evaluation of their porcine pancreatic elastase inhibitory activity.[376]
3. Separation of phenylacetic acid, 6-aminopenicillanic acid, and penicillin G with electrodialysis under constant voltage.[377]
4. Novel synthesis of 6,6-dibromo-2′-Z-chloromethyl and 2′-Z-bromomethyl anhydropenicillins from 6,6-dibromo 2β-(chloromethyl) and 2β-(bromomethyl)-2α-methyl-penam-3α-carboxylic acid *via* anhydropenicillin rearrangement.[378]
6. Synthesis and antibacterial activity of monocyclic β-lactams related to cephalosporin and penicillin sulfones.[379]
7. Synthesis and antibacterial activity of monocyclic β-lactams related to cephalosporin and penicillin sulfones.[380]
8. Bioactive polymers; synthesis, characterization, release and antimicrobial property of macromolecular prodrug of ampicillin.[381]
9. Synthesis of 6-[1-[4-(benzoxazol-2-yl)thiobutyl]-1,2,3-triazole-4-yl]methylenepenam as β-lactamase inhibitors.[382]
10. Penicillin V benzhydryl ester sulfoxide monohydrate.[383]

Cephalosporins

1. Synthesis of a radiolabeled new oral cephalosporin, E1101.[384]
2. Synthesis and antibacterial activities of novel C(7)-catechol-substituted cephalosporins (I).[385]
3. Studies on β-lactam antibiotics: synthesis and antibacterial activity of novel C-3 alkyne-substituted cephalosporins.[386]
4. Aerobic oxidation of 3-iodomethyl-Δ^3-cephem-4-carboxylate to 3-formyl-Δ^3-cephem-4-carboxylate through 3-hydroperoxymethyl-Δ^3-cephem-4-carboxylate.[387]
5. Structure-activity relationships between linkage style at the C-3 position and nuclear analogues of C(7)-hydroxyminoaminothiazol cephalosporins against methicillin-resistant *Staphylococcus aureus*.[388]
6. Structure-activity relationships of cephalosporin derivatives against methicillin-resistant *Staphylococcus aureus*.[389]
7. Cycloaddition reactions of a 3-(1,3-butadienyl)cephalosporin and antibacterial activity of new cephem derivatives.[390]

8. Synthesis of 3-methoxymethyl cephalosporin prodrug diastereomers (IV).[391]
9. Synthesis of cephalosporin derivatives utilizing the cephem triflate. 2. Introduction of 3-position substituents by the reaction with enamines.[392]
10. Synthesis and antibacterial activities of novel C(3)-pyrimidine(thiol)-substituted cephalosporins.[393]
11. Synthesis and structure-activity relationship of C-3 quaternary ammonium cephalosporins exhibiting anti-MRSA activities.[394]
12. Synthesis and structure-activity relationship of C-3 benzoyloxymethyl cephalosporins exhibiting anti-MRSA activities.[395]
13. Synthesis and biological activity of C-3 pyridinylethene-substituted cephalosporins.[396]
14. Synthesis and biological activity of quaternary (ammoniopropenyl) cephalosporins with hydroxylated alicyclic or aliphatic amines.[397]
15. An efficient synthesis of 3-(E)-hydroxypropenyl cephem derivatives, key intermediates for 3-(E)-ammoniopropenylcephalosporin antibiotics.[398]
16. Synthesis and biological activity of C-3 direct heterocyclylcarbon-substituted novel cephalosporins.[399]
17. Studies on 3′-quaternary ammonium cephalosporins (IV). Synthesis and antibacterial activity of 3′-(2-alkyl-3- aminopyrazolium) cephalosporins related to FK037.[400]
18. Studies on 3′-quaternary ammonium cephalosporins – III. Synthesis and antibacterial activity of 3′-(3-aminopyrazoilum) cephalosporins.[401]
19. Synthesis and structure-activity relationship of C-3 substituted triazolylthiomethyl cephems.[402]
20. Synthesis of 3-thiosubstituted cephalosporins.[403]
21. Synthesis of cephalosporin oligopeptides as potential proteinase inhibitors.[404]
22. Structural studies on an iron(III) complex containing (Z)-2-(2-aminothiazol-4-yl)-*N*-(2-hydroxyethyl)-2-(hydroxyimino)acetamide, a model compound for a cephalosporin antibiotic cefdinir.[405]
23. Structural studies on copper(II) complex containing (Z)-2-(2-aminothiazol-4-yl)-*N*-(2-hydroxyethyl)-2-(hydroxyimino)acetamide, a model compound for a cephalosporin antibiotic cefdinir.[406]

Trinems and Related Compounds

1. Stereoselective synthesis of a key intermediate of sanfetrinem by means of a chelated tin(IV) enolate.[407]

References

1. (*a*) F. Kunst, N. Ogasawara, I. Moszer, A. M. Albertini, G. Alloni, V. Azevedo, M. G. Bertero, P. Bessieres, A. Bolotin, S. Borchert, R. Borriss, L. Boursier, A. Brans, M. Braun, S. C. Brignell, S. Bron, S. Brouillet, C. V. Bruschi, B. Caldwell,

V. Capuano, N. M. Carter, S. K. Choi, J. J. Codani, I. F. Connerton, N. J. Cummings, R. A. Daniel, F. Denizot, K. M. Devine, A. Dusterhoft, S. D. Ehrlich, P. T. Emmerson, K. D. Entian, J. Errington, C. Fabret, E. Ferrari, D. Foulger, C. Fritz, M. Fujita, Y. Fujita, S. Fuma, A. Galizzi, N. Galleron, S. Y. Ghim, P. Glaser, A. Goffeau, E. J. Golightly, G. Grandi, G. Guiseppi, B. J. Guy, K. Haga, *et al.*, *Nature*, 1997, **390(6657)**, 249; (*b*) R. W. Newbert, B. Barton, P. Greaves, J. Harper, and G. Turner, *J. Ind. Microbiol. Biotechnol.*., 1997, **19**, 18.

2. A. Fang, D. L. Pierson, S. K. Mishra, D. W. Koenig, and A. L. Demain, *J. Ind. Microbiol. Biotechnol.*, 1997, **18**, 22.
3. J. Romero, J. F. Martin, P. Liras, A. L. Demain, and N. Rius, *J. Ind. Microbiol. Biotechnol.*, 1997, **18**, 241.
4. J. L. de la Fuente, A. Rumbero, J. F. Martin, and P. Liras, *Biochem. J.*, 1997, **327**, 59.
5. J. Kennedy and G. Turner, *Mol. Gen. Genet.*, 1996, **253**, 189.
6. H. B. A. Theilgaard, K. N. Kristiansen, C. M. Henriksen, and J. Nielsen, *Biochem. J.*, 1997, **327**, 185.
7. C.-Y. Shiau, M. F. Byford, R. T. Aplin, J. E. Baldwin, and C. J. Schofield, *Biochemistry*, 1997, **36**, 8798.
8. P. L. Roach, I. J. Clifton, C. M. H. Hensgens, N. Shibata, A. J. Long, R. W. Strange, S. S. Hasnain, C. J. Schofield, J. E. Baldwin, *et al.*, *Eur. J. Biochem.*, 1996, **242**, 736.
9. P. L. Roach, I. J. Clifton, C. M. H. Hensgens, N. Shibta, C. J. Schofield, J. Hajdu, and J. E. Baldwin, *Nature*, 1997, **387(6635)**, 827.
10. I. Borovok, O. Landman, R. Kreisberg, Y. Aharonowitz, and G. Cohen, *Biochemistry*, 1996, **35**, 1981.
11. D. S. H. Tan and T.-S. Sim, *J. Biol. Chem.*, 1996, **271**, 889.
12. M. Sami, T. J. N. Brown, P. L. Roach, C. J. Schofield, and J. E. Baldwin, *FEBS Lett.*, 1997, **405**, 191.
13. O. Landman, I. Borovok, Y. Aharonowitz, and G. Cohen, *FEBS Lett.*, 1997, **405**, 172.
14. (*a*) J. E. Baldwin, R. M. Adlington, D. G. Marquess, A. R. Pitt, M. J. Porter, and A. T. Russell, *Tetrahedron*, 1996, **52**, 2515; (*b*) J. E. Baldwin, R. M. Adlington, D. G. Marquess, A. R. Pitt, M. J. Porter, and A. T. Russell, *Tetrahedron*, 1996, **52**, 2537.
15. F. Cavelier, A. T. Russell, C. J. Schofield, and J. E. Baldwin, *J. Chem. Res., Synop.*, 1997, 200.
16. N. Shibata, M. D. Lloyd, J. E. Baldwin, and C. J. Schofield, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 1579.
17. P. A. Whiteman and E. P. Abraham, *FEBS Lett.*, 1996, **394**, 31.
18. J. M. Fernandez-Canon and J. M. Luengo, *J. Antibiot.*, 1997, **50**, 45.
19. A. Roa, M. L. Goble, J. L. Garcia, C. Acebal, and R. Virden, *Biochem. J.*, 1996, **316**, 409.
20. N. C. R. Van Straten, H. I. Duynstee, E. De Vroom, G. A. Van der Marel, and J. H. Van Boom, *Liebigs Ann./Recl.*, 1997, 1215.
21. T. F. Favino, G. Fronza, C. Fuganti, D. Fuganti, P. Grasselli, and A. Mele, *J. Org. Chem.*, 1996, **61**, 8975.
22. N. W. Fadnavis, M. Sharfuddin, S. K. Vadivel, and U. T. Bhalerao, *J. Chem. Soc., Perkin Trans. 1*, 1997, 3577.
23. G. Cardillo, A. Tolomelli, and C. Tomasini, *J. Org. Chem.*, 1996, **61**, 8651.
24. H. Waldmann, A. Heuser, and S. Schulze, *Tetrahedron Lett.*, 1996, **37**, 8725.
25. H. Waldmann and S. Gabold, *Chem. Commun.*, 1997, 1861.

26. M. G. Kim and S. B. Lee, *J. Mol. Catal. B: Enzym.*, 1996, **1**, 181.
27. M. G. Kim and S. B. Lee, *J. Mol. Catal. B: Enzym.*, 1996, **1**, 201.
28. M. Fite, M. Capellas, M. D. Benaiges, G. Caminal, P. Clapes, and G. Alvaro, *Biocatal. Biotransform.*, 1997, **14**, 317.
29. T. C. Huang, D. H. Chen, and S. S. Wang, *Chem. Eng. Commun.*, 1996, **147**, 99.
30. A. Illanes, C. Altamirano, and A. Ruiz, *Ann. N. Y. Acad. Sci.*, 1996, **799**, 472.
31. S. Ospina, E. Barzana, O. T. Ramirez, and A. Lopez-Munguia, *Prog. Biotechnol.*, 1996, **11**, 464.
32. S. G. Senthilvel and J. S. Pai, *Biotechnol. Tech.*, 1996, **10**, 611.
33. Y.-S. Lee, H.-C. Yang, and S.-S. Park, *J. Microbiol. Biotechnol.*, 1996, **6**, 375.
34. H. Yamada, Y. Ishii, Y. Noguchi, T. Miura, T. Mori, and Y. Saito, *Ann. N. Y. Acad. Sci.*, 1996, **799**, 74.
35. R. Fernandez-Lafuente, J. M. Guisan, M. Pregnolato, and M. Terreni, *Tetrahedron Lett.*, 1997, **38**, 4693.
36. O. H. Justiz, R. Fernandez-Lafuente, J. M. Guisan, P. Negri, G. Pagani, M. Pregnolato, and M. Terreni, *J. Org. Chem.*, 1997, **62**, 9099.
37. B. Thanomsub, C. Boonchird, W. Panbangred, and V. Meevootisom, *Microb. Util. Renewable Resour.*, 1996, **9**, 395.
38. G. Carrea, A. Corcelli, G. Palmisano, and S. Riva, *Biotechnol. Bioeng.*, 1996, **52**, 648.
39. W. W. Lorenz and J. Wiegel, *J. Bacteriol.*, 1997, **179**, 5436.
40. F. J. Perez-Llarena, P. Liras, A. Rodriguez-Garcia, and J. F. Martin, *J. Bacteriol.*, 1997, **179**, 2053.
41. J. Pitlik and C. A. Townsend, *Chem. Commun.*, 1997, 225.
42. J. E. Thirkettle, J. E. Baldwin, J. Edwards, J. P. Griffin, and C. J. Schofield, *Chem. Commun.*, 1997, 1025.
43. J. E. Baldwin, J. M. Charnock, C. D. Garner, Y. Fujishima, A. Hassan, C. J. Schofield, and H. Edwards, *Protein Eng.*, 1997, **10**, 45.
44. J. E. Baldwin, R. M. Adlington, J. S. Bryans, M. D. Lloyd, T. J. Sewell, C. J. Schofield, K. H. Baggaley, and R. Cassels, *Tetrahedron*, 1997, **53**, 7011.
45. L. A. Egan, R. W. Busby, D. Iwata-Reuyl, and C. A. Townsend, *J. Am. Chem. Soc.*, 1997, **119**, 2348.
46. M. C. Di Giovanni, D. Misiti, C. Villani, and G. Zappia, *Tetrahedron: Asymmetry*, 1996, **7**, 2277.
47. S. J. McGowan, *et al. Mol. Microbiol*, 1996, **22**, 415.
48. N. O. Concha, B. A. Rasmussen, K. Bush, and O. Herzberg, *Structure*, 1996, **4(7)**, 823.
49. N. O. Concha, B. A. Rasmussen, K. Bush, and O. Herzberg, *Protein Sci.*, 1997, **6**, 2671; (*b*) A. Carfi, S. Pares, E. Duee, M. Galleni, C. Duez, J. M. Frere, and O. Dideberg, *EMBO J.*, 1995, **14**, 4914.
50. M. W. Crowder, Z. Wang, S. L. Franklin, E. P. Zovinka, and S. J. Benkovic, *Biochemistry*, 1996, **35**, 12126.
51. M. Hernandez Valladares, A. Felici, G. Weber, H. W. Adolph, M. Zeppezauer, G. M. Rossolini, G. Amicosante, J.-M. Frere, and M. Galleni, *Biochemistry*, 1997, **36**, 11534.
52. M. Goto, T. Takahashi, F. Yamashita, A. Koreeda, H. Mori, M. Ohta, and Y. Arakawa, *Biol. Pharm. Bull.*, 1997, **20**, 1136.
53. D. J. Payne, J. H. Bateson, B. C. Gasson, T. Khushi, D. Proctor, S. C. Pearson, and R. Reid, *FEMS Microbiol. Lett.*, 1997, **157**, 171.
54. D. J. Payne, J. H. Bateson, B. C. Gasson, D. Proctor, T. Khushi, T. H. Farmer,

D. A. Tolson, D. Bell, P. W. Skett, A. C. Marshall, R. Reid, L. Ghosez, Y. Combret, and J. Marchand-Brynaert, *Antimicrob. Agents Chemother.*, 1997, **41**, 135.
55. M. W. Walter, A. Felici, M. Galleni, R. P. Soto, R. M. Adlington, J. E. Baldwin, J. M. Frere, M. Gololobov, and C. J. Schofield, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 2455.
56. M. W. Walter, R. M. Adlington, J. E. Baldwin, and C. J. Schofield, *Tetrahedron*, 1997, **53**, 7275.
57. A. Dubus, P. Ledent, J. Lamotte-Brasseur, and J.-M. Frere, *Proteins: Struct., Funct., Genet.*, 1996, **25**, 473.
58. T. Palzkill, W. Huang, and J. Petrosino, *Tech. Protein Chem. VIII, [Symp. Protein Soc.], 10th*, 1997.
59. X. Raquet, V. Lounnas, J. Lamotte-Brasseur, J. M. Frere, and R. C. Wade, *Biophys. J.*, 1997, **73**, 2416.
60. R. F. Pratt, M. Dryjanski, E. S. Wun, and V. M. Marathias, *J. Am. Chem. Soc.*, 1996, **118**, 8207.
61. N. C. J. Strynadka, M. Eisenstein, E. Katchalski-Katzir, B. K. Shoichet, I. D. Kuntz, R. Abagyan, M. Totrov, J. Janin, J. Cherfils, *et al.*, *Nat. Struct. Biol.*, 1996, **3**, 233.
62. F. Perez-Llarena, J. F. Martin, M. Galleni, J. J. R. Coque, J. L. Fuente, J.-M. Frere, and P. Liras, *J. Bacteriol.*, 1997, **179**, 6035.
63. H. Dietz, D. Pfeifle, and B. Wiedemann, *Antimicrob. Agents Chemother.*, 1997, **41**, 2113.
64. R. P. A. Brown, R. T. Aplin, and C. J. Schofield, *Biochemistry*, 1996, **35**, 12421.
65. R. P. A. Brown, R. T. Aplin, C. J. Schofield, and C. H. Frydrych, *J. Antibiot.*, 1997, **50**, 184.
66. C. N. Eid, N. G. Halligan, T. I. Nicas, D. L. Mullen, T. F. Butler, R. J. Loncharich, J. W. Paschal, C. J. Schofield, *et al.*, *J. Antibiot.*, 1997, **50**, 283.
67. E. Setti, N. A. V. Reddy, O. A. Phillips, D. P. Czajkowski, K. Atchison, H. Atwal, R. G. Micetich, S. N. Maiti, C. Kunugita, and A. Hyodo, *J. Antibiot.*, 1996, **49**, 944.
68. D. P. Czajkowski, A. V. N. Reddy, E. L. Setti, O. A. Phillips, R. G. Micetich, C. Kunmugita, and S. N. Maiti, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 11.
69. S. Paik and E. H. White, *Tetrahedron*, 1996, **52**, 5303.
70. S. Biagini, V. C. Gibson, M. R. Giles, E. L. Marshall, and M. North, *Chem. Commun.*, 1997, 1097.
71. W. D. Detlefsen and B. P. Branchaud, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 2289.
72. P. A. M. van der Klein, J. Castelijns, H. C. J. Hirs, J. Verweij, and E. de Vroom, *Recl. Trav. Chim. Pays-Bas*, 1996, **115**, 185.
73. A. Lavoie, A. M. Cantin, and E. Escher, *Pept.: Chem., Struct. Biol., Proc. Am. Pept. Symp., 14th*, 1996.
74. A. R. Leach, *J. Mol. Graphics Modell.*, 1997, **15**, 158.
75. E. G. Mata, *Tetrahedron Lett.*, 1997, **38**, 6335.
76. J. Frau, J. Donoso, F. Munoz, and F. G. Blanco, *Theochem*, 1997, **390**, 255.
77. G. Mukherjee and T. Ghosh, *Proc. Indian Acad. Sci., Chem. Sci.*, 1996, **108**, 371.
78. T. Kupka, *Spectrochim. Acta, Part A*, 1997, **53A**, 2649.
79. M. Wendeler, J. Fattah, J. M. Twyman, A. J. Edwards, C. M. Dobson, S. J. Heyes, and K. Prout, *J. Am. Chem. Soc.*, 1997, **119**, 9793.
80. T. Oberhauser and V. Meduna, *Tetrahedron*, 1996, **52**, 7691.
81. T. Fekner, J. E. Baldwin, R. M. Adlington, and C. J. Schofield, *Chem. Commun.*, 1996, 1989.

82. H. Tanaka, Y. Yamaguchi, S.-i. Sumida, and S. Torii, *Chem. Commun.*, 1996, 2705.
83. H. Tanaka, S.-i. Sumida, Y. Kameyama, K. Sorajo, I. Wada, and S. Torii, *Bull. Chem. Soc. Jpn.*, 1996, **69**, 3651.
84. H. Tanaka, R. Kikuchi, and S. Torii, *Bull. Chem. Soc. Jpn.*, 1996, **69**, 1391.
85. H. Tanaka, S.-i. Sumida, Y. Nishioka, N. Kobayashi, Y. Tokumaru, Y. Kameyama, and S. Torii, *J. Org. Chem.*, 1997, **62**, 3610.
86. J. D. Copp and G. A. Tharp, *Org. Process Res. Dev.*, 1997, **1**, 92.
87. M. Botta, M. Crucianelli, R. Saladino, C. Mozzetti, and R. Nicoletti, *Tetrahedron*, 1996, **52**, 10205.
88. F. Bonadies, F. De Angelis, L. Locati, and A. Scettri, *Tetrahedron Lett.*, 1996, **37**, 7129.
89. N. Grigan, D. Musel, G. A. Veinberg, and E. Lukevics, *Synth. Commun.*, 1996, **26**, 1183.
90. A. I. Hashem, A. M. Youssef, R. A. Aziz, A. M. Ali, and G. A. Nasser, *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.*, 1997, **36**, 196.
91. J. M. I. Neeft, J. Verweij, H. G. J. Hirs, and E. de Vroom, *Tetrahedron*, 1996, **52**, 11905.
92. J. R. Hwu, A. A. Moshfegh, S.-C. Tsay, C. C. Lin, W. N. Tseng, A. Azaripour, H. Mottaghian, and G. H. Hakimelahi, *J. Med. Chem.*, 1997, **40**, 3434.
93. W. Cabri, M. Botta, F. Corelli, and F. Messiana, *Tetrahedron Lett.*, 1997, **38**, 3291.
94. M. Curini, O. Rosati, P. Barontini, R. Di Falco, M. Riscazzi, and S. Brusco, *Gazz. Chim. Ital.*, 1997, **127**, 195.
95. C. G. Gourdoupis and I. K. Stamos, *J. Heterocycl. Chem.*, 1996, **33**, 987.
96. V. Farina, S. I. Hauck, and R. A. Firestone, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 1613.
97. H. Tanaka, S.-i. Sumida, and S. Torii, *J. Org. Chem.*, 1997, **62**, 3782.
98. K. I. Choi, J. H. Cha, M. J. Kim, Y. S. Cho, Y. Kim, M. H. Chang, and H. Y. Koh, *Korean J. Med. Chem.*, 1997, **7**, 11.
99. R. L. Elliott, N. H. Nicholson, F. E. Peaker, A. K. Takle, C. M. Richardson, J. W. Tyler, J. White, M. J. Pearson, D. S. Eggleston, and R. C. Haltiwanger, *J. Org. Chem.*, 1997, **62**, 4998.
100. R. Hara, H. Itahana, K. Sakamoto, H. Hiusamichi, and N. Nagano, *J. Antibiot.*, 1996, **49**, 1182.
101. H.-Y. Kang, S. H. Lee, K. I. Choi, and H. Y. Koh, *Tetrahedron Lett.*, 1996, **37**, 7549.
102. J. D. Buynak, A. S. Rao, G. P. Ford, C. Carver, G. Adam, B. Geng, B. Bachmann, S. Shobassy, and S. Lackey, *J. Med. Chem.*, 1997, **40**, 3423.
103. S. N. Maiti, D. P. Czajkowski, N. A. V. Reddy, P. Spevak, J. Kaleta, and R. G. Micetich, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 823.
104. R. P. Alexander, R. W. Bates, A. J. Pratt, and J. A. E. Kraunsoe, *Tetrahedron*, 1996, **52**, 5983.
105. E. Metais, L. E. Overman, M. I. Rodriguez, and B. A. Stearns, *J. Org. Chem.*, 1997, **62**, 9210.
106. S. Oumoch and G. Rousseau, *Heterocycles*, 1996, **43**, 2615.
107. S. Oumoch and G. Rousseau, *Bull. Soc. Chim. Fr.*, 1996, **133**, 997.
108. M. A. Ciufolini and Q. Dong, *Chem. Commun.*, 1996, 881.
109. L. C. Blaszczak, C. N. Eid, J. Flokowitsch, G. S. Gregory, S. A. Hitchcock, G. W. Huffman, D. R. Mayhugh, M. J. Nesler, D. A. Preston, and M. Zia-Ebrahimi, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2261.
110. H. Tanaka, Y. Tokumaru, Y. Kameyama, and S. Torii, *Chem. Lett.*, 1997, 1221.

111. H. R. Pfaendler and C. Medicus, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 623.
112. H. R. Pfaendler and C. Medicus, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 757.
113. H. R. Pfaendler, R. Weishaupt, and C. Medicus, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2217.
114. R. Tanaka, Y. Oyama, S. Imajo, S. Matsuki, and M. Ishiguro, *Bioorg. Med. Chem.*, 1997, **5**, 1389.
115. M. Ishiguro, R. Tanaka, K. Namikawa, T. Nasu, H. Inoue, T. Nakatsuka, Y. Oyama, and S. Imajo, *J. Med. Chem.*, 1997, **40**, 2126.
116. A. Allsop, G. Brooks, P. D. Edwards, A. C. Kaura, and R. Southgate, *J. Antibiot.*, 1996, **49**, 921.
117. B. Furman, S. Molotov, R. Thurmer, Z. Kaluza, W. Voelter, and M. Chmielewski, *Tetrahedron*, 1997, **53**, 5883.
118. B.-l. Furman, Z. Kaluza, and M. Chmielewski, *J. Org. Chem.*, 1997, **62**, 3135.
119. C. Belzecki, R. Urbanski, Z. Urbanczyk-Lipkowska, and M. Chmielewski, *Tetrahedron*, 1997, **53**, 14153.
120. D. Andreotti, T. Rossi, and C. Marchioro, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 2589.
121. D. Andreotti, S. Biondi, R. Di Fabio, D. Donati, E. Piga, and T. Rossi, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 2019.
122. C. Marchioro, G. Pentassuglia, A. Perboni, and D. Donati, *J. Chem. Soc., Perkin Trans. 1*, 1997, 463.
123. S. Hanessian and M. J. Rozema, *J. Am. Chem. Soc.*, 1996, **118**, 9884.
124. R. Di Fabio, D. Andreotti, S. Biondi, G. Gaviraghi, and T. Rossi, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 2025.
125. B. Bertani, R. Di Fabio, C. Ghiron, A. Perboni, T. Rossi, R. J. Thomas, and P. Zarantonello, *Tetrahedron*, 1997, **53**, 15011.
126. S. Gehanne, E. Piga, D. Andreotti, S. Biondi, and D. Pizzi, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 2791.
127. M. E. Tranquillini, G. L. Araldi, D. Donati, G. Pentassuglia, A. Pezzoli, and A. Ursini, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 1683.
128. S. Hanessian, A. M. Griffin, and M. J. Rozema, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1857.
129. M. Panunzio, R. Camerini, R. Pachera, D. Donati, C. Marchioro, and A. Perboni, *Tetrahedron: Asymmetry*, 1996, **7**, 2929.
130. S. M. Sakya, T. W. Strohmeyer, S. A. Lang, and Y.-I. Lin, *Tetrahedron Lett.*, 1997, **38**, 5913.
131. B. Alcaide, C. Polanco, and M. A. Sierra, *Tetrahedron Lett.*, 1996, **37**, 6901.
132. B. K. Banik, G. V. Subbaraju, M. S. Manhas, and A. K. Bose, *Tetrahedron Lett.*, 1996, **37**, 1363.
133. S. Pippich, H. Bartsch, and T. Erker, *J. Heterocycl. Chem.*, 1997, **34**, 823.
134. O. Sakurai, T. Ogiku, M. Takahashi, M. Hayashi, T. Yamanaka, H. Horikawa, and T. Iwasaki, *J. Org. Chem.*, 1996, **61**, 7889.
135. O. Sakurai and H. Horikawa, *Tetrahedron Lett.*, 1996, **37**, 7811.
136. S. R. Martel, R. Wisedale, T. Gallagher, L. D. Hall, M. F. Mahon, R. H. Bradbury, and N. J. Hales, *J. Am. Chem. Soc.*, 1997, **119**, 2309.
137. S. R. Martel, D. Planchenault, R. Wisedale, T. Gallagher, and N. J. Hales, *Chem. Commun.*, 1997, 1897.
138. M. E. Jung and B. T. Vu, *J. Org. Chem.*, 1996, **61**, 4427.
139. P. A. Jacobi, S. Murphree, F. Rupprecht, and W. Zheng, *J. Org. Chem.*, 1996, **61**, 2413.
140. S. G. Davies and D. R. Fenwick, *Chem. Commun.*, 1997, 565.

141. S. Roland, M. Savignac, J.-O. Durand, and J.-P. Genet, *Bull. Soc. Chim. Fr.*, 1996, **133**, 945.
142. L. Banfi, G. Guanti, and M. T. Zannetti, *Tetrahedron Lett.*, 1996, **37**, 521.
143. H. Kwon, M. Lee, I. Lee, S. Lee, T. Yoong, and T. Hwang, *Bull. Korean Chem. Soc.*, 1997, **18**, 475.
144. H. Kwon, M. Lee, I. Lee, S. Lee, T. Yoon, and T. Hwang, *Bull. Korean Chem. Soc.*, 1997, **18**, 463.
145. Y. M. Goo, M. H. Seo, and Y. Y. Lee, *Bull. Korean Chem. Soc.*, 1996, **17**, 909.
146. (*a*) M. H. Seo, Y. Y. Lee, and Y. M. Goo, *Bull. Korean Chem. Soc.*, 1996, **17**, 314; (*b*) G. Kim and Y. Ryu, *Bull. Korean Chem. Soc.*, 1996, **17**, 493.
147. T. L. Gilchrist, A. Lemos, and C. J. Ottaway, *J. Chem. Soc., Perkin Trans. 1*, 1997, 3005.
148. Y. M. Goo, M. H. Seo, and Y. Y. Lee, *Bull. Korean Chem. Soc.*, 1997, **18**, 1032.
149. K. Oda and A. Yoshida, *Chem. Pharm. Bull.*, 1997, **45**, 1439.
150. T. Yamanaka, M. Seki, T. Kuroda, H. Ohmizu, and T. Iwasaki, *Tetrahedron Lett.*, 1996, **37**, 4967.
151. M. Seki, T. Yamanaka, T. Miyake, and H. Ohmizu, *Tetrahedron Lett.*, 1996, **37**, 5565.
152. M. Seki, T. Miyake, T. Yamanaka, and H. Ohmizu, *Synlett*, 1996, 455.
153. K. Kondo, M. Seki, T. Kuroda, T. Yamanaka, and T. Iwasaki, *J. Org. Chem.*, 1997, **62**, 2877.
154. M. Seki, K. Kondo, and T. Iwasaki, *J. Chem. Soc., Perkin Trans. 1*, 1996, 2851.
155. K. Nozaki, H. Kumobayashi, T. Horiuchi, H. Takaya, T. Saito, A. Yoshida, K. Matsumura, Y. Kato, T. Imai, and T. Miura, *J. Org. Chem.*, 1996, **61**, 7658.
156. H. Ishibashi, K. Kodama, C. Kameoka, H. Kawanami, and M. Ikeda, *Tetrahedron*, 1996, **52**, 13867.
157. H. Ishibashi, C. Kameoka, K. Kodama, H. Kawanami, M. Hamada, and M. Ikeda, *Tetrahedron*, 1997, **53**, 9611.
158. H. Ishibashi, C. Kameoka, K. Kodama, and M. Ikeda, *Tetrahedron*, 1996, **52**, 489.
159. M. Ihara, K. Satoh, Y. Ishida, N. Taniguchi, Y. Tokunaga, M. Takemura, and K. Fukumoto, *Heterocycles*, 1996, **42**, 437.
160. J. C. Arnould, R. N. Illingworth, W. W. Nichols, and R. G. Wilson, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 2449.
161. N. Ohtake, H. Imamura, H. Kiyonaga, H. Jona, M. Ogawa, S. Okada, A. Shimizu, M. Moriya, H. Sato, Y. Tominaga, K. Yamada, M. Nakano, R. Ushijima, and S. Nakagawa, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1617.
162. N. Ohtake, O. Okamoto, S. Kato, R. Ushijima, H. Fukatsu, and S. Nakagawa, *J. Antibiot.*, 1997, **50**, 586.
163. N. Ohtake, K. Yamada, E. Mano, O. Okamoto, R. Ushijima, and S. Nakagawa, *J. Antibiot.*, 1997, **50**, 567.
164. N. Ohtake, O. Okamoto, R. Mitomo, Y. Kato, K. Yamamoto, Y. Haga, H. Fukatsu, and S. Nakagawa, *J. Antibiot.*, 1997, **50**, 598.
165. J. D. Armstrong, III, J. L. Keller, J. Lynch, T. Liu, F. W. Hartner, Jr., N. Ohtake, S. Okada, Y. Imai, O. Okamoto, R. Ushijima, S. Nakagawa, and R. P. Vonante, *Tetrahedron Lett.*, 1997, **38**, 3203.
166. M. Sunagawa, H. Matsumura, A. Sasaki, H. Yamaga, Y. Sumita, and H. Nouda, *J. Antibiot.*, 1996, **49**, 1175.
167. J.-e. Igarashi, T. Nishimura, A. Sasaki, and M. Sunagawa, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 1881.
168. K. Oda and A. Yoshida, *Tetrahedron Lett.*, 1997, **38**, 5687.

169. K. Oda and T. Nishi, *J. Antibiot.*, 1997, **50**, 446.
170. Y. Narukawa, K. Nishi, and H. Onoue, *Tetrahedron Lett.*, 1996, **37**, 2589.
171. K. Nishi, Y. Narukawa, and H. Onoue, *Tetrahedron Lett.*, 1996, **37**, 2987.
172. M. Miyauchi, O. Kanno, and I. Kawamoto, *J. Antibiot.*, 1997, **50**, 794.
173. M. Sunagawa, H. Matsumura, A. Sasaki, H. Yamaga, Y. Kitamura, Y. Sumita, and H. Nouda, *J. Antibiot.*, 1997, **50**, 621.
174. K. M. J. Brands, G. Marchesini, J. M. Williams, U. H. Dolling, and P. J. Reider, *Tetrahedron Lett.*, 1996, **37**, 2919.
175. H.-W. Lee, E.-N. Kim, H.-J. Son, S.-K. Ahn, K.-H. Chung, J.-W. Kim, and C.-R. Lee, *Chem. Pharm. Bull.*, 1996, **44**, 2326.
176. Y. Iso, T. Irie, Y. Nishino, K. Motokawa, and Y. Nishitani, *J. Antibiot.*, 1996, **49**, 199.
177. Y. Iso, T. Irie, T. Iwaki, M. Kii, Y. Sendo, K. Motokawa, and Y. Nishitani, *J. Antibiot.*, 1996, **49**, 478.
178. G. Burton, G. J. Clarke, J. D. Douglas, A. J. Eglington, C. H. Frydrych, J. D. Hinks, N. W. Hird, E. Hunt, S. F. Moss, A. Naylor, N. H. Nicholson, and M. J. Pearson, *J. Antibiot.*, 1996, **49**, 1266.
179. G. Burton, N. J. C. Clear, A. J. Eglington, A. W. Guest, E. Hunt, M. A. Naylor, M. J. Pearson, and A. K. Takle, *J. Antibiot.*, 1996, **49**, 1258.
180. N. J. C. Clear, J. S. Davies, A. J. Eglington, S. C. M. Fell, J. D. Hinks, N. W. Hird, E. Hunt, S. F. Moss, and M. J. Pearson, *J. Antibiot.*, 1997, **50**, 237.
181. Y.-I. Lin, P. Bitha, S. M. Sakya, S. A. Lang, Jr., Y. Yang, W. J. Weiss, P. J. Petersen, K. Bush, and R. T. Testa, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 3063.
182. K. H. Nam, C. H. Oh, J. K. Cho, H. J. Kim, K. S. Lee, and J. H. Cho, *Arch. Pharm.*, 1996, **329(10)**, 443.
183. S. M. Sakya, T. W. Strohmeyer, P. Bitha, S. A. Lang, Jr., and Y.-I. Lin, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1805.
184. A. Yamada, K. Hattori, S. Kuroda, T. Chiba, T. Kamimura, and K. Sakane, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 2705.
185. C. Palomo, J. M. Aizpurua, A. Mielgo, and A. Linden, *J. Org. Chem.*, 1996, **61**, 9186.
186. C. Palomo, J. M. Aizpurua, M. Legido, and R. Galarza, *Chem. Commun.*, 1997, 233.
187. C. Palomo, J. M. Aizpurua, M. Legido, R. Galarza, P. M. Deya, J. Dunogues, J. P. Picard, A. Ricci, and G. Seconi, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1239.
188. C. Palomo, J. M. Aizpurua, M. Legido, A. Mielgo, and R. Galarza, *Chem.-Eur. J.*, 1997, **3**, 1432.
189. C. Palomo, J. M. Aizpurua, J. M. Garcia, R. Galarza, M. Legido, R. Urchegui, P. Roman, A. Luque, J. Server-Carrio, and A. Linden, *J. Org. Chem.*, 1997, **62**, 2070.
190. M. Jayaraman, V. Srirajan, A. R. A. S. Deshmukh, and R. M. Bhawal, *Tetrahedron*, 1996, **52**, 3741.
191. V. Srirajan, A. R. A. S. Deshmukh, and B. M. Bhawal, *Tetrahedron*, 1996, **52**, 5585.
192. M. Jayaraman, A. R. Deshmukh, and B. M. Bhawal, *Tetrahedron*, 1996, **52**, 8989.
193. M. Jayaraman, V. G. Puranik, and B. M. Bhawal, *Tetrahedron*, 1996, **52**, 9005.
194. V. Srirajan, V. G. Puranik, A. R. A. S. Deshmukh, and B. M. Bhawal, *Tetrahedron*, 1996, **52**, 5579.
195. T. Gunda and F. Sztaricskai, *Tetrahedron*, 1997, **53**, 7985.
196. E. Bandini, G. Martelli, G. Spunta, and M. Panunzio, *Synlett*, 1996, 1017.
197. B. Ruhland, A. Bhandari, E. M. Gordon, and M. A. Gallop, *J. Am. Chem. Soc.*, 1996, **118**, 253.

198. B. Ruhland, A. Bombrun, and M. A. Gallop, *J. Org. Chem.*, 1997, **62**, 7820.
199. J. Podlech, *Synlett*, 1996, 582.
200. J. Podlech and M. R. Linder, *J. Org. Chem.*, 1997, **62**, 5873.
201. E. Rossi, G. Abbiati, and E. Pini, *Tetrahedron*, 1997, **53**, 14107.
202. G. Wu and W. Tormos, *J. Org. Chem.*, 1997, **62**, 6412.
203. T. Asahi, M. Nakamura, J. Kobayashi, F. Toda, and H. Miyamoto, *J. Am. Chem. Soc.*, 1997, **119**, 3665.
204. G. Cainelli, D. Giacomini, P. Galletti, and M. Dacol, *Tetrahedron: Asymmetry*, 1997, **8**, 3231.
205. R. Hayakawa, M. Shimizu, and T. Fujisawa, *Tetrahedron Lett.*, 1996, **37**, 7533.
206. M. J. Miller and S. K. Richardson, *J. Mol. Catal. B: Enzym.*, 1996, **1**, 161.
207. R. N. Patel, A. Banerjee, and L. J. Szarka, J. Am. Oil Chem. Soc., 1996, **73**, 1363.
208. A. K. Bose, M. Jayaraman, A. Okawa, S. S. Bari, E. W. Robb, and M. S. Manhas, *Tetrahedron Lett.*, 1996, **37**, 6989.
209. M. Kidwai, P. Kumar, Y. Goel, and K. Kumar, *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.*, 1997, **36B**, 175.
210. Y. M. Goo, J. H. Lee, J. S. Cho, and Y. Y. Lee, *Bull. Korean Chem. Soc.*, 1996, **17**, 985.
211. J. N. Junior and A. Kascheres, *J. Heterocycl. Chem.*, 1997, **34**, 341.
212. R. Annunziata, M. Cinquini, F. Cozzi, V. Molteni, and O. Schupp, *Tetrahedron*, 1996, **52**, 2573.
213. R. Annunziata, M. Benaglia, M. Cinquini, F. Cozzi, O. Martini, and V. Molteni, *Tetrahedron*, 1996, **52**, 2583.
214. R. Annunziata, M. Cinquini, F. Cozzi, V. Molteni, and O. Schupp, *J. Org. Chem.*, 1996, **61**, 8293.
215. G. Barbaro, A. Battaglia, A. Guerrini, and C. Bertucci, *Tetrahedron: Asymmetry*, 1997, **8**, 2527.
216. C. Kashima, K. Fukusaka, and K. Takahashi, *J. Heterocycl. Chem.*, 1997, **34**, 1559.
217. C. Kashima, K. Takahashi, I. Fukuchi, and K. Fukusaka, *Heterocycles*, 1997, **44**, 289.
218. G. Cainelli, D. Giacomini, and P. Galletti, *Synthesis*, 1997, 886.
219. B. Furman, Z. Kaluza, and M. Chmielewski, *Tetrahedron*, 1996, **52**, 6019.
220. O. Neuss, B. Furman, Z. Kaluza, and M. Chmielewski, *Heterocycles*, 1997, **45**, 265.
221. Z. Kaluza and S. H. Park, *Synlett*, 1996, 895.
222. Z. Kaluza and R. Lysek, *Tetrahedron: Asymmetry*, 1997, **8**, 2553.
223. Y. Taguchi, T. Tsuchiya, A. Oishi, and I. Shibuya, *Bull. Chem. Soc. Jpn.*, 1996, **69**, 1667.
224. K. Karupaiyan, V. Srirajan, A. R. A. S. Deshmukh, and B. M. Bhawal, *Tetrahedron Lett.*, 1997, **38**, 4281.
225. A. D'Annibale, A. Pesce, S. Resta, and C. Trogolo, *Tetrahedron*, 1997, **53**, 13129.
226. A. D'Annibale, A. Pesce, S. Resta, and C. Trogolo, *Tetrahedron Lett.*, 1997, **38**, 1829.
227. A. D'Annibale, A. Pesce, S. Resta, and C. Trogolo, *Tetrahedron Lett.*, 1996, **37**, 7429.
228. N. De Kimpe, K. A. Tehrani, and G. Fonck, *J. Org. Chem.*, 1996, **61**, 6500.
229. N. De Kimpe, D. De Smaele, and Z. Sakonyi, *J. Org. Chem.*, 1997, **62**, 2448.
230. M. E. Piotti and H. Alper, *J. Am. Chem. Soc.*, 1996, **118**, 111.
231. M. Sadakane, R. Vahle, K. Schierle, D. Kolter, and E. Steckhan, *Synlett*, 1997, 95.
232. Y. H. Lee, C.-h. Lee, and W. S. Choi, *Bull. Korean Chem. Soc.*, 1996, **17**, 290.

233. Y. Nagao, T. Kumagai, S. Tamai, H. Matsunaga, T. Abe, and Y. Inoue, *Heterocycles*, 1996, **42**, 849.
234. C. Dartiguelongue, S. Payan, O. Duval, L. M. Gomes, and R. D. Waigh, *Bull. Soc. Chim. Fr.*, 1997, **134**, 769.
235. D.-C. Fang and X.-Y. Fu, *Int. J. Quantum Chem.*, 1996, **57**, 1107.
236. J. Frau, J. Donoso, F. Munoz, B. Vilanova, and F. Garcia-Blanco, *Helv. Chim. Acta*, 1997, **80**, 739.
237. R. Lopez, M. F. Ruiz-Lopez, D. Rinaldi, J. A. Sordo, and T. L. Sordo, *J. Phys. Chem.*, 1996, **100**, 10600.
238. M. V. Roux, P. Jimenez, J. Z. Davalos, O. Castano, M. T. Molina, R. Notario, M. Herreros, and J. L. M. Abboud, *J. Am. Chem. Soc.*, 1996, **118**, 12735.
239. X. Creary, C. Zhu, and Z. Jiang, *J. Am. Chem. Soc.*, 1996, **118**, 12331.
240. D. J. A. De Ridder, K. Goubitz, C. A. Reiss, H. Schenk, and H. Hiemstra, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 1996, **C52**, 1473.
241. F. Ercan, D. Ulku, and V. Guner, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 1996, **C52**, 1779.
242. Z. Mandic, R. Kiralj, B. Kojic-Prodic, and M. Kovacevic, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 1996, **C52**, 988.
243. A. Mousser, S. Badeche, H. Merazig, J. Benhaoua, and D. A. Sidhoum, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 1996, **C52**, 201.
244. D. Ulku, F. Ercan, and V. Guner, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 1997, **C53**, 1945.
245. J. H. Vyas, A. J. Baxi, and A. R. Parikh, *J. Inst. Chem.*, 1996, **68(4)**, 101.
246. S. D. Sorathiya, V. B. Patel, and A. R. Parikh, *J. Inst. Chem.*, 1996, **68(6)**, 177.
247. V. B. Patei, S. D. Sobathiya, and A. R. Parikh, *J. Inst. Chem.*, 1997, **69(1)**, 9.
248. A. Sapi, J. Fetter, K. Lempert, M. Kajtar-Peredy, and G. Czira, *Tetrahedron*, 1997, **53**, 12729.
249. L. Hazai and M. Kajtar-Peredy, *J. Chem. Res., Synop.*, 1996, 374.
250. Y. D. Kulkarni, D. Srivastava, A. Bishnoi, and P. R. Dua, *J. Indian Chem. Soc.*, 1996, **73**, 173.
251. L. T. Giang, J. Fetter, K. Lempert, M. Kajtar-Peredy, and A. Gomory, *Tetrahedron*, 1996, **52**, 10169.
252. J. Fetter, F. Bertha, M. Kajtar-Peredy, K. Lempert, and A. Sapi, *J. Chem. Res., Synop.*, 1997, 118.
253. J. C. Lancelot, C. Saturnino, H. El-Kashef, D. Perrine, C. Mahatsekake, H. Prunier, and M. Robba, *J. Heterocycl. Chem.*, 1996, **33**, 427.
254. D. Hesk, C. Bowlen, S. Hendershot, D. Koharski, P. McNamara, D. Rettig, and S. Saluja, *J. Labelled Compd. Radiopharm.*, 1996, **38**, 1039.
255. G. V. Shustov and A. Rauk, *Tetrahedron: Asymmetry*, 1996, **7**, 699.
256. H. S. P. Rao and B. Bharathi, *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.*, 1996, **35B**, 167.
257. W. F. J. Karstens, F. P. J. T. Rutjes, and H. Hiemstra, *Tetrahedron Lett.*, 1997, **38**, 6275.
258. C. Henneuse, T. Boxus, L. Tesolin, G. Pantano, and J. Marchand-Brynaert, *Synthesis*, 1996, 495.
259. R. M. Adlington, J. E. Baldwin, W. McCoull, G. J. Pritchard, C. J. Schofield, and N. J. Westwood, *Synth. Commun.*, 1997, **27**, 3803.
260. B. Alcaide, M. F. Aly, and M. A. Sierra, *J. Org. Chem.*, 1996, **61**, 8819.
261. B. Alcaide, Y. Martin-Cantalejo, J. Perez-Castells, M. A. Sierra, and A. Monge, *J. Org. Chem.*, 1996, **61**, 9156.

262. J. R. Bellettini and M. J. Miller, *J. Org. Chem.*, 1996, **61**, 7959.
263. M. Jayaraman, M. S. Manhas, and A. K. Bose, *Tetrahedron Lett.*, 1997, **38**, 709.
264. A. C. O'Leary, A. D. Neary, C. M. Waldron, and M. J. Meegan, *J. Chem. Res., Synop.*, 1996, 368.
265. J.-P. Genet, J.-O. Durand, S. Roland, M. Savignac, and F. Jung, *Tetrahedron Lett.*, 1997, **38**, 69.
266. T. Ishihara, K. Ichihara, and H. Yamanaka, *Tetrahedron*, 1996, **52**, 255.
267. A. G. M. Barrett, S. P. D. Baugh, V. C. Gibson, M. R. Giles, E. L. Marshall, and P. A. Procopiou, *Chem. Commun.*, 1996, 2231.
268. A. G. M. Barrett, S. P. D. Baugh, V. C. Gibson, M. R. Giles, E. L. Marshall, and P. A. Procopiou, *Chem. Commun.*, 1997, 155.
269. A. G. M. Barrett, S. P. D. Baugh, D. C. Braddock, K. Flack, V. C. Gibson, and P. A. Procopiou, *Chem. Commun.*, 1997, 1375.
270. K. Tsuji and H. Ishikawa, *Synth. Commun.*, 1997, **27**, 595.
271. B. K. Banik, M. S. Manhas, and A. K. Bose, *Tetrahedron Lett.*, 1997, **38**, 5077.
272. B. K. Banik, O. Zegrocka, M. S. Manhas, and A. K. Bose, *Heterocycles*, 1997, **46**, 173.
273. A. Basak, H. M. M. Bdour, and G. Bhattacharya, *Tetrahedron Lett.*, 1997, **38**, 2535.
274. A. Amougay, O. Letsch, J.-P. Pete, and O. Piva, *Tetrahedron*, 1996, **52**, 2405.
275. Y. Kita, N. Shibata, N. Kawano, N. Yoshida, K. Matsumoto, and Y. Takebe, *J. Chem. Soc., Perkin Trans. 1*, 1996, 2321.
276. S. Pippich, H. Bartsch, and W. Holzer, *Tetrahedron*, 1997, **53**, 8439.
277. K. Oda, T. Nakano, H. Morimoto, and N. Takamura, *Heterocycles*, 1996, **42**, 577.
278. C. Palomo, J. M. Aizpurua, I. Ganboa, B. Odriozola, R. Urchegui, and H. Gorls, *Chem. Commun.*, 1996, 1269.
279. C. Palomo, M. Oiarbide, and A. Esnal, *Chem. Commun.*, 1997, 691.
280. C. Palomo, I. Ganboa, B. Odriozola, and A. Linden, *Tetrahedron Lett.*, 1997, **38**, 3093.
281. M. P. Doyle, Q. L. Zhou, H. Simonsen Stanley, and V. Lynch, *Synlett*, 1996, 697.
282. F. Fulop, M. Palko, G. Bernath, and P. Sohar, *Synth. Commun.*, 1997, **27**, 195.
283. K.-M. Cheung and P. M. Shoolingin-Jordan, *Tetrahedron*, 1997, **53**, 15807.
284. R. Joyeau, A. Felk, S. Guilaume, M. Wakselman, I. Vergely, C. Doucet, N. Boggetto, and M. Reboud-Ravaux, *J. Pharm. Pharmacol.*, 1996, **48**, 1218.
285. R. J. Cvetovich, M. Chartrain, F. W. Hartner, Jr., C. Roberge, J. S. Amato, and E. J. J. Grabowski, *J. Org. Chem.*, 1996, **61**, 6575.
286. M. P. Kirkup, R. Rizvi, B. Shankar, B. Shankar, S. Dugar, J. W. Clader, S. W. McCombie, S.-I. Lin, N. Yumibe, *et al.*, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 2069.
287. J. W. Clader, D. A. Burnett, M. A. Caplen, M. S. Domalski, S. Dugar, W. Vaccaro, R. Sher, M. E. Browne, H. Zhao, *et al.*, *J. Med. Chem.*, 1996, **39**, 3684.
288. L.-Y. Chen, A. Zaks, S. Chackalamannil, and S. Dugar, *J. Org. Chem.*, 1996, **61**, 8341.
289. B. B. Shankar, M. P. Kirkup, S. W. McCombie, J. W. Clader, and A. K. Ganguly, *Tetrahedron Lett.*, 1996, **37**, 4095.
290. R. M. Adlington, J. E. Baldwin, B. Chen, S. L. Cooper, W. McCoull, G. J. Pritchard, T. J. Howe, G. W. Becker, R. B. Hermann, A. M. McNulty, and B. L. Neubauer, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1689.
291. K. J. Cutrona and P. E. J. Sanderson, *Tetrahedron Lett.*, 1996, **37**, 5045.
292. H.-O. Kim, C. Lum, and M. S. Lee, *Tetrahedron Lett.*, 1997, **38**, 4935.
293. M. N. Qabar and M. Kahn, *Tetrahedron Lett.*, 1996, **37**, 965.

294. Z. Wu, G. I. Georg, B. E. Cathers, and J. V. Schloss, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 983.
295. J. Pitlik and C. A. Townsend, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 3129.
296. L. Banfi, A. Basso, and G. Guanti, *Tetrahedron*, 1997, **53**, 3249.
297. D. H. Kim and K.-R. Kim, *Bull. Korean Chem. Soc.*, 1996, **17**, 34.
298. A. S. Gajare, S. B. Bhawsar, D. B. Shinde, and M. S. Shingare, *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.*, 1997, **36B**, 449.
299. M. H. Khan and Nizamuddin, *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.*, 1997, **36B**, 625.
300. P. Arndt, C. Lefeber, R. Kempe, A. Tillack, and U. Rosenthal, *Chem. Ber.*, 1996, **129**, 1281.
301. M. D. G. Garcia-Martin, M. V. De Paz, and J. A. Galbis, *Macromol. Chem. Phys.*, 1997, **198**, 219.
302. F. L. Carrasquero, M. Garcia-Alvarez, J. J. Navas, C. Aleman, and S. Munoz-Guerra, *Macromolecules*, 1996, **29**, 8449.
303. S. J. Danishefsky, J. J. Masters, W. B. Young, J. T. Link, L. B. Snyder, T. V. Magee, D. K. Jung, R. C. A. Isaacs, W. G. Bornmann, *et al.*, *J. Am. Chem. Soc.*, 1996, **118**, 2843.
304. I. Ojima, J. C. Slater, P. Pera, J. M. Veith, A. Abouabdellah, J.-P. Begue, and R. J. Bernacki, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 133.
305. I. Ojima and J. C. Slater, *Chirality*, 1997, **9**, 487.
306. K. Uoto, S. Ohsuki, H. Takenoshita, T. Ishiyama, S. Iimura, Y. Hirota, I. Mitsui, H. Terasawa, and T. Soga, *Chem. Pharm. Bull.*, 1997, **45**, 1793.
307. J. Nieschalk and E. Schaumann, *Liebigs Ann.*, 1996, 141.
308. G. Cascio, L. Lorenzi, D. Caglio, E. Manghisi, F. Arcamone, G. Guanti, G. Satta, G. Morandotti, and R. Sperning, *Farmaco*, 1996, **51**, 189.
309. G. Barbaro, A. Battaglia, C. Bruno, P. Giorgianni, and A. Guerrini, *J. Org. Chem.*, 1996, **61**, 8480.
310. A. R. Chowdhury, V. V. Kumar, R. Roy, and A. P. Bhaduri, *J. Chem. Res., Synop.*, 1997, 254.
311. P. R. Dave, *J. Org. Chem.*, 1996, **61**, 5453.
312. A. P. Marchand, K. C. V. Ramanaiah, S. G. Bott, J. C. Gilbert, and S. Kirschner, *Tetrahedron Lett.*, 1996, **37**, 8101.
313. D. Glasl, G. Rihs, and H. H. Otto, *Helv. Chim. Acta*, 1997, **80**, 671.
314. T. R. Mihova, A. Linden, and H. Heimgartner, *Helv. Chim. Acta*, 1996, **79**, 2067.
315. M. Alajarin, P. Molina, and A. Vidal, *Tetrahedron Lett.*, 1996, **37**, 8945.
316. D. A. Neel, R. E. Holmes, and J. W. Paschal, *Tetrahedron Lett.*, 1996, **37**, 4891.
317. M. F. Gordeev, E. M. Gordon, and D. V. Patel, *J. Org. Chem.*, 1997, **62**, 8177.
318. J. R. Bellettini and M. J. Miller, *Tetrahedron Lett.*, 1997, **38**, 167.
319. A. A. Brakhage, *FEMS Microbiol. Lett.*, 1997, **148**, 1.
320. R. Lal, R. Khanna, H. Kaur, M. Khanna, N. Dhingra, S. Lal, K.-H. Gartemann, R. Eichenlaub, and P. K. Ghosh, *Crit. Rev. Microbiol.*, 1996, **22**, 201.
321. J. Nosek, R. Radzio, and U. Kuck, *Chem. Unserer Zeit*, 1997, **31**, 172.
322. A. S. Paradkar, S. E. Jensen, and R. H. Mosher, *Drugs Pharm. Sci.*, 1997, **82**, 241.
323. P. L. Skatrud, T. Schwecke, H. Van Liempt, and M. B. Tobin, *Biotechnology*, 1997, 247.
324. M. Bonafede and L. B. Rice, *J. Lab. Clin. Med.*, 1997, **130**, 558.
325. O. G. Brakstad and J. A. Maeland, *Apmis*, 1997, **105**, 264.
326. J.-M. Ghuysen, *Int. J. Antimicrob. Agents*, 1997, **8**, 45.
327. H. Hachler, *BIOspektrum*, 1997, 21.

328. R. Hakenbeck, *Verh. – K. Ned. Akad. Wet., Afd. Natuurkd., Tweede Reeks*, 1997, **96**, 225.
329. M. Hedberg and C. E. Nord, *J. Chemother. (Florence)*, 1996, **8**, 3.
330. J. Lessel, *Pharm. Unserer Zeit*, 1996, **25**, 17.
331. D. M. Livermore, *Clin. Microbiol. Infect.*, 1997, **3**, 4S10.
332. D. M. Livermore, *J. Antimicrob. Chemother.*, 1997, **39**, 673.
333. A. A. Medeiros, *Clin. Microbiol. Infect.*, 1997, **3**, 4S2.
334. A. A. Medeiros, *Clin. Infect. Dis.*, 1997, **24**, S19.
335. F. Moosdeen, *Clin. Infect. Dis.*, 1997, **24**, 487.
336. I. Massova and S. Mobashery, *Acc. Chem. Res.*, 1997, **30**, 162.
337. P. Munoz, F. Garcia-Garrote, and E. Bouza, *Int. J. Antimicrob. Agents*, 1996, **7**, S9.
338. M. H. Nicolas-Chanoine, *J. Antimicrob. Chemother.*, 1997, **40**, 1.
339. H. Ogawara, *Actinomycetologica*, 1996, **10**, 104.
340. T. Palzkill, W. Huang, and J. Petrosino, *Tech. Protein Chem. VIII, [Symp. Protein Soc.], 10th*, 1997, p. 827.
341. P. Pflieger, P. Angehrn, M. Bohringer, K. Gubernator, I. Heinze-Krauss, C. Hubschwerlen, C. Oefner, M. G. P. Page, R. Then, and F. Winkler, *Spec. Publ. R. Soc. Chem.*, 1997, **198**, 101.
342. G. M. Rossolini, T. Walsh, and G. Amicosante, *Microb. Drug Resist. (Larchmont, N. Y.)*, 1996, **2**, 245.
343. F. Sanschagrin, C. Therrien, E. Theriault, N. Bejaoui, D. Dubois, and R. C. Levesque, *Antibiot. Resist.*, 1996, p. 99.
344. M. Szydlowska and Z. Markiewicz, *Acta Microbiol. Pol.*, 1997, **46**, 233.
345. B. Wiedemann and H. Deitz, *BIOspektrum*, 1997, 24.
346. B. K. Banik, M. Jayaraman, V. Srirajan, M. S. Manhas, and A. K. Bose, *J. Indian Chem. Soc.*, 1997, **74**, 943.
347. A. H. Berks, *Tetrahedron*, 1996, **52**, 331.
348. S. Biondi, *Spec. Publ. – R. Soc. Chem.*, 1997, **198**, 86.
349. M. Chmielewski, Z. Kaluza, and B. Furman, *Chem. Commun.*, 1996, 2689.
350. J. Demonty, *Rev. Med. Liege*, 1996, **51**, 47.
351. T. Fujisawa and M. Shimizu, *Rev. Heteroat. Chem.*, 1996, **15**, 203.
352. C. Ghiron and T. Rossi, *Targets Heterocycl. Syst.*, 1997, **1**, 161.
353. A. C. Ghosh, M. M. Bora, and N. N. Dutta, *Bioseparation*, 1996, **6**, 91.
354. J.-M. Ghuysen, P. Charlier, J. Coyette, C. Duez, E. Fonze, C. Fraipont, C. Goffin, B. Joris, and M. Nguyen-Disteche, *Microb. Drug Resist.* (*Larchmont, N. Y.*), 1996, **2**, 163.
355. E. M. Gordon, M. A. Gallop, and D. V. Patel, *Acc. Chem. Res.*, 1996, **29**, 144.
356. P. R. Guzzo and M. J. Miller, *Adv. Nitrogen Heterocycl.*, 1996, **2**, 1.
357. A. J. Hutt and J. O'Grady, *J. Antimicrob. Chemother.*, 1996, **37**, 7.
358. D. H. Kim, Z.-H. Li, S. S. Lee, K. R. Kim, S. J. Chung, and E.-J. Kim, *Pure Appl. Chem.*, 1996, **68**, 849.
359. Y. Kita and N. Shibata, *Synlett*, 1996, 289.
360. A. F. Mayer, *Fortschr.-Ber. VDI, Reihe 3*, 1996, **426**, 1.
361. (*a*) I. Ojima and F. Delaloge, *Chem. Soc. Rev.*, 1997, **26**, 377; (*b*) C. Palomo, J. M. Aizpurua and I. Ganboa, in *Conference Proceedings: Enantiosel. Synth. β-Amino Acids*, 1997, 279-357
362. M. Panunzio, P. G. Cozzi, C. M. Kretz, E. Bandini, and G. Martelli, *Top. Heterocycl. Syst.: Synth., React. Prop.*, 1996, **1**, 119.
363. J. Parrick and L. K. Mehta, *Prog. Heterocycl. Chem.*, 1996, **8**, 66.
364. M. E. C. Polywka, *Spec. Chem.*, 1996, **16**, 5S.

365. J. E. Thirkettle, C. J. Schofield, and M. W. Walter, *Amino Acids, Pept., Proteins*, 1997, **28**, 281.
366. A.-M. W. H. Thunnissen and B. W. Dijkstra, *Nat. Struct. Biol.*, 1996, **3**, 218.
367. S. Torii and H. Tanaka, *Yuki Gosei Kagaku Kyokaishi*, 1996, **54**, 941.
368. F. Alfani, M. Cantarella, N. Cutarella, A. Gallifuoco, P. Golini, and D. Bianchi, *Biotechnol. Lett.*, 1997, **19**, 175.
369. A. D'Acunzo, E. de Alteriis, F. Maurano, E. Battistel, and P. Parascandola, *J. Ferment. Bioeng.*, 1996, **81**, 138.
370. G. Del Rio, A. Lopez-Munguia, and X. Soberon, *Ann. N. Y. Acad. Sci.*, 1996, **799**, 61.
371. C. Fargues, S. Chanel, and G. Grevillot, *Bioseparation*, 1996, **6**, 343.
372. R. Fernandez-Lafuente, C. M. Rosell, B. Piatkowska, and J. M. Guisan, *Enzyme Microb. Technol.*, 1996, **19**, 9.
373. V. C. Sonawane, R. S. Jolly, and R. M. Vohra, *Biotechnol. Lett.*, 1996, **18**, 965.
374. S.-C. Tsay, Z. Ramezani, S. Hakimelahi, S.-M. Liao, and G. H. Hakimelahi, *Bull. Inst. Chem., Acad. Sin.*, 1997, **44**, 1.
375. N. Artizzu, L. Bonsignore, F. Cottiglia, and G. Loy, *Boll. Chim. Farm.*, 1996, **135**, 225.
376. C. E. Boschetti, E. G. Mata, O. A. Mascaretti, J. A. Cricco, G. Coux, A. Lodeyro, and O. A. Rovert, *J. Braz. Chem. Soc.*, 1996, **7**, 285.
377. D.-H. Chen, S.-S. Wang, and T.-C. Huang, *Sep. Sci. Technol.*, 1996, **31**, 839.
378. G. O. Danelon, E. G. Mata, and O. A. Mascaretti, *Tetrahedron Lett.*, 1996, **37**, 4431.
379. R. Koncki, E. Leszczynska, A. Cybulska, and S. Glab, *Anal. Chim. Acta*, 1996, **321**, 27.
380. J. Marchand-Brynaert, *Bull. Soc. Chim. Belg.*, 1997, **106**, 585.
381. H. Patel, D. A. Raval, and Madamwar, *Indian J. Pharm. Sci.*, 1997, **59**, 153.
382. C. Im, C. B. Yim, J. S. Oh, and S. B. Yoon, *Arch. Pharmacal Res.*, 1997, **20**, 647.
383. T.-S. Yoon and W. Shin, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 1996, **C52**, 3142.
384. S. Negi, Y. Komatsu, A. Tsuruoka, and Y. Uemura, *J. Labelled Compd. Radiopharm.*, 1996, **38**, 1.
385. Y.-Z. Kim, J.-C. Lim, J.-H. Yeo, C.-S. Bang, S.-S. Kim, T. H. Lee, S. H. Oh, Y.-C. Moon, and C.-S. Lee, *J. Antibiot.*, 1996, **49**, 496.
386. D. Barrett, T. Terasawa, S. Okuda, K. Kawabata, N. Yasuda, T. Kamimura, K. Sakane, and T. Takaya, *J. Antibiot.*, 1997, **50**, 100.
387. H. Tanaka, R. Kikuchi, and S. Torii, *Bull. Chem. Soc. Jpn.*, 1996, **69**, 220.
388. H. Hanaki, H. Akagi, T. Otani, and K. Hiramatsu, *J. Infect. Chemother.*, 1996, **2**, 143.
389. H. Hanaki, A. Hiroshi, S. Nomura, N. Unemi and K. Hiramatsu, *J. Antibiot.*, 1996, **49**, 402.
390. R. Hara, K. Sakamoto, H. Hisamichi, and N. Nagano, *J. Antibiot.*, 1996, **49**, 1162; R. Hara, H. Itahana, H. Hisamichi, S. Fukuoka, and N. Nagano, *J. Antibiot.*, 1996, **49**, 1179.
391. M. H. Jung, K.-W. Cho, J.-S. Oh, and J.-G. Park, *Korean J. Med. Chem.*, 1997, **7**, 23.
392. H.-Y. Kang, S. H. Lee, K. Choi, II, and H. Y. Koh, *Bull. Korean Chem. Soc.*, 1997, **18**, 1242.
393. Y.-Z. Kim, J.-C. Lim, J.-H. Yeo, C.-S. Bang, S.-S. Kim, T. H. Lee, S. H. Oh, Y.-C. Moon, and C.-S. Lee, *Korean J. Med. Chem.*, 1996, **6**, 15.

394. O. K. Kim, T. W. Hudyma, J. D. Matiskella, Y. Ueda, J. J. Bronson, and M. M. Mansuri, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2753.
395. O. K. Kim, Y. Ueda, M. M. Mansuri, J. W. Russell, and V. W. Bidwell, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1945.
396. S. B. Ko, Y. S. Lee, J. Y. Lee, W. H. Ham, and H. Park, *Arch. Pharmacal Res.*, 1996, **19**, 411.
397. J. Y. Lee, Y. S. Lee, D. W. Suk, E.-R. Woo, B. Y. Chung, and H. Park, *J. Antibiot.*, 1996, **49**, 1286.
398. Y. S. Lee, J. Y. Lee, J.-H. Jeong, and H. Park, *Arch. Pharmacal Res.*, 1997, **20**, 288.
399. Y. S. Lee, Y. S. Lee, and H. Park, *J. Antibiot.*, 1996, **49**, 405.
400. H. Ohki, K. Kawabata, Y. Inamoto, S. Okuda, T. Kamimura, and K. Sakane, *Bioorg. Med. Chem.*, 1997, **5**, 1685.
401. H. Ohki, K. Kawabata, Y. Inamoto, S. Okuda, T. Kamimura, and K. Sakane, *Bioorg. Med. Chem.*, 1997, **5**, 557.
402. R. Singh, C. Fiakpui, J. Galpin, J. Stewart, M. P. Singh, and R. G. Micetich, *Eur. J. Med. Chem.*, 1996, **31**, 301.
403. M. Valencic, M. Japelj, and E. de Vroom, *Acta Chim. Slov.*, 1996, **43**, 181.
404. J. Pitlik, P. Bagossi, J. Jeko, and J. Tozser, *Pharmazie*, 1996, **51**, 700.
405. S. Deguchi, M. Fujioka, Y. Okamoto, T. Yasuda, N. Nakamura, K. Yamaguchi, and S. Suzuki, *J. Chem. Soc., Dalton Trans.*, 1996, 1967.
406. S. Deguchi, Y. Shibahara, M. T. Mooney, K. Yamamoto, T. Tada, M. Fujioka, Y. Okamoto, T. Yasuda, and S. Suzuki, *J. Inorg. Biochem.*, 1997, **65**, 191.
407. T. Rossi, C. Marchioro, A. Paio, R. J. Thomas, and P. Zarantonello, *J. Org. Chem.*, 1997, **62**, 1653.

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